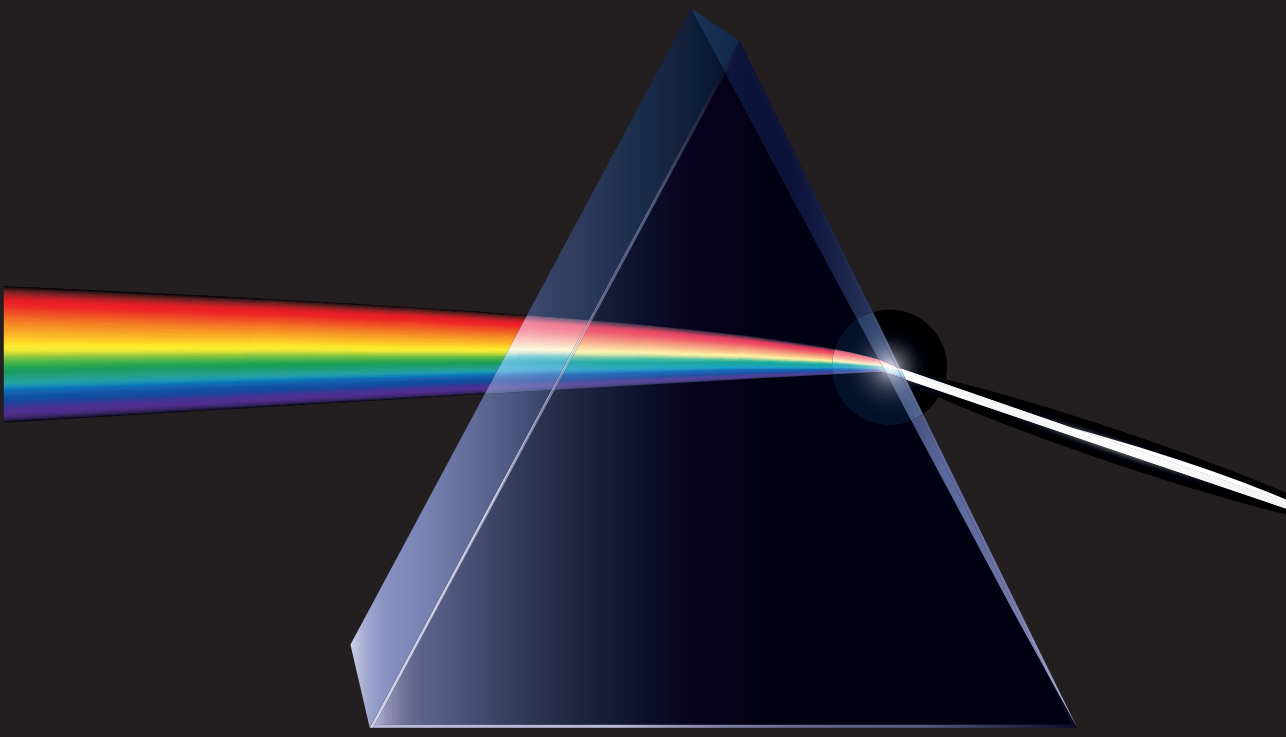
Unraveling the Genetic Architecture of Pain

Exploring the genetic factors contributing to rare/multifactorial pain disorder and pain treatment outcome



Song Li
李崧

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Unraveling the Genetic Architecture of Pain

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SONG LI
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Promotoren:
Prof. dr. B. Franke
Prof. dr. K.C.P. Vissers

Copromotoren:
Dr. M.J.H. Coenen (Erasmus Medisch Centrum)
Dr. R.L.M. van Boekel

Manuscriptcommissie:
Prof. dr. B.J.F. van den Bemt
Dr. H. Adams
Prof. dr. W.F.F.A. Buhre (Universiteit Utrecht)

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SONG LI
born on November 23, 1992
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PhD supervisors:
Prof. dr. B. Franke
Prof. dr. K.C.P. Vissers

PhD co-supervisors:
Dr. M.J.H. Coenen (Erasmus Medical Center)
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Manuscript Committee:
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Chapter 1

Introduction

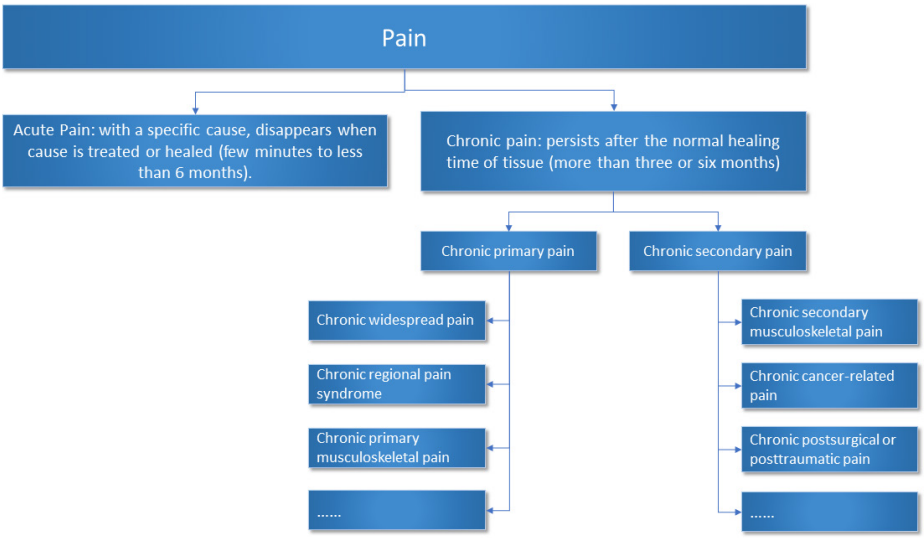

Figure 1. General structure of pain classification.

The prevalence of chronic pain in adults is approximately 20% worldwide [1], and it increases with age [2]. Pain usually starts as acute but can become chronic as a result of various factors, such as disease, surgery, and physical or mental overload [3]. According to the International Association for the Study of Pain (IASP) definition, *pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage*. Acute pain usually has a specific cause and disappears whenever the underlying cause is treated or healed. However, chronic pain persists beyond the normal healing time of tissues and typically lasts more than three months [3] or even longer than six months [4]. Chronic pain often becomes the sole or predominant clinical problem in some patients (Figure 1). In my thesis, I focused on chronic pain.

Chronic pain

Chronic pain is a significant global concern, imposing an enormous burden on society and personal health. In the Netherlands, the prevalence of chronic pain is in line with the prevalence observed worldwide [5]. Chronic pain is one of the leading causes of years lived with disability [6, 7], and it can contribute to the development of other health conditions, such as disability, depression, sleep disturbances [8], and reduced quality of life [9]. The estimated annual cost of chronic pain in the U.S.

reached up to $635 billion in 2010, attributable to healthcare costs related to pain directly and the costs associated with reduced worker productivity [10].

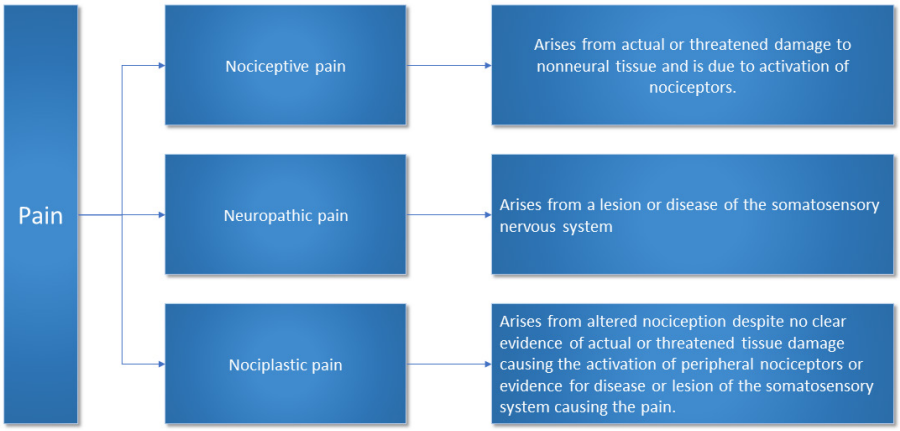

Figure 2. Classification of pain by the nature of pain.

Chronic pain can be primary or secondary. Chronic primary pain is a distinct condition not attributable to any particular classified illness (e.g., chronic widespread pain), whereas chronic secondary pain emerges as a symptom originating from another classified disease (e.g., chronic pain associated with osteoarthritis) (Figure 1). Moreover, pain can be classified as nociceptive pain (pain that arises from non-neural tissue damage and is due to activation of nociceptors) and neuropathic pain (pain that arises from a lesion or disease of the somatosensory nervous system). Recently, the term nociplastic pain has been proposed to describe clinically and psychophysically altered pain experience that cannot directly be linked to nociceptive or neuropathic pain (Figure 2) [11]. By IASP definition, *nociplastic pain arises from altered nociception without clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or pain without evidence for disease or lesion of the somatosensory system causing the pain*. An example of nociplastic pain is irritable bowel syndrome.

Multifactorial nature of chronic pain

The development of chronic pain is multifactorial and can be attributed to multiple risk factors associated with physical, psychological, and social factors. Demographic factors associated with chronic pain include age, gender, and BMI . Older age, female

gender, and obesity are linked to an increased risk for chronic pain [12-14]. However, this is not the same for all types of chronic pain, as younger age is a risk factor for chronic postsurgical pain (CPSP) [15]. In terms of lifestyle and behavior risk factors, examples of risk factors include smoking and alcohol intake [16]. Smokers are more likely to suffer from chronic pain than non-smokers [17]. Alcohol consumption has an inverse association with the occurrence of chronic pain. Plausible mechanisms could explain this protective effect, e.g., expectations about the analgesic effects of alcohol can affect pain perception. However, other explanations, including reverse causation, are also probable [18]. The most important clinical risk factor for the development of chronic pain is the presence of another site of acute or chronic pain within the body. The greater the severity and the greater the number of sites affected by pain, the more likely it is that chronic will develop [19, 20]. In addition, patients with co-morbid physical (e.g., diabetes) [17] and mental chronic diseases (e.g., depression) [21] are more likely to suffer from chronic pain than those without.

Adapted from *Sarah E.E. Mills. et al. 2019.*

Figure 3. The associated risk factors with pain.

Besides the demographic and clinical risk factors for chronic pain, increasing evidence suggests that genetic factors influence pain sensitivity and the susceptibility of developing chronic pain [22, 23]. This is also reflected in heritability estimates ranging from 25% to 50% for different types of pain, as revealed in twin studies [24]. Genetics plays different roles in Mendelian pain disorders and multifactorial pain disorders, but both contribute to our understanding of the genetic architecture of pain disorders. An overview of the factors associated with chronic pain development can be found in Figure 3.

Multidimensionality of chronic pain

Chronic pain is a multidimensional experience that extends beyond pain itself, profoundly impacting many aspects of a patient's life as well as the lives of their significant others. It compromises physical and emotional function, affecting a patient's levels of activity (ability to work at home and job and engage in social and recreational pursuits) [25, 26]. Thus, effectively managing chronic pain requires a comprehensive, multimodal approach that addresses its physical, emotional, and socio-economic dimensions to improve overall patient outcomes and quality of life. This comprehensive, multimodal approach is not yet fully developed, not even for a type of chronic pain that mainly arises after surgery and hospital stay: chronic postsurgical pain.

Chronic postsurgical pain

Chronic postsurgical pain (CPSP) develops or increases in intensity after a surgical procedure and persists beyond the normal healing process, i.e., three months [27]. The occurrence of CPSP ranges widely from 5% to 85%, depending on the surgical site, type of surgery, duration, the likelihood of nerve damage, and perioperative factors [27]. The burden of CPSP is enormous, as 310 million operations are performed annually worldwide. Conservative estimates indicate that 23 million individuals experience CPSP annually [28]. Severe CPSP can affect patients' physical and psychological well-being, leading to reduced quality of life, limitations in physical activities, emotional distress, and sleep disturbances [29, 30]. Additionally, CPSP can lead to prolonged pain medication use, particularly opioids, which in turn contributes to the issues of opioid overuse, misuse, and addiction [31].

Despite the significant impact of CPSP, it is still underdiagnosed and undertreated [32]. The diagnosis remained limited due to a lack of appropriate diagnostic phenotyping tools and categories in the International Classification of Diseases (ICD-10). However, recently, it was included in ICD-11. Improper recognition and diagnosis of CPSP might negatively affect treatment [33]. The management of CPSP might be improved by using individualized risk prediction for clinical decision-making by healthcare providers [34]; identifying risk factors is crucial to prediction model development. Several previously identified CPSP risk factors have been included in risk prediction models, for instance, demographic characteristics (age and sex), clinical factors (psychosocial factors, preceding pain) [15, 35], and intraoperative variables [36].

However, adequate prediction of CPSP in patients has still not been achieved in clinical practice due to a lack of validation and clinical impact analyses, suboptimal sensitivity (true positive), and specificity (true negative) of the models [37]. The absence of validation stems from limited generalizability (as most models were tailored to specific populations and surgical procedures), variability in the tools employed to assess CPSP, and diverse pain measurement scales [37]. Additionally, the multidimensionality of the experience contributes to this [38]. Current models use a single pain measurement that might not truly reflect the patients' pain experiences. The discriminatory ability (sensitivity and specificity) might be improved by incorporating genetic risk factors into the models. However, the genetic factors and the underlying biological mechanism of CPSP development have not been fully elucidated, which is the subject of this thesis. Hereby, it is important to make a distinction between Mendelian pain disorders and multifactorial pain disorders, such as CPSP.

Mendelian pain disorders

Mendelian pain disorders are inherited rare pain disorders within families, including conditions such as congenital insensitivity to pain caused by loss-of-function mutations and amplification of pain caused by gain-of-function mutations. In Mendelian pain disorders, genes were identified by linkage analysis in the past, while sequencing is used nowadays. Genetic mutations identified in these disorders typically have a rare allele frequency and a strong effect size. Although the incidence of such pain disorders is rare, altered pain conditions have helped unravel the function of nociceptive neurons and highlighted the potential to target affected genes for pain treatment. More than 20 genes have been identified as causing Mendelian pain disorders [39-41]. A summary is presented in Table 1 (adapted from [39]). A classic example of a Mendelian pain disorder is primary erythermalgia, where rare mutations in the *Sodium Voltage-Gated Channel Alpha Subunit 9* (*SCN9A*) gene cause the disease. This gene encodes a voltage-gated sodium channel, which plays a significant role in nociception signaling. Primary erythermalgia is an autosomal dominant disease characterized by recurrent episodes of severe pain associated with redness and warmth in the feet or hands.

Table 1. Identified genes in patients with Mendelian pain disorders characterized by painful manifestations or painlessness. * indicates a gene ID, and # indicates a disease ID.

Gene	OMIM gene ID	Disease	OMIM disease ID	Inheritance	Pain-related manifestation
CSNK1D	*600864	FASPS2	#615224	AD	Migraine with/without aura
TRPA1	*604775	FEPS1	#615040	AD	Episodic pain in the upper body
SCN10A	*604427	FEPS2	#615551	AD	Episodic burning pain affecting distal lower extremities and hands; Hyperalgesia
SCN11A	*604385	FEPS3	#615552	AD	Episodic pain localized to the distal extremities
		HSAN7	#615548	AD	Insensitivity to pain
SCN9A	*603415	Primary erythermalgia	#133020	AD	Painful episodic reddish skin discoloration; Myalgia; Episodic burning pain in the hands and feet; itching
		CIP	#243000	AR	Painless fractures; Distal painless ulcers; Isolated absence of pain sensation
		Paroxysmal extreme pain disorder	#167400	AD	Episodic mandibular and submandibular pain triggered by eating and yawning; Episodic ocular pain; Episodico rectal pain triggered by defecation; Painful micturition; Episodic reddish discoloration associated with pain; Episodic skin flushing associated with pain; Episodic burning pain
NLRP3	*606416	FCAS1	#120100	AD	Episodic arthralgia; Episodic myalgia; Episodic headache
NLRP12	*609648	FCAS2	#611762	AD	Episodic abdominal pain; Episodic arthralgias; Episodic arthritis; Episodic myalgia; Episodic headache
NLRC4	*606831	FCAS4	#616115	AD	Episodic arthralgia
NTRK1	*191315	CIPA	#256800	AR	Diffuse pain insensitivity (including visceral pain)
ZFHX2	*617828	MARSIS	#147430	AD	Painless fractures; Painless cutaneous thermal burns; Pain insensitivity
SPTLC1	*605712	HSAN1A	#162400	AD	Distal painless ulcers due to sensory neuropathy; Distal sensory loss of pain; Sharp, lightning-like pain

Table 1. Continued

Gene	OMIM gene ID	Disease	OMIM disease ID	Inheritance	Pain-related manifestation
SPTLC2	*605713	HSAN1C	#613640	AD	Distal painless ulcers due to sensory neuropathy; Distal sensory loss of pain
WNK1	*605232	HSAN2A	#201300	AR	Painless fractures due to injury; Impaired pain sensation in distal extremities
FAM134B	*613114	HSAN2B	#613115	AR	Impaired pain sensation in distal extremities
ELP1	*603722	HSAN3	#223900	AR	Decreased pain perception
NGF	*162030	HSAN5	#608654	AR	Distal pain insensitivity
DST	*113810	HSAN6	#614653	AR	Decreased pain response
PRDM12	*616458	HSAN8	#616488	AR	Recurrent infections due to painless trauma and ulceration; Ulcerating painless lesions of distal extremities, tongue, lips; Insensitivity to pain
ATL1	*606439	HSN1D	#613708	AD	Distal painless ulcers due to sensory neuropathy; Distal sensory loss of pain; Occasional lancinating pain
DNMT1	*126375	HSN1E	#614116	AD	Sensory neuropathy affecting pain sensation in the lower/ upper limbs; Occasional lancinating pain
ATL3	*609369	HSN1F	#615632	AD	Distal painless ulcers due to sensory neuropathy; Distal sensory impairment to pain
KIF1A	*601255	HSN2C	#614213	AR	Ulceration and amputation of fingers and toes due to sensory loss; Panmodal distal sensory loss; Spontaneous pain
ATP1A2	*182340	FHM2	#602481	AD	Migraine with/without aura
CACNA1A	*601011	FHM1	#141500	AD	Migraine with/without aura
KCNK18	*613655	MGR13	#613656	AD	Migraine headache with/ without visual aura, lateralized or holocranial headache
PRRT2	*614386	BFIS2	#605751	AD	Migraine
SCN1A	*182389	FHM3	#609634	AD	Migraine with/without aura
SLC2A1	*138140	DYT9	#601042	AD	Migraine, headache

Multifactorial pain disorders

The other type of pain disorder is a multifactorial pain disorder, such as chronic postsurgical pain. Multifactorial pain disorders result from a combination of genetic, environmental, and psychological factors. Genetic risk factors play a significant role in influencing pain perception and susceptibility, with the combined effect of many single nucleotide polymorphisms (SNPs) with common allele frequency (> 5%) having a small effect size. In general, SNPs are identified in large populations of unrelated individuals with or without the condition (cases/controls) using either a targeted selection of candidate SNPs or a genome-wide panel of SNPs (genome-wide association analysis).

Research on genetic variants associated with CPSP is still in its initial phase. Two recent systematic literature reviews on genetic association studies of (chronic) postsurgical pain showed that only three variants in three genes (*OPRM1* rs1799971, *COMT* rs4680, and *KCNS1* rs734784) remained significantly associated with CPSP after meta-analysis [42, 43]. The majority of genetic studies on pain have primarily focused on acute pain, such as analgesic requirements and pain score rating after an operation, and previous studies are mostly candidate gene studies [44, 45], which might overlook the beyond-known mechanisms. Hypothesis-free methods, such as genome-wide association studies on large cohorts, are needed to discover the genetic background of CPSP further.

Pain treatment

Pain management differs for nociceptive, neuropathic, or nociplastic pain. The treatment of nociceptive pain follows the WHO three-step analgesic ladder [46]: the first treatment step is non-opioid analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs); the second step is weak opioids for mild to moderate pain, such as tramadol; the third step is strong opioids for moderate to severe pain, such as morphine. However, drugs effective for nociceptive pain may not work for neuropathic pain due to the different underlying mechanisms between nociceptive and neuropathic pain. This is reflected in the recommended first-line treatments for neuropathic pain, primarily based on antidepressants and antiepileptic drugs. Since this thesis focuses on the treatment of nociceptive pain using the WHO three-step analgesic ladder, guidelines for other pharmacological treatments of pain, such as medications for neuropathic pain, can be found elsewhere [47].

Besides the genetic predisposition for pain susceptibility, genetic factors can also contribute to pain treatment response. The interindividual variance of analgesic responsiveness and side effect profiles are at least partly determined by genetics [48, 49]. For instance, genetic variation in the *cytochrome P450 2D6* (*CYP2D6*) gene significantly impacts pain management outcomes by converting parent drugs like codeine or tramadol into their active metabolites. The Dutch Pharmacogenetics Working Group recommends *CYP2D6* genotyping for codeine as "beneficial", suggesting testing before or shortly after starting treatment [50].

Research on genetic associations with pain treatment still has room for optimization. Most studies focus on acute pain treatment outcomes, such as analgesic requirements or pain relief scores after surgery [51, 52], but long-term pain treatment outcomes are less frequently investigated. Additionally, current studies are limited by small gene panels and sample sizes, often reporting contradictory results. The most studied genetic variant is the A to G base pair change at coding position 118 in the *OPRM1* gene (rs1799971), with the G allele showing inconsistent results across different studies [53, 54]. Therefore, definitive conclusions on these genetic associations cannot be drawn yet, and a non-hypothesis-driven approach in a large population is needed.

Genetic research methodology and selection considerations

For Mendelian (pain) disorders, as previously mentioned, the hypothesis is that these conditions are caused by genetic variants with low frequency but high penetrance (the proportion of individuals carrying a specific genetic variant who exhibit the symptoms of a genetic disorder) [55]. To identify the disease-causing variants, linkage analysis was traditionally the most common method before sequencing technologies became the primary choice. Linkage analysis locates a disease-causing gene by identifying chromosomal regions that are co-inherited with the known gene markers or trait of interest. Currently, whole-exome sequencing (WES) or whole-genome sequencing (WGS) is most commonly method to detect potential causal genetic variants in Mendelian disorders.

For multifactorial (pain) disorders, according to the "common disease, common variants" hypothesis, if a genetically influenced disease is common in the population (with a prevalence greater than 1–5%), then genetic variations of moderate frequency and relatively low penetrance collectively contributing to

genetic susceptibility [56]. Therefore, screening variants in large populations at low cost is optimal, making genome-wide association studies (GWAS) the preferred approach for studying multifactorial disorders. A summary of the methodologies used in genetic studies can be found in Figure 4.

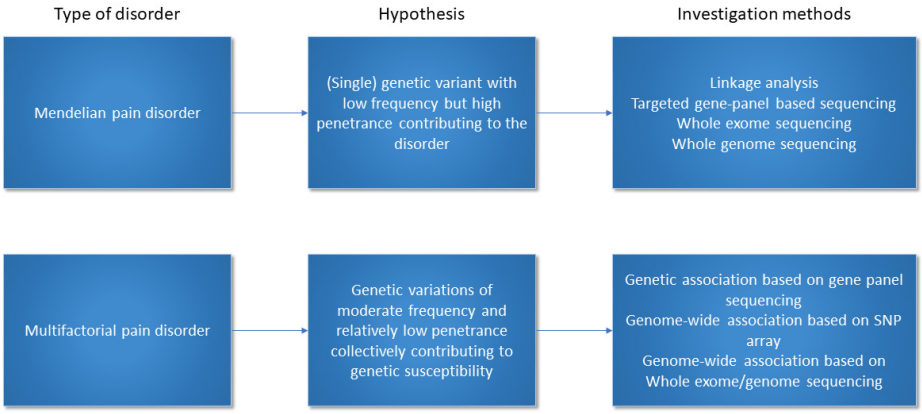

Figure 4. Hypotheses and method selection for investigating different types of disorders.

The aim of this thesis

This thesis aimed to increase the knowledge of genetic factors associated with pain and pain treatment response.

Specifically, we aimed to

1) identify genetic variants associated with the Mendelian pain disorder, primary erythermalgia,

2) review and identify genetic variants associated with chronic postsurgical pain using a GWAS approach,

3) identify genetic variants associated with pain treatment outcome,

4) present a research protocol that serves as an example for further research on chronic postsurgical pain. The identified genetic variants might serve as a tool to optimize chronic pain management.

Outline of the thesis

In **Chapter 2**, we present two families with primary erythermalgia where pathogenic variants in the *SCN9A* gene were ruled out, and no other disease-causing mutation was found with linkage analysis by SNP array and whole exome sequencing analysis. In this chapter, further examination was conducted to uncover the disease-causing gene(s) by whole genome sequencing. Six patients presenting the disease and two without symptoms were included in the whole genome sequencing analysis. The pathogenicity of identified variants was examined with Integrative Genomics Viewer inspection, and a relevance check was conducted in the dbSNP database. This study explored possible candidate genes, other than *SCN9A*, for primary erythermalgia.

In **Chapter 3**, we systematically reviewed and summarized genome-wide association studies (GWASes) investigating the associations between genetic variants and pain or pain-related phenotypes (nociception, neuropathy) in humans. We reviewed 57 full-text articles and identified 30 loci reported in more than 1 study. To check whether genes identified in this review are associated with (other) pain phenotypes, we searched two pain genetic databases, the Human Pain Genetics Database, and the Mouse Pain Genetics Database. Finally, we give recommendations concerning the most interesting genes related to pain for validation.

To investigate the genetic background of multifactorial pain disorders, we focused on chronic postsurgical pain. As the genetic background of chronic postsurgical pain remains largely unclear, we aimed to investigate this further by performing a GWAS including participants from the UK Biobank who underwent surgery. In **Chapter 4**, we aimed to identify SNPs associated with CPSP development after abdominal surgery, one of the most common surgeries. The identified loci were selected for further validation (RNA expression analysis) in clinical samples of adhesions from patients with and without pain. This study provided preliminary evidence for genetic risk factors implicated in CPSP following abdominal surgery.

In **Chapter 5**, we expanded the surgical procedures selection to all major and minor surgeries and conducted a GWAS in subjects from UKB. In this analysis, we hypothesized that pain experience will overlap between subjects undergoing major and minor surgeries (i.e., the true effect size of SNPs is the same between CPSP development after major and minor surgeries). In addition, we aimed to explore the genetic correlations of CPSP development after different surgical procedures. This study provided a foundation to examine the function of the identified risk variants

and offered summary statistics for future investigations into the mechanisms underlying CPSP.

In **Chapter 6**, we describe an ongoing genetic study for CPSP at our research center. This is a prospective, observational study. Patients undergoing elective surgery will be recruited to a sample size of approximately 10,000. The primary objective of this study is to identify specific genetic risk factors for acute and chronic postoperative pain development, followed by constructing a prediction model facilitating more personalized postoperative pain management for each individual. The secondary objectives are to build a databank enabling researchers to identify other risk factors for postoperative pain, for instance, demographic and clinical outcome indicators; provide insight into (genetic) factors that predict pharmacological pain relief; and investigate the relationship between acute and chronic postoperative pain. This protocol can serve as an example for future research on CPSP, aiming to reduce heterogeneity in pain measurements considering the multifactorial and multidimensional nature of pain.

In **Chapter 7**, we investigated the genetic component of treatment outcome differences, and we performed a GWAS in participants with musculoskeletal pain from the UK Biobank. The phenotype was NSAID vs. opioid users as a reflection of the treatment outcome of NSAIDs. Pathway and network analyses identified immunity-related processes and a (putative) central role of EGFR. This study shed light on the genetic factors influencing long-term pain treatment outcomes.

In **Chapter 8**, a general discussion is presented to summarize the findings across different chapters, integrate them with literature, and provide directions for future research, followed by a summary in **Chapter 9**.

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1

Chapter 2

Investigating Genetic Variants in Primary Erythermalgia Patients without SCN9A Mutations: findings from Whole Genome Sequencing

Song Li [1], Joost P.H. Drenth [2], Angelien Heister [1], Rene te Morsche [3], Marieke J.H. Coenen [1,4], Barbara Franke [1]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[2] Department of Gastroenterology and Hepatology, Amsterdam UMC, The Netherlands

[3] Department of Gastroenterology and Hepatology, Radboud University Medical Center, Nijmegen, The Netherlands

[4] Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands.

Abstract

Erythermalgia (EM) is a rare disorder characterized by recurrent attacks of red, warm, and painful swollen extremities. EM can be primary, due to gain-of-function missense mutations in the sodium voltage-gated channel alpha subunit 9 (*SCN9A*) gene. This primary type of EM is inherited in an autosomal dominant manner. EM can also present as secondary EM, stemming from an underlying disease or medication use. This study presents two families with primary EM where pathogenic variants in *SCN9A* were ruled out, and no other disease-causing mutations were found with linkage, SNP array, and whole exome sequencing analysis.

In this study, further examination was conducted in the two families to uncover the disease-causing gene by whole genome sequencing. Six patients presenting primary erythermalgia and two without symptoms were included in the whole genome sequencing analysis. The pathogenicity of identified variants was examined with Integrative Genomics Viewer inspection, and a relevance check was conducted in dbSNP.

Seventeen single nucleotide variants (SNVs) were present in affected members of both families, but none were considered pathogenic. After excluding intergenic and non-coding RNA variants for copy number variants and structural variants, 97 overlapping genes harboring potentially disease-causing mutations were identified. Further filtering focusing on neurology, nociception, and pain-related gene functions resulted in ten candidate genes. However, none of the genes and variants could be linked to the disease with certainty.

The challenge of pinpointing the causal gene for EM in these families highlights the complexity of the underlying genetic cause of this disorder.

Keywords
Primary Erythermalgia, Whole Genome Sequencing, SCN9A

Introduction

Erythermalgia (EM) is a rare disorder, with an incidence of 0.25 to 1.3 per 100,000 persons a year [1, 2]. EM is characterized by symmetrical recurrent attacks of red, warm, and painful swollen extremities [3-5]. The symptoms are provoked by warmth or exercise and can be intermittent or constantly present [6]. A range of therapeutic options has been proposed, including medications used for other pain conditions, such as topical capsaicin cream, selective serotonin reuptake inhibitors, anticonvulsants, calcium channel blockers, tricyclic antidepressants, and mexiletin [5, 7]. However, treating EM remains unsatisfactory due to variable treatment responses, and it is challenging to achieve consistent and lasting relief of symptoms [8]. Targeted treatments, such as selective Nav1.7 inhibitors targeting the affected proteins, could potentially offer new hope for managing primary EM [9, 10].

EM can manifest as either primary (OMIM 133020, ORPHA 90026) or secondary (ORPHA 529864). Both were previously classified under the same name; however, a distinction was made recently [5]. In both primary and secondary EM, individuals may experience the simultaneous presence of vasculopathy and neuropathy. Primary EM is inherited in an autosomal dominant way, as shown in families with multiple affected members[5]. Sometimes, it occurs as a sporadic disease without any relevant family history [11]. It is unclear yet whether secondary EM can only be sporadic or might run in the family as well [12]. Although primary and secondary EM were previously classified as one disease, they show some different characteristics. Primary EM has a more symmetrical symptom distribution and younger onset age (in the first decade) than secondary EM [5]. Primary EM is caused by gain-of-function missense mutations in *SCN9A*. *SCN9A* encodes the voltage-gated sodium channel subunit alpha Nav1.7, and mutations in this gene can lead to a significant hyperpolarizing shift in the voltage dependence of activation (facilitating channel opening), slowed channel deactivation (keeping the channel open for longer time period once activated), and an increased ramp current (causing an increase in amplitude of the current produced in response to slow, small depolarizations) [13]. Recently, genetic heterogeneity was found for primary EM [14], which shows that *SCN9A* might not be the only causal gene for primary EM. Some studies showed that primary EM can be caused by mutations in other sodium channel families, such as Nav1.8 [15, 16] and Nav1.9 [17]. Compared with primary EM, secondary EM has a more asymmetrical distribution and can begin at any age. The cause of secondary EM can result from an underlying disease (such as essential thrombocythemia, which is the most frequent cause of secondary EM) or from medication use (such as verapamil) [13].

Here, we present findings from two families diagnosed with primary EM, which had been extensively investigated before. Pathogenic variants in *SCN9A* had been ruled out in both families through Sanger sequencing. Sequencing results for one of the families were previously reported [18]. Furthermore, linkage analysis (using SNP arrays) and whole exome sequencing (WES) had been performed in both families. Unfortunately, none these efforts had led to the identification of the pathogenic variants in the families. In this study, we aimed to uncover the pathogenic variants in these two families through whole genome sequencing (WGS).

Methods

Subjects

The pedigrees of both families can be found in Figure 1. The diagnosis of primary EM in the families was confirmed by the patient's history and the clinical findings. The onset age ranged from 21 years to 76 years for family 2. More details of clinical assessment for family 2 can be found elsewhere [14].

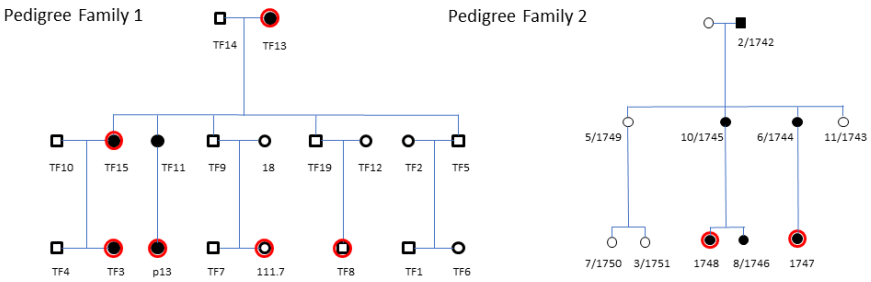

Figure 1. Pedigree of the Pain Family. Black symbols indicate affected individuals, while white symbols indicate unaffected individuals. Squares represent males, and circles represent females. The combination of letters and numbers alone indicates each subject's pseudonymized ID.

Whole Genome Sequencing

Four affected family members from Family 1 and two from Family 2 were selected for WGS, and two unaffected subjects from Family 1 were also sequenced for reference. Family members included for sequencing are indicated in red in Figure 1. WGS was performed by BGI (Hong Kong, China) on a BGISeq500 using a 2x 100 bp paired-end module, with a minimal median coverage per genome of 30-fold. BWA V.2.2.1 and Qualimap V.2.2.1 were used for read mapping along the hg38 reference genome (GRCh38/hg38) and bam quality control, respectively.

Single nucleotide variant (SNV) calling was carried out using xAtlas V.0.1, and variants were annotated using an in-house developed pipeline. This variant annotation was performed using the Variant Effect Predictor (VEP V.91) and Gencode V.34lift37 basic gene annotations. Frequency information was added from GnomAD V.2.1.1 and an in-house database. In-house gene panel information was added for those genetic variants within a known disease gene. Additional annotations were included, such as CADD score V.1.6 [19], spliceAI [20], OMIM [21], and KEGG pathways [22].

Structural variants (SVs) were called using Manta Structural Variant Caller V.1.1.0 (Illumina), following a paired-end and split read evidence approach for SV identification. Copy number variants (CNVs) were called using Control-FREEC V.11.6, which detects copy number changes and allelic imbalances based on read depth. SVs and CNVs were annotated using an in-house developed pipeline. This pipeline was based on ANNOVAR and Gencode V.34lift37 basic gene annotations. Additional frequency information was added from GnomAD V.2.1, 1000G V.8, and the GoNL SV database.

Variant prioritization

Since EM is considered an autosomal dominant disease, we used a common approach to find the pathogenetic variants for such disorders: the overlapping strategy [23]. That is to identify the same genetic variants (the same variant type and genomic coordinate) shared by all affected individuals in both families, while excluding those variants found in unaffected controls. We refer to the identified shared variants as "overlapping variants" in the following text.

Besides overlapping variants, it is possible that the exact same pathogenic variant is not shared between two families, but both families may harbor different pathogenic variants in the same gene. This can be different types of variants or the same variant type at different genomic coordinates). Therefore, we expanded our search to identify sharing of mutated genes by first identifying genetic variants present only in affected individuals but absent in unaffected controls within each family. After that, we went on to find a mutated gene was shared across two families although the variant type and/or genomic coordinate of the variant differed between families. We refer to these identified genes as "overlapping genes" in the following text.

After variant calling and annotation, variants were prioritized using the following criteria. For SNV, the criteria were as follows: total reads greater than 5, variation

percentage ranging from 15 to 85, synonymous variants were excluded, and the allele frequency of an SNV was less than 0.0001 in all databases (gnomAD-G, gnomAD-G NFE, inhouse WGS). For CNV prioritization, variants were kept if they passed the following filters: heterozygous variants were included (as primary erythermalgia is considered an autosomal dominant disease), allele frequency less than 0.0001, and variant OVERLAP less than 95% in all databases (INHOUSE CNV database, Decipher). A CNV was considered benign if it overlapped with known CNVs in the database (95% overlap was used as cutoff range to filter out benign CNV). For SV, the following prioritization was followed: variants were kept if the quality score was greater or equal to 100, GQ (Phred-scaled Probability that the call is incorrect) greater or equal to 15, heterozygous genotype, paired reads greater than 8, and split reads greater than 0.15 (paired-end reads generated from both ends of a DNA fragment in paired-end sequencing; split reads occur when a single DNA fragment maps to multiple distinct genome locations, indicating the presence of structural variations such as deletions, insertions, or translocations. Both are parameters for the quality control of SV).

Given our assumption of complete penetrance for EM, i.e., individuals carrying the mutation will present the disease, we selected mutations that present solely in affected cases (and thus absent in unaffected family members) for further analysis. First, variants present in both families were investigated. To identify overlapping variants, variants of the same type, occurring in the same gene and at the same genomic coordinates were examined across both families. Finally, to identify overlapping genes, we analyzed all variants, regardless of variant type, within the same genes but with differing genomic coordinates between the two families. Intergenic and non-coding RNA variants were excluded for CNV and SV in the last analysis step. Results of overlapping genes are presented in different manners based on variant type. SNVs were selected and checked for presence in genes linked to neurology, nociception, and pain. For CNV and SV prioritization, the start and end genomic coordinate position can differ among patients/controls. An R package convaq was used to identify the overlapping segments [24].

Additionally, shared variants falling into prioritized genomic regions from previously performed analysis and literature were examined. The first region on chromosome 2 spans from genomic position 166145185 to 166425944. This region is the *SCN9A region + 50 Kb*, to check whether mutations in *SCN9A* have been missed in our previous analysis. The other regions were previously identified through linkage analysis in our earlier exploration. Regions with positive LOD scores in both families (chr2 36536219 – 38001525, chr6 77293888 - 98809851) were selected. Thus far, no

disease-causing regions or genes were detected with our previously performed linkage and WES analyses. In these previous analyses, all variants were examined, including the variants in the intergenic regions and non-coding RNA.

Results

An overview of the number of variants in affected persons per family and the overlapping variants after variant prioritization is depicted in Figure 2. Among the variants found to be present in affected persons, 17 overlapping SNVs were identified in both families (data not shown). However, none of these variants were considered as pathogenic. Fourteen of the 17 SNVs were present in controls upon inspection of the sequence data using the Integrative Genomics Viewer (IGV) [25], and three SNVs lacked relevance when checked in the dbSNP database. No shared CNVs or SVs in the same genomic region were identified.

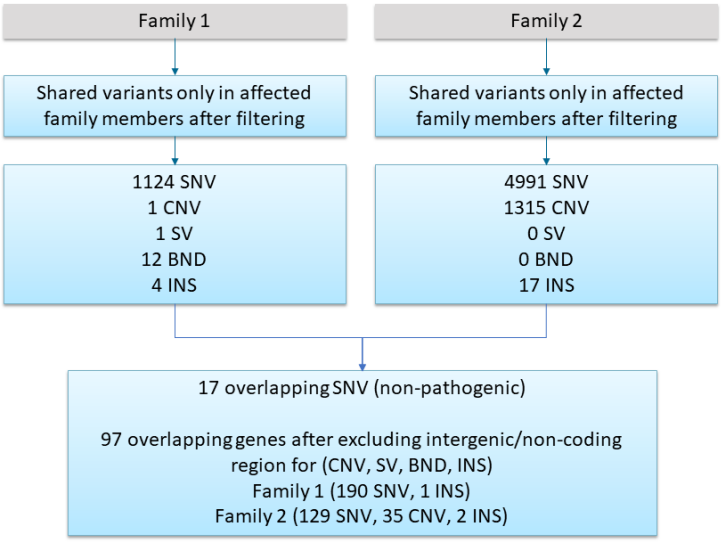

Figure 2. Workflow to find the overlapping genes between Family 1 and Family 2. Firstly, when selecting the overlapping variants in each family, the box at the bottom presents the genes shared between the two families. SNV: Single Nucleotide Variant. CNV: Copy Number Variant. SV: Structural Variant. BND: Breakend. INS: Insertion.

After excluding intergenic and non-coding RNA variants in CNV and SV, we identified 97 overlapping genes. These overlapping genes carried possible disease causing variants, however, the variants were not the same in both families. To highlight the most relevant variants, further filtering was applied by focusing on overlapping

genes with SNVs (thus excluding overlap with CNV and SV) and functions related to neurology, nociception, and pain. Ten genes remained after this filtering, and SNVs in these genes are presented in Table 1. Although none of the overlapping genes were directly involved in nociception or pain, they are implicated in neurological functions. Specifically, the functions of these genes include nervous system development (*ALK*, *NBEA*, *NDST1*, *PIGK*), neuron development (*DGKG*), synaptic transmission (*GABRB3*, *NRXN3*), the neurotrophin signaling pathway (*MAPK9*, *NTRK3*), and neuroactive ligand-receptor interaction (*TSHR*).

Table 1. Genes overlapping between two families after prioritization based on functions related to neurology, nociception, and pain. CHR: chromosome. Start: Start position. REF: Reference. ALT: alternative variant. SNV: single nucleotide variant. NA: not available. GO: gene ontology. OMIM: online Mendelian inheritance in man. KEGG: Kyoto Encyclopedia of Genes and Genomes. AF: allele frequency.

Gene name	CHR	Start	REF	ALT	Gene component	GO description
ALK	chr2	29471741	NA	T	INTRON_REGION	[transmembrane receptor protein tyrosine kinase signaling pathway]; [brain development]; [protein amino acid N-linked glycosylation]; [nervous system development]; [protein amino acid phosphorylation];
		29494846	GT	NA	INTRON_REGION	
		29795032	T	A	INTRON_REGION	
DGKG	chr3	186243897	NA	TT	INTRON_REGION	[neuron development]; [activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway]; [intracellular signaling cascade];
		186175908	A	C	INTRON_REGION	
		186225065	C	A	INTRON_REGION	
GABRB3	chr15	26603266	A	G	INTRON_REGION	[synaptic transmission, GABAergic]; [ion transport]; [signal transduction];
		26732305	GATG	NA	INTRON_REGION	

OMIM DISEASE	KEGG Name	rs number	AF	Family
[Neuroblastoma, susceptibility to], 613014	Pathways in cancer - Homo sapiens (human); Non-small cell lung cancer - Homo sapiens (human);	NA	-1	Family 1
		NA	-1	Family 1
		rs1201692614	6.57E-06	Family 2
NA	Glycerolipid metabolism - Homo sapiens (human); Glycerophospholipid metabolism - Homo sapiens (human); Metabolic pathways - Homo sapiens (human); Phosphatidylinositol signaling system - Homo sapiens (human); Phospholipase D signaling pathway - Homo sapiens (human); Choline metabolism in cancer - Homo sapiens (human);	NA	-1	Family 1
		rs939473866	6.57E-06	Family 2
		rs968648100	6.61E-06	Family 2
[Epilepsy, childhood absence, susceptibility to], 612269 ; Developmental and epileptic encephalopathy, 617113 , Autosomal dominant	Neuroactive ligand-receptor interaction - Homo sapiens (human); Retrograde endocannabinoid signaling - Homo sapiens (human); Serotonergic synapse - Homo sapiens (human); GABAergic synapse - Homo sapiens (human); Morphine addiction - Homo sapiens (human); Nicotine addiction - Homo sapiens (human);	rs899064503	1.32E-05	Family 1
		NA	-1	Family 2

Table 1. Continued

Gene name	CHR	Start	REF	ALT	Gene component	GO description
MAPK9	chr5	180243200	T	C	INTRON_REGION	[positive regulation of gene expression]; [positive regulation of macrophage derived foam cell differentiation]; [response to cadmium ion]; [protein amino acid phosphorylation]; [induction of apoptosis in response to chemical stimulus]; [response to stress]; [JNK cascade];
		180291974	CCG	NA	5'UTR	

OMIM DISEASE	KEGG Name	rs number	AF	Family
NA	Endocrine resistance - Homo sapiens (human); MAPK signaling pathway - Homo sapiens (human); ErbB signaling pathway - Homo sapiens (human); Ras signaling pathway - Homo sapiens (human); cAMP signaling pathway - Homo sapiens (human); FoxO signaling pathway - Homo sapiens (human); Sphingolipid signaling pathway - Homo sapiens (human); Mitophagy - animal - Homo sapiens (human); Autophagy - animal - Homo sapiens (human); Protein processing in endoplasmic reticulum - Homo sapiens (human); Apoptosis - Homo sapiens (human); Apoptosis - multiple species - Homo sapiens (human); Necroptosis - Homo sapiens (human); Wnt signaling pathway - Homo sapiens (human); Osteoclast differentiation - Homo sapiens (human); Focal adhesion - Homo sapiens (human); Tight junction - Homo sapiens (human); Toll-like receptor signaling pathway - Homo sapiens (human); NOD-like receptor signaling pathway - Homo sapiens (human); RIG-I-like receptor signaling pathway - Homo sapiens (human); C-type lectin receptor signaling pathway - Homo sapiens (human); IL-17 signaling pathway - Homo sapiens (human); Th1 and Th2 cell differentiation - Homo sapiens (human); Th17 cell differentiation - Homo sapiens (human); T cell receptor signaling pathway - Homo sapiens (human); Fc epsilon RI signaling pathway - Homo sapiens (human); TNF signaling pathway - Homo sapiens (human); Neurotrophin signaling pathway - Homo sapiens (human); Retrograde endocannabinoid signaling - Homo sapiens (human); Dopaminergic synapse - Homo sapiens (human); Inflammatory mediator regulation of TRP channels - Homo sapiens (human); Insulin signaling pathway - Homo sapiens (human); GnRH signaling pathway - Homo sapiens (human); Progesterone-mediated oocyte maturation - Homo sapiens (human); Prolactin signaling pathway - Homo sapiens (human); Adipocytokine signaling pathway - Homo sapiens (human); Relaxin signaling pathway - Homo sapiens (human) K1; Type II diabetes mellitus - Homo sapiens (human); Insulin resistance - Homo sapiens (human); Non-alcoholic fatty liver disease (NAFLD) - Homo sapiens (human); AGE-RAGE signaling pathway in diabetic complications - Homo sapiens (human); Epithelial cell signaling in Helicobacter pylori infection - Homo sapiens	rs540936543 NA	1.97E-05 -1	Family 1 Family 2

Table 1. Continued

Gene name	CHR	Start	REF	ALT	Gene component	GO description
MAPK9	chr5	180243200	T	C	INTRON_REGION	[positive regulation of gene expression]; [positive regulation of macrophage derived foam cell differentiation]; [response to cadmium ion]; [protein amino acid phosphorylation]; [induction of apoptosis in response to chemical stimulus]; [response to stress]; [JNK cascade];
		180291974	CCG	NA	5'UTR	
NBEA	chr13	34953650	G	A	INTRON_REGION	[protein localization];
		35046281	A	G	INTRON_REGION	
		35404831	A	G	INTRON_REGION	
		35447677	T	C	INTRON_REGION	
		35478159	TGTTT	NA	INTRON_REGION	
		35566283	C	T	INTRON_REGION	
		35317243	C	G	INTRON_REGION	
		35652691	NA	T	INTRON_REGION	
NDST1	chr5	150516979	GT	NA	INTRON_REGION	[MAPKKK cascade]; [polysaccharide biosynthetic process]; [respiratory gaseous exchange]; [fibroblast growth factor receptor signaling pathway]; [embryonic development]; [glycosaminoglycan metabolic process]; [forebrain development]; [midbrain development]; [embryonic viscerocranium morphogenesis]; [heparan sulfate proteoglycan biosynthetic process]; [inflammatory response]; [smoothened signaling pathway]; [organ morphogenesis]; [embryonic neurocranium morphogenesis];
		150516990	NA	GT	INTRON_REGION	

OMIM DISEASE	KEGG Name	rs number	AF	Family
NA	(human); Shigellosis - Homo sapiens (human); Salmonella infection - Homo sapiens (human); Pertussis - Homo sapiens (human); Chagas disease (American trypanosomiasis) - Homo sapiens (human); Toxoplasmosis - Homo sapiens (human); Tuberculosis - Homo sapiens (human); Hepatitis C - Homo sapiens (human); Hepatitis B - Homo sapiens (human); Influenza A - Homo sapiens (human); Kaposi's sarcoma-associated herpesvirus infection - Homo sapiens (human); Herpes simplex infection - Homo sapiens (human); Epstein-Barr virus infection - Homo sapiens (human); Pathways in cancer - Homo sapiens (human); Colorectal cancer - Homo sapiens (human); Pancreatic cancer - Homo sapiens (human); Choline metabolism in cancer - Homo sapiens (human); Fluid shear stress and atherosclerosis - Homo sapiens (human);	rs540936543	1.97E-05	Family 1
		NA	-1	Family 2
Neurodevelopmental disorder with or without early-onset generalized epilepsy, 619157 , Autosomal dominant	NA	rs1429864198	6.57E-06	Family 1
		rs1290703161	6.57E-06	Family 1
		rs972482395	3.29E-05	Family 1
		rs1479006126	6.57E-06	Family 1
		rs1457183516	6.58E-06	Family 1
		rs1267536159	6.57E-06	Family 1
		rs531691818	0	Family 2
		NA	-1	Family 2
Mental retardation, autosomal recessive, 616116 , Autosomal recessive	Glycosaminoglycan biosynthesis - heparan sulfate / heparin - Homo sapiens (human); Metabolic pathways - Homo sapiens (human);	NA	-1	Family 1
		NA	-1	Family 2

Table 1. Continued

Gene name	CHR	Start	REF	ALT	Gene component	GO description
NRXN3	chr14	79027667	T	C	INTRON_REGION	[synaptic transmission]; [neurotransmitter secretion]; [synapse assembly]; [axon guidance]; [cell adhesion]; [cell adhesion];
		79048459	G	A	INTRON_REGION	
		79162110	T	NA	INTRON_REGION	
		79370748	A	G	INTRON_REGION	
		79777985	C	A	INTRON_REGION	
		79783309	A	C	INTRON_REGION	
		79819213	A	G	INTRON_REGION	
		79852770	T	C	INTRON_REGION	
		78374771	AAAAAAAA	NA	INTRON_REGION	
		78487747	ATAA	NA	INTRON_REGION	
		78537970	T	C	INTRON_REGION	
		78549057	T	C	INTRON_REGION	
		78641451	G	T	INTRON_REGION	
		79685961	C	T	INTRON_REGION	
NTRK3	chr15	88039121	AC	NA	INTRON_REGION	[mechanoreceptor differentiation]; [protein amino acid autophosphorylation]; [positive regulation of axon extension involved in regeneration]; [transmembrane receptor protein tyrosine kinase signaling pathway]; [multicellular organismal development]; [nervous system development]; [cell differentiation];
		88159947	NA	ACACAC	INTRON_REGION	
PIGK	chr1	77219723	NA	TTTATTTA	NA	[proteolysis]; [attachment of GPI anchor to protein]; [attachment of GPI anchor to protein]; [protein thiol-disulfide exchange];
		77219723	NA	TTTATTTA	NA	
TSHR	chr14	81039394	G	A	INTRON_REGION	[adult locomotory behavior]; [regulation of locomotion]; [positive regulation of multicellular organism growth]; [G-protein signaling, coupled to cyclic nucleotide second messenger]; [cell-cell signaling]; [B cell differentiation]; [positive regulation of cell proliferation]; [signal transduction]; [activation of adenylate cyclase activity by G-protein signaling pathway];
		80978102	NA	ACAC	INTRON_REGION	
		81005926	T	C	INTRON_REGION	

Analyses of the genomic regions identified in our previous research did not identify shared variants (SNVs, CNVs, SVs) in any of the families. In addition, a close examination of the *SCN9A* region did not lead to the identification of previously undetected genetic variation.

OMIM DISEASE	KEGG Name	rs number	AF	Family
NA	Cell adhesion molecules (CAMs) - Homo sapiens (human);	NA	-1	Family 1
		rs1407449498	1.97E-05	Family 1
		NA	-1	Family 1
		rs1193439836	1.97E-05	Family 1
		NA	-1	Family 1
		NA	-1	Family 1
		rs1207314736	6.57E-06	Family 1
		rs1017007059	6.57E-05	Family 1
		NA	-1	Family 2
		NA	-1	Family 2
		rs756218970	1.31E-05	Family 2
		rs759042437	3.28E-05	Family 2
		NA	-1	Family 2
		rs944484643	4.60E-05	Family 2
NA	Neurotrophin signaling pathway - Homo sapiens (human); Central carbon metabolism in cancer - Homo sapiens (human);	NA	-1	Family 1
		NA	-1	Family 2
Neurodevelopmental disorder with hypotonia and cerebellar atrophy, with or without seizures, 618879 , Autosomal recessive	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis - Homo sapiens (human); Metabolic pathways - Homo sapiens (human);	NA	-1	Family 1
		NA	-1	Family 2
Hyperthyroidism, familial gestational, 603373 , Autosomal dominant; Hyperthyroidism, nonautoimmune, 609152 , Autosomal dominant; Thyroid adenoma, hyperfunctioning, somatic ; Thyroid carcinoma with thyrotoxicosis ; Hypothyroidism, congenital, nongoitrous, 275200 , Autosomal recessive	cAMP signaling pathway - Homo sapiens (human); Neuroactive ligand-receptor interaction - Homo sapiens (human); Thyroid hormone synthesis - Homo sapiens (human); Regulation of lipolysis in adipocytes - Homo sapiens (human); Autoimmune thyroid disease - Homo sapiens (human);	rs1019482148	5.93E-05	Family 1
		NA	-1	Family 2
		NA	-1	Family 2

Discussion

In this study, we investigated likely pathogenic variants in two families with primary EM without *SCN9A* mutations; while these families had been extensively investigated with conventional genetic methods before, no causal variants had been identified. By analyzing the whole genome sequencing data, we prioritized exact same genetic variants and same genes with different genetic variation in the two families studied. Despite strict variant prioritization efforts, we ended up with a lengthy list of variants in 97 genes, including overlapping genes but not same genetic variation in the two families. Although we could not pinpoint a likely disease-causal variant, we prioritized gene functions implicated in neurology, nociception, or pain, ultimately narrowing down the list to ten promising genes.

The genes in the list were mainly implicated in neurological functions that might contribute to pain development, as pain and neurological function are known to be closely related. The nervous system plays a crucial role in the perception and modulation of pain, and many genes that are essential for the development and function of the nervous system can influence pain pathways. For instance, ALK plays an important role in brain development and affects specific neurons in the central nervous system [26]. Recently, the ALKAL2/ALK signaling axis was found to work as a central regulator of nociceptor-induced sensitization [27]. Another example is DGKG, which is implicated in neuron development. Although there is no direct evidence showing the involvement of DGKG in pain, an isoform of the gene family (i.e. DGKI) is involved in processes related to pruritogenic (itch-causing) and algogenic (pain-causing) stimuli [28]. Similarly, genes implicated in the neurotrophin signaling pathway contribute to neuron development and may be implicated in pain. For example, NTRK3 has been associated with areas of pain and/or pain intensity [29]. In addition to genes that can be linked to pain pathways, several of the prioritized genes are implicated in synaptic transmission and neuroactive ligand-receptor interaction. The process of synaptic transmission is involved in synaptic plasticity and can modulate pain pathways by affecting the excitability of neurons [30]. For example, *GABRB3* encodes receptors for neurotransmitters, such as gamma-aminobutyric acid (GABA), which may be involved in antinociception [31].

We faced challenges identifying the likely causal variants for several reasons. First, while the patients in our study are diagnosed with primary EM, there may be a probability that they actually have secondary EM rather than primary EM. The distinction between primary and secondary EM is based solely on the age of onset and the symmetry of symptoms. However, this classification lacks rigid boundaries,

with criteria that are not absolute. For instance, primary EM can manifest at a late age, but late onset is most likely related to secondary EM [32]. The patients from family 2 indeed showed late disease onset and thus might have secondary EM, and the heritability and genetic architecture of secondary EM is still not clarified.

If not the same but different variants within the same gene cause the disease in different families – as we assumed in the present study, complexity of the analysis naturally increases, especially given the small sample size. As we have seen, the gene list increases, making it even more challenging to pinpoint potentially causal variants without biological validation. The overlapping variants in each family are often (family-specific) variants of uncertain clinical significance [33] instead of actual pathogenic variants. Additionally, as the identified variants by the WGS results could just be false-positive findings [34], biological validation (such as Sanger sequencing and functional studies) is needed for pinpointing the candidate variants. Such validation is challenging when considering different variants within the same gene yielding a lengthy list of candidate variants. However, we could not further narrow the lengthy candidate gene list down by excluding the family-specific variants especially since we only sequenced two controls from Family 1. Moreover, we did not explore the possibility that pathogenic genes might differ between the families. Assuming the same pathogenic gene for both families already resulted in 97 candidate genes, if we were to consider different candidate genes in each family, it would likely produce an even longer list, making further prioritization even more challenging. To gain more insight into this, it would be important to sequence more (affected and unaffected) family members to allow analysis of each individual family to narrow down candidate variants more easily.

In this study, we investigated overlapping genes across for the two families and identified 97 genes, but we only presented the results of the SNVs. However, other types of variants than SNVs might also be involved. We chose to present only the overlapping genes from SNVs, as they are more interpretable and provide a good starting point for validation. Overlap between SNVs and CNVs/SVs was not presented because many are present in intergenic regions or non-coding RNA, whose functions are not yet clearly understood. Although we have explored the shared genetic variants between these two families using WES before, it is still possible to find shared variants in WGS that are not identified by WES because WGS is more powerful to detect variants in terms of detection rates and quality [34].

Additionally, although it is assumed that mutations in *SCN9A* show complete penetrance for EM, a survey conducted by the Erythromelalgia Association revealed

a lower-than-expected penetrance for EM [13]. Hence, it is possible that pathogenic variants (in *SCN9A* or other likely pathogenic variants) with "incomplete" or "reduced" penetrance underlie EM in the families investigated, i.e., some individuals who carry the pathogenic variant manifest the clinical condition while others do not. Our analysis focused on complete penetrance, but it could be that some of the unaffected members do carry the mutation. To get a clear picture, it would be of added value to analyze the complete families with WGS. However, this was not possible due to high costs.

For future research, we suggest a clear definition of EM. Although several distinctions have been proposed between primary and secondary EM, the up-to-date definitions of EM still do not include this distinction [35]. This hampers genetic analysis in primary erythermalgia, as genes are not expected to be the sole strong disease-causing factors in secondary erythermalgia. [5]. However, distinguishing between primary and secondary EM can be challenging. Although the symptoms distribution and onset age between primary and secondary EM might be an indication, biopsy specimens from patients with conditions show non-specific results [36]. While a thorough physical examination may offer some clues, a detailed patient history is crucial for accurate differentiation, a detailed patient history is crucial for accurate differentiation. Furthermore, our understanding of the genetics of primary EM is expanded through the screening of variants in genes encoding diverse voltage-gated sodium channels, particularly the SCN10A and SCN11A genes. [15, 37]. Although we have not identified a shared voltage-gated sodium channel gene that can be clearly linked to the disease for both families in our study, other voltage-gated sodium channel genes than *SCN9A* could be included in the candidate gene screening panel for EM. Moreover, EM has been investigated as a monogenic disorder (Mendelian disease), and the current genetic model for Mendelian disease assumes that it is caused by rare variants in a single gene with a strong effect [38]. However, it is possible that the combined effect of multiple genetic variants and non-genetic risk factors can also cause Mendelian disease. An example is rhegmatogenous retinal detachment (RRD), which can be monogenic and multifactorial [39]. This perhaps also applies to EM. Therefore, to fully understand the genetic architecture of EM, conducting a genome-wide association analysis might be beneficial. Additionally, exploring environmental factors is also crucial for a comprehensive understanding of EM that cannot be solely attributed to genetic causes. More importantly, collaboration through a consortium is essential to better understand EM. Consortia enable data sharing, pooling of resources, and interdisciplinary collaboration. Successful examples are SolveRD [40] and The Undiagnosed Diseases Network International (UDNI) [41]. To

the best of our knowledge, there is currently no consortium for EM listed on orpha.net. Establishing such an infrastructure will enhance our understanding of EM's etiology, improve diagnostics for patients with EM, and facilitate the prediction of individual disease risk and the rate of disease progression.

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Chapter 3

A systematic review of genome-wide associated studies for pain, nociception, neuropathy, and pain treatment responses

Song Li [1], Annika Brimmers[1], Regina L.M. van Boekel [2], Kris C.P. Vissers [2], Marieke J.H. Coenen [1*]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands.

[2] Department of Anesthesiology, Pain and Palliative Medicine, Radboud university medical center, Nijmegen, The Netherlands.

[*] Current address: Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands

Corresponding author:
Marieke J.H. Coenen, Ph.D. Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands. M.Coenen@erasmusmc.nl

Abstract

Pain is the leading cause of disability worldwide, imposing an enormous burden on personal health and society. Pain is a multifactorial and multidimensional problem. Currently, there is (some) evidence that genetic factors could partially explain individual susceptibility to pain and interpersonal differences in pain treatment response. To better understand the underlying genetic mechanisms of pain, we systematically reviewed and summarized a genome-wide association study (GWAS) investigating the associations between genetic variants and pain/pain-related phenotypes in humans. We reviewed 57 full-text articles and identified 30 loci reported in more than one study. To check whether genes described in this review are associated with (other) pain phenotypes, we searched two pain genetic databases, Human Pain Genetics Database and Mouse Pain Genetics Database. Six GWAS-identified genes/loci were also reported in those databases, mainly involved in neurological functions and inflammation. These findings demonstrate an important contribution of genetic factors to the risk of pain and pain-related phenotypes. However, replication studies with consistent phenotype definitions and sufficient statistical power are required to validate further these pain-associated genes. Our review also highlights the need for bioinformatic tools to elucidate the function of identified genes/loci. We believe that a better understanding of the genetic background of pain will shed light on the underlying biological mechanisms of pain and benefit patients by improving the clinical management of pain.

Keywords
Pain, Nociception, Neuralgia, Genome-wide association studies, Systematic review

Introduction

Pain, especially chronic pain, is a condition that greatly impacts the quality of life. The prevalence of chronic pain in adults is about 20% [1, 2], and it increases with age [3]. Chronic painful conditions are the leading causes of years lived with disability [4], and they can contribute to the development of other health conditions, such as disability, depression, and sleep disturbances [2]. Besides the burden for patients, chronic pain can have enormous socioeconomic consequences directly or indirectly, e.g., due to absenteeism. For example, the total costs associated with only low back pain in European countries are estimated to be 0.1-2% of gross domestic product [5, 6].

Pain is a subjective experience with very heterogeneous presentations. Pain can be acute or chronic; acute pain is usually associated with tissue damage and generally eases with the healing of tissue [7], whereas chronic pain persists or recurs for more than three months [7]. Chronic pain can develop without a clear etiology or pathophysiology (chronic primary pain, e.g., fibromyalgia) or secondary to an underlying disease (chronic secondary pain, e.g., chronic pain associated with osteoarthritis). There is a distinction between nociceptive pain -pain from ongoing tissue inflammation or damage- and neuropathic pain -pain caused by nerve damage. More recently, the term nociplastic pain has been proposed to describe the clinically and psychophysically altered nociception that cannot directly be linked to nociceptive or neuropathic pain [8]. Nociplastic pain is defined as *pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain* (IASP terminology).Examples of nociplastic pain are fibromyalgia and irritable bowel syndrome. Although there are differences in the pathways leading to the different types of pain, part of the underlying mechanisms may be shared, such as structural changes in the brain [9], central sensitization [10], and neurochemical imbalances in the central nervous system [11].

The risk of developing pain can be attributed to sociodemographic factors (e.g., age, female gender, and occupation) [12, 13], psychological factors (e.g., depression) [14], clinical factors (e.g., chronic disease) [15], and lifestyle factors [16]. In addition, pre-existing pain is related to the development of other types of pain. For instance, acute postoperative pain is a risk factor for chronic pain development after surgery [17]. Besides these factors, genetic susceptibility could also contribute to the development of pain. Indeed, heritability estimates for different pain phenotypes range from 30 to 70%, indicating that genetics contributes [18]. For

instance, the heritability of neuropathic pain, low back pain, and neck pain are estimated to be ~37%, 52-68%, and 35-58%, respectively [19, 20].

Although numerous genetic risk factors have been described for pain development and unsatisfied pain treatment response, the underlying genetic mechanisms remain elusive. One reason might be that most published studies use a hypothesis-driven approach, thus focusing on specific genes/pathways with known functions, which might be biased by previous knowledge of the etiology of pain [21]. The two most investigated genes related to pain are *COMT* (involved in neurotransmission) and *OPRM1* (encoding opioid receptor) [22, 23]. However, no consistent associations with pain have been observed for both genes from candidate gene studies [21] [24]. Hypothesis-free methods like genome-wide association studies (GWASes) are more appropriate for finding additional genes beyond known mechanisms. Indeed, GWASes have identified many putative causal genes other than the previously described candidate genes, which shed new light on the mechanism of pain development [25]. Unfortunately, most candidate and genome-wide association studies on pain report inconsistent results, which is partly due to the low statistical power of the studies. Therefore, few findings are convincing enough to be investigated further.

Besides contributing to pain development, current evidence suggests that genetic variabilities can also contribute to pain treatment response differences in terms of efficacy and side effects [32][119]. To date, several studies investigated the association between genetic variants and treatment efficacy and adverse event in the two most common drug categories for pain management, non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics [26]. A clear example is codeine treatment outcome and genetic variants in the drug-metabolizing gene *CYP2D6* [12; 24].

In this systematic review, we aimed to summarize GWASes investigating pain, nociception, neuropathy, and pain treatment responses in humans to provide an overview of the potential genetic risk factors for pain. In addition, the overlap of the identified genes in all included studies is summarized, aiming to fill the knowledge gap on the shared genetic background of pain syndromes. To provide additional evidence for the role of the identified genes in pain, we examined whether the genes identified in this systematic review were linked to pain by other studies using the human and mouse pain genetics database.

Method

This systematic review was conducted and reported following Preferred Reporting Items for Systematic Reviews and MetaAnalyses guidelines (PRISMA)[27]. This study should be viewed as a descriptive review, and we did not conduct a meta-analysis considering the broad pain phenotypes included in this study.

Systematic search

A systematic literature search was performed to assess all available literature on GWAS of pain, nociception, neuropathy, and pain treatment response. Headaches and migraine were excluded as the underlying biological mechanisms differ from other pain phenotypes. A search term including four elements was built. The first three terms were included to capture pain, and the fourth term identifies GWASes. The following terms were used: (1) pain, pain perception, or pain threshold, describing "*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (IASP terminology)*; (2) nociception or nociceptor, describing "*the neural process of encoding noxious stimuli, which pain sensation is not necessarily implied*" (IASP terminology); Nociception (pain sensitivity) was included as it has predictive value in postoperative pain [28] and pain treatment outcome [29]. (3) neuralgia or peripheral nervous system diseases or neuropathy, describing "*a disturbance of function or pathological change in a nerve: in one nerve, mononeuropathy; in several nerves, mononeuropathy multiplex; if diffuse and bilateral, polyneuropathy*" (IASP terminology); neuropathy is included because it is one of the underlying cause of neuropathic pain (4) genome-wide association study, a hypothesis-free method to scan associations between genetic variants and phenotypes. A hypothesis-free method is mainly data-driven or discovery-driven without pre-set hypotheses, e.g., testing all genetic markers on a genotyping array or whole exome/genome sequencing. The first three elements were connected by "OR" and then connected to the last element by "AND" as shown in Figure 1. Science libraries MEDLINE and Embase were searched for relevant literature using MeSH and Emtree terms, respectively (See Table S1). The literature search was performed on February 21st, 2022. To validate our search terms, we examined all publications of pain, nociception, and peripheral neuropathy traits in the GWAS Catalog to determine whether we missed relevant publications.

After removing duplicates, all identified articles were assessed by two independent reviewers (A.B. and S.L.), and paper selection was conducted independently in Rayyan [30]. A third independent reviewer evaluated papers with conflict decisions to reach a consensus (M.C. and R.v.B). As we focused on the pain itself other

than the background diseases triggering pain in this review, we excluded papers investigating the diverse background disease causing pain. Paper selection criteria details can be found in Table S2.

The review was not pre-registered as the first intention was to write a narrative review. However, after we performed the search, the number of papers was manageable to write a systematic review. Based on this, we decided to switch to a systematic review.

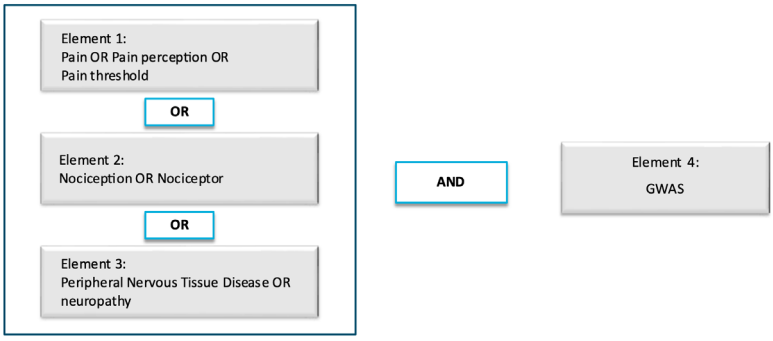

Figure 1. Search strategy for systematic review. GWAS: genome-wide association study.

Quality Assessment

The quality of studies was assessed by checking compliance with the "STrengthening the Reporting of Genetic Association studies" (STREGA) guidelines that include 30 key items in 6 categories: title and abstract, introduction, method, results, discussion, and funding information (Table S3) [31]. The quality score was calculated for each study based on the sum of each assessed item. Higher scores represent studies of higher quality. No quality score threshold was set to select papers.

Data extraction

For each paper included in this review, the following information was extracted: PMID, first author, publication year, outcome phenotype, phenotype variable type (e.g., continuous, discrete, time-to-event, or binary), study characteristics (sample size, ethnicity, P-value threshold applied in the original paper, number of significant loci) of discovery, replication, and meta-analysis phase, and SNPs associated with the investigated phenotype. The phenotypes investigated in the included papers as defined by the authors of the original publication can be found in Table S4.

To reduce reporting of possible false positive findings, an upper boundary of P < 1E-5 was set for associated SNPs. We referred to this threshold as a suggestively significant threshold. The genome-wide significance mentioned in the following paragraphs is defined as the conventional value of GWAS threshold, 5E-8. If multiple SNPs within the same loci/gene were identified, only the most statistically significantly associated SNP was extracted for inclusion in this paper. The following information was extracted for selected SNPs: rsid; allele frequency, effect size, and standard error of effect allele; and the P-value in the discovery, replication, and meta-analysis phase if applicable. For effect size, values from the meta-analysis phase were prioritized to report whenever available. If an odds ratio was reported, it was converted to effect size by natural logarithm to make results comparable.

When the included papers indicated that they aimed to replicate GWAS-identified loci from previous studies we included this in the review. We describe this in each phenotype section using the following wording "replication" or "replicated". We use the word “overlap” to indicate our own search for overlap between the studies as described in the paragraph below and in section 3.11.

Follow-up research

As genomic position and (nearby) genes of extracted loci were not always reported, and different papers use different reference genome versions for annotation, all extracted variants were reannotated to genes by wANNOWAR [32] and Ensembl Variant Effect Predictor (VEP) [33]. If a variant was located in an intergenic region, it was mapped to the closest genes (upstream and downstream). Chromosome band was obtained in the UCSC genome browser [34]. All annotations were based on homo sapiens (human) genome assembly GRCh37 (hg19).

To investigate whether the identified loci/genes from included papers overlap with the same pain phenotypes or between different pain phenotypes, we first examined the linkage disequilibrium (LD) of extracted variants with LD matrix [35] in CEU ancestry. Besides checking LD, all SNPs were mapped to (closest) genes (see above), and the mapped gene symbols were checked for overlap.

All mapped genes from the included GWASes were queried in the human [36] and mouse [37] pain genetics database to find additional evidence that the genes contribute to pain phenotypes. The Human Pain Genetic Database (HPGDB) is a comprehensive variant-focused inventory of genetic contributors to human pain summarized from both candidate gene studies and GWASes. This database was updated until July 2021. Before querying, papers already included in this review were removed from the

HPGDB. The mouse pain genetic database included 434 genes involved in acute or tonic nociception, injury- or stimulus-induced hypersensitivity (i.e., allodynia or hyperalgesia), or drug- or stress-induced inhibition of nociception (i.e., analgesia) in the mouse. This database only contains the results of papers published before 2015.

Results

Literature search

A literature search in MEDLINE and Embase resulted in the identification of 579 articles. Figure 2 illustrates the paper selection workflow and reasons for exclusion. During the screening process, 32 duplicates were removed, and after screening titles and abstracts, 474 articles were excluded as they were not in line with our research question. The full text of the 73 remaining studies was reviewed, which led to the exclusion of 16 papers because of the outcome (n = 9), study design (n = 5), or publication type (n = 2). Details concerning the reason for exclusion are described in Table S5. To ensure that no papers were missed, the GWAS catalog was checked using the phenotype keywords "pain" and "neuropathy". Five additional papers were identified but not included in this review as they did not meet the inclusion criteria (Table S5).

Included studies

The characteristics of the 57 included papers are summarized in Table 1. The STREGA quality score of the studies ranged from 16 to 29 (see Table S6). Most studies reported on participants with European ancestry (including Hispanic) (n=44). Thirty-two studies had a relatively small sample size (<1000 samples), while the studies with a large sample size mainly included data from the UK Biobank (UKB) (n=12). Twenty-four studies (42%) did not include a replication cohort (see Table S7 for replication and meta-analysis information of included papers).

We followed the eleventh revision of the International Classification of Diseases (ICD-11) of chronic pain (see Figure 3) to define the phenotypes reported in the identified papers. Papers were categorized based on anatomical sites. Cancer pain was investigated most (n=17), followed by musculoskeletal pain (n=14) and neuropathic pain (n=9). Pain sensitivity is the least investigated phenotype with only one paper.

Below is a summary of the included studies focusing on overlapping findings between the studies. Details of all loci meeting the inclusion criteria are provided in Supplementary Data S1. Phenotype definitions were added in each general section (cancer pain, musculoskeletal pain, neuropathic pain, postoperative pain, visceral

pain, and orofacial pain), mostly from ICD-11. The definitions of pain sensitivity and pain treatment response were not added as the diverse phenotypes were used in different studies and lacked an official definition. Definitions used in the included studies may sometimes differ from the official definitions, the definitions used in the included papers can be found in Table S4.

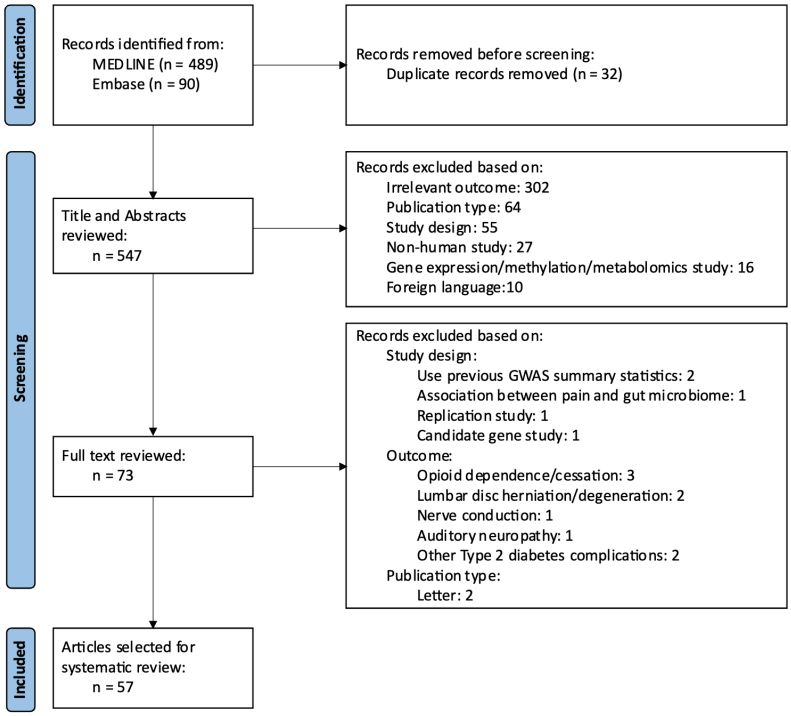

Figure 2. Systematic literature search and assessment process according to PRISMA principles.

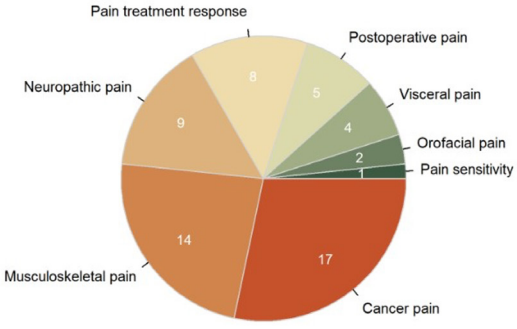

Figure 3. The number of published studies on different pain conditions included in this review. Note: the total number of studies in this figure is 60 rather than the total number of papers (n=57), as three papers investigated different pain phenotypes that are not in the same category.

Table 1. Characteristics of included studies in this review.

PMID	Author, year	Outcome phenotype(s)	Phenotype category	Phenotype variable type
33802509	Adjei, 2021	Paclitaxel; paclitaxel and carboplatin; or oxaliplatin receipt induced sensory symptoms	Cancer pain	Continuous
22843789	Baldwin, 2012	Paclitaxel-induced peripheral neuropathy: the maximum grade neuropathy observed	Cancer pain	Discrete
		Paclitaxel-induced peripheral neuropathy: the cumulative dose level	Cancer pain	Time-to event
28317148	Campo, 2017	Bortezomib-induced peripheral neuropathy	Cancer pain	Binary
32562552	Chua, 2020	Microtubule targeting agents induced peripheral neuropathy	Cancer pain	Time-to event
24909733	Cook-Sather, 2014	Acute postoperative pain	Postoperative pain	Binary
		Opioid analgesia	Pain treatment response	Continuous
25710658	Diouf, 2015	Vincristine-induced peripheral neuropathy	Cancer pain	Binary
24582949	Docampo, 2014	Fibromyalgia	Musculoskeletal pain	Binary
28611204	Dolan, 2017	Cisplatin-induced peripheral neuropathy	Cancer pain	Oridinal
32681239	Dunbar, 2020	Constant-severe pain in chronic pancreatitis	Visceral pain	Binary
34924555	Fontanillas, 2021	Cold pressor test	Pain sensitivity	Time to event
		Pain sensitivity questionnaire score	Pain sensitivity	Continuous
30747904	Freidin, 2019	Chronic back pain	Musculoskeletal pain	Binary
33021770	Freidin, 2021	Chronic back pain	Musculoskeletal pain	Binary
21622719	Galvan, 2011	Opioid analgesia	Pain treatment response	Discrete
27605156	García-Sanz, 2017	Bortezomib and thalidomide induced peripheral neuropathy	Cancer pain	Binary
27143689	Hertz, 2016	Docetaxel-induced peripheral sensory neuropathy	Cancer pain	Time to event
29855537	Hirata, 2018	Dysmenorrhoea pain	Visceral pain	Discrete
28025368	Janicki, 2016	Complex regional pain syndrome	Musculoskeletal pain	Binary
31194737	Johnston, 2019	Multisite chronic pain	Musculoskeletal pain	Discrete

	Discovery study design			
	Sample size	**Ethnicity**	**P-value threshold**	**No. of significant locus**
	692	EA; AA; Asian; American Indian or Alaska Native; others	1.00E-06	3
	855	EA	1.00E-05	4
	855	EA	1.00E-05	7
	cases 102, controls 544	German	1.00E-05	4
	469	EA	1.00E-05	0
	In EA, cases 132, controls 136 In AA, cases 103, controls 118	EA; AA	1.00E-05	2 in EA
	EA 277, AA 241	EA; AA	1.00E-05	3 in EA; 9 in AA
	cases 89, controls 232	EA; AA; Asian; Hispanic; others	1.00E-05	5
	cases 300, controls 203	White Spanish	1.00E-05	9
	680	EA; others	1.00E-05	13
	cases 787, controls 570	EA	1.00E-05	1
	6853	EA	1.00E-06	1
	25321	EA	1.00E-06	3
	cases 91100, controls 258900	EA	5.00E-08	5
	In males, cases 35705, controls 166372 In females, cases 43230, controls 194524	EA	5.00E-08	7 in females; 2 in males
	438	EA	1.00E-05	8
	cases 40, controls 132	Not report	1.00E-05	3
	623	EA	1.05E-05	3
	11348	Japanese	5.50E-09	2
	cases 230, controls 230	EA; AA; Hispanics	2.50E-07	0
	387649	EA	5.00E-08	39

Table 1. Continued

PMID	Author, year	Outcome phenotype(s)	Phenotype category	Phenotype variable type
33830993	Johnston, 2021	Multisite chronic pain	Musculoskeletal pain	Discrete
27454463	Jones, 2016	Dysmenorrhoea pain	Visceral pain	Discrete
34391895	Kanai, 2021	Oxaliplatin induced peripheral sensory neuropathy: Grade2/3 vs Grade 0	Cancer pain	Binary
		Oxaliplatin induced peripheral sensory neuropathy: Grade2/3 vs Grade 0/1	Cancer pain	Binary
19207018	Kim, 2009	Acute postoperative pain	Postoperative pain	Discrete
		NSAID analgesia	Pain treatment response	Continuous
26015512	Komatsu, 2015	Paclitaxel-induced sensory peripheral neuropathy	Cancer pain	Binary
23776197	Leandro-García, 2013	Paclitaxel-induced peripheral sensory neuropathy	Cancer pain	Time-to event
31196165	Lee, 2019	Acute post-radiation therapy pain	Postoperative pain	Binary
24554482	Leger, 2014	Stavudine and didanosine induced peripheral neuropathy	Neuropathic pain	Binary
27764105	Lemmela, 2016	Sciatica	Neuropathic pain	Binary
28447608	Li, 2017	Dysmenorrhoea pain	Visceral pain	Binary
30506673	Li, 2019	Vincristine-induced peripheral neuropathy	Cancer pain	Time-to event in discovery cohort; discrete in replication cohort
27060151	Magrangeas, 2016	Bortezomib-induced peripheral neuropathy	Cancer pain	Binary
24974787	Meng, 2015 #1	Diabetic neuropathic pain	Neuropathic pain	Binary
26629533	Meng, 2015 #2	Diabetic neuropathic pain	Neuropathic pain	Binary
31482140	Meng, 2019	Chronic knee pain	Musculoskeletal pain	Binary
32246137	Meng, 2020	Shoulder and neck pain	Musculoskeletal pain	Binary
26566055	Mieda, 2016	Opioid analgesia	Pain treatment response	Continuous
23183491	Nishizawa, 2014	Opioid analgesia	Pain treatment response	Continuous

Discovery study design			
Sample size	**Ethnicity**	**P-value threshold**	**No. of significant locus**
178556 males, 209093 females	EA	5.00E-08	10 in females; 5 in males
11891	EA	1.00E-06	6
cases 233, controls 49	Japanese	1.00E-05	2
cases 383, controls 605	Japanese	1.00E-05	7
112	EA	3.30E-08	0
112	EA	3.30E-08	1
cases 24, controls 121	Asian	1.00E-05	4
144	EA	1.05E-05	10
cases 326, controls 786	African American; Hispanic Whites; non-Hispanic Whites; others	1.00E-05	3
cases 90, controls 164	EA; AA; Hispanic	1.05E-05	5
cases 291, controls 3671	Finnish	5.00E-08	2
cases 2404, controls 2920	Chinese	5.00E-08	0
1068	EA	1.05E-05	2
cases 155, controls 314	EA; others	1.05E-05	4
cases 572, controls 2491	EA	1.00E-06	1
In males, cases 470, controls 2021 In females, cases 491, controls 1239	EA	1.00E-06	1 in overall; 1 in females; 1 in males
cases 22204, controls 149312	EA	5.00E-08	2
cases 53994, controls 149312	EA	5.00E-08	3
350	Japanese	Fisrt and second stage P<0.05; final stage Q < 0.05	1
355	Japanese	Fisrt and second stage P<0.05; final stage Q < 0.05	1

Table 1. Continued

PMID	Author, year	Outcome phenotype(s)	Phenotype category	Phenotype variable type
29207912	Nishizawa, 2018	Opioid analgesia	Pain treatment response	Continuous
33685280	Nishizawa, 2021	Chronic pain	Musculoskeletal pain	Binary
		Postherpetic neuralgia	Neuropathic pain	Binary
22956598	Peters, 2012	Chronic widespread pain	Musculoskeletal pain	Binary
33926923	Rahman, 2021	Chronic widespread pain	Musculoskeletal pain	Binary
27670397	Reyes-Gibby, 2016	Severe pre-treatment cancer pain	Cancer pain	Binary
29884837	Reyes-Gibby, 2018	Neuropathy	Neuropathic pain	Binary
28081371	Sanders, 2017	Temporomandibular disorder	Orofacial pain	Binary
26138065	Schneider, 2015	Paclitaxel induced peripheral neuropathy	Cancer pain	Binary
30431558	Smith, 2019	Temporomandibular disorder	Orofacial pain	Binary
29278617	Sucheston-Campbell, 2018	Paclitaxel induced peripheral neuropathy	Cancer pain	Binary
30261039	Suri, 2018	Chronic back pain	Musculoskeletal pain	Binary
33729212	Suri, 2021	Chronic back pain	Musculoskeletal pain	Binary
29502940	Takahashi, 2018	Opioid analgesia	Pain treatment response	Continuous
31127053	Tang, 2019	Diabetic peripheral neuropathy	Neuropathic pain	Binary
32587327	Tsepilov, 2020	Genetic components of multisite chronic pain	Musculoskeletal pain	Continuous
31903573	van Reij, 2020	Chronic postoperative pain	Postoperative pain	Binary
34854908	Veluchamy, 2021	Neuropathic pain	Neuropathic pain	Binary
28051079	Warner, 2017	Chronic postoperative neuropathic pain	Postoperative pain	Binary
34975738	Winsvold, 2021	Idiopathic polyneuropathy	Neuropathic pain	Binary
22020760	Won, 2012	Oxaliplatin-induced chronic peripheral neuropathy	Cancer pain	Binary
30277654	Yokoshima, 2018	Opioid analgesia	Pain treatment response	Discrete

PMID, publication PubMed ID. EA, European ancestry; AA, African American.

Discovery study design			
Sample size	**Ethnicity**	**P-value threshold**	**No. of significant locus**
350	Japanese	Fisrt and second stage P<0.05; final stage Q < 0.05	2
cases 191, controls 282	Japanese	1.86E-07	0
cases 89, controls 282	Japanese	1.86E-07	1
cases 1308, controls 5791	EA	1.00E-05	10
cases 6914, controls 242929	EA	5.00E-08	3
cases 148, controls 810	EA	5.00E-08	0
cases 130, controls 913	EA	5.00E-08	4
cases 769, controls 9384	Hispanic; Latino	5.00E-08	1 in overall; 2 in females
cases 727, controls 843	EA; AA; others	5.00E-05	1 in EA; 1 in AA
cases 999, controls 2031	EA; AA; others	5.00E-08	1 in overall; 2 in females; 1 in males.
cases 178, controls 1230	EA; AA	5.00E-08	0
cases 29531, controls 128494	EA	5.00E-07	4
cases 49182, controls 51629	EA	5.00E-08	0
355	Japanese	Fisrt and second stage P<0.05; final stage Q < 0.05	2
cases 4384, controls 784	EA	1.00E-05	13
265000	EA	1.30E-08	9
cases 34, controls 296	EA	1.00E-05	11
In stage 1, cases 1244, controls 2832, In stage 2, cases 3268, controls 425 657	EA	5.00E-08	1
cases 109, controls 504	Not report	1.00E-05	4
cases 2093, controls 445256	EA	5.00E-08	0
cases 39, controls 57	Korean	1.00E-05	0
71	Japanese	5.00E-08	2

Cancer pain

"Chronic cancer-related pain is chronic pain caused by primary cancer itself or metastases (chronic cancer pain) or its treatment (chronic postcancer treatment pain)." [38]

Severe pre-treatment pain

Reyes-Gibby *et al.* [39] conducted a GWAS on severe pre-treatment pain in untreated cancer patients to exclude pain associated with cancer treatment. They identified one genome-wide significant intergenic variant near *OR13G1/OR6F1* in the combined analysis of the discovery and replication (n= 958) phase.

Chemotherapy-induced peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is caused by oral or intravenous chemotherapy. Common chemotherapy agents that cause peripheral neuropathy include taxanes (paclitaxel and docetaxel), platinum-based drugs (cisplatin and oxaliplatin), vinca alkaloids (vincristine), thalidomide, and proteasome inhibitors (bortezomib).

Baldwin *et al.* [40] conducted the first GWAS on CIPN in patients receiving paclitaxel treatment in the CALGB 40101 cohort (n=855). This study identified 11 suggestively significant loci associated with paclitaxel-induced peripheral neuropathy. Seven GWASes [41-47] were performed to identify genetic variants associated with taxane-induced peripheral neuropathy. Only one genome-wide significant association (an intronic locus in *TMEM150C*) was identified in the study by Adjei *et al*. (n=692)[46], but this locus could not be replicated in the same study.

There are eight GWASes for other chemotherapies, including platinum-induced peripheral neuropathy [48-50], vincristine-induced peripheral neuropathy [51, 52], and bortezomib-induced peripheral neuropathy [53-55]. Only in the analysis focusing on vincristine-induced peripheral neuropathy (n=321) [51], a genome-wide significant intergenic region (*LOC100996325/CEP72*) was identified. All the other studies reported only suggestively significant results.

Acute post-radiation therapy pain

Lee *et al.* [56] conducted a GWAS on post-radiotherapy pain in breast cancer patients (n=1112). They identified three suggestively significant loci (an intronic variant in *ABCC4*, an intergenic variant near *LINC01203/EGFL6*, and a non-coding transcript variant in *RFFL*) without a replication cohort.

Overlap between studies on cancer pain

Although only a limited number of genome-wide significant hits have been reported, the studies focusing on CIPN reported three suggestively associated loci that showed overlap between studies investigating different drugs: an intergenic region near *LRP12/ZFPM2*, an intronic locus in *FGD4*, and an intergenic region near *LINC00290*. Interestingly, the gene *LRP12* is involved in the internalization of lipophilic molecules, and *FGD4* is known to cause a peripheral nervous system disorder (Charcot-Marie-Tooth disease).

Musculoskeletal pain

Chronic musculoskeletal pain is defined as *chronic pain arising from musculoskeletal structures such as bones or joints* [57].

Chronic back pain

Back pain is the leading cause of disabling conditions worldwide [58]. Back pain may appear as a new (acute) episode, or it develops as persistent (chronic) back pain if individuals fail to recover from acute episodes [59]. The estimated heritability of back pain ranges from 30%-68%, indicating a genetic predisposition [60] [20, 61, 62].

Four GWASes have been conducted on (chronic) back pain. In 2018, Suri *et al.* performed the first GWAS focusing on self-reported chronic back pain by combining two cohorts (the UKB and the CHARGE consortium) in a meta-analysis (n=158025) [63]. Four loci were identified: three n intronic regions (*DCC*, *DIS3L2*, and *SOX5*) and one in an intergenic region near *CCDC26/GSDMC*. A later study by Freidin *et al. [64]* also used the UKB cohort for a GWAS on back pain (n=350000); the main phenotype definition difference with Suri *et al.* [63] is that they had no limitations on the duration of back pain. Besides *CCDC26/GSDMC* and *SOX5*, they identified three additional loci, two in the intronic region of *C8orf34* and *HTRA1* and one in the intergenic region near *SPOCK2/CHST3*.

In another study on chronic back pain [65], no genome-wide significant loci were identified using samples from eMERGE Phase 3 (eMERGE3) and Geisinger (n = 100811, in total). However, this study replicated results from the two studies described above. The variants rs12310519 (*SOX5*) and rs7814941 (*CCDC26/GSDMC*) were replicated (P = 0.011, P = 0.005, respectively), with a very similar magnitude and direction of effect as the initial studies. The previously reported association of rs3180 (near *SPOCK2/CHST3*) was not statistically significant in this study but had a similar effect magnitude and direction.

The genetic architecture for chronic pain might be sex-specific as the prevalence of chronic pain is sex-related (i.e., females are more frequently affected), even after adjustment for many socioeconomic, demographic, and clinical risk factors (hormone profiles) [66, 67]. A sex-stratified GWAS on chronic back pain in the UKB identified two loci in males (n = 202077) and seven loci in females (n = 237754)[68]. One of the loci identified in males (rs1678626 in the intronic region of *SPOCK2*) was replicated in the same study. One locus identified in females also showed a significant P-value in the replication (rs62327819 in the intronic region of the *SLC10A7* gene (P = 0.0048)) but showed an opposite direction of effect.

Chronic knee pain

Knee pain can be localized (use of 1 or 2 fingers to point to a specific location), regional (use of all of the fingers or the whole hand to cover a more extensive region), or diffuse/unable to identify pain as localized or regional in nature [69]. Genetics studies have focused on knee pain caused by osteoarthritis and less on knee pain in general [70]. The only GWAS that we identified studying chronic knee pain was performed by Meng *et al.* [70]. In their study, two genome-wide significant loci associated with general knee pain were identified using data from the UKB (n= 171516): one variant in the 5'-UTR region of *GDF5* and the other is in the intergenic region near *KIF12/COL27A1*. These results were supported by two independent replication cohorts of knee osteoarthritis in the same study.

Chronic shoulder and neck pain

Neck or shoulder pain is often described as a single entity [71] as pain in the cervicobrachial area with shared etiology [72, 73], and lesions in the neck can lead to pain in the shoulder and vice versa [74]. Also for this phenotype, only one GWAS was identified [75]. In this study, three loci were identified to be associated with neck and should pain in the UKB (n= 203309): two intergenic variants (one near *FOXP2*, one near *CA10/LINC01982*), and one variant in the non-coding RNA *LINC01572*. A replication included in the same paper showed a weak association for the *FOXP2* and *LINC01572* loci in the GS:SFHS cohort but not in the TwinsUK cohort. All three loci showed genome-wide significance in the joint meta-analysis.

Chronic widespread pain, fibromyalgia, and multisite chronic pain

Chronic widespread pain (CWP) is defined as "*diffuse musculoskeletal pain in at least four of five body regions and at least three or more body quadrants (as defined by upper-lower/left-right side of the body) and axial skeleton (neck, back, chest, and abdomen)*" [76]. Fibromyalgia is considered more severe and at the end of the spectrum of CWP. Fibromyalgia is often accompanied by sleep disorders, cognitive

dysfunction, and somatic symptoms [76], but CWP and fibromyalgia syndrome are sometimes used interchangeably.

Two early papers conducted a GWAS on chronic widespread pain (n=7099) [21] and fibromyalgia (n=503) [77], respectively. However, both papers were low in statistical power, with only suggestively significant results. More recently, Rahman *et al.* [78] conducted the largest GWAS on CWP using the UKB as a discovery cohort (n=249843) and six independent replication cohorts (n= 57257). Three genome-wide significant loci were identified; two were in the intronic region of *RNF123* and *ATP2C1,* and one was in the 3'-UTR region of *COMT*. Only the *RNF123* locus was successfully replicated.

Two studies carried out a GWAS on the number of localized chronic pain sites in the UKB (see Table S4 for more details concerning the phenotype definition). These two studies unraveled a shared genetic background between CWP and multisite chronic pain (MCP). Johnston *et al.* [79] identified 39 genome-wide significant loci with diverse gene functions (n=387649). Many identified genes were implicated in nervous-system development, neural connectivity, and neurogenesis. In a later sex-stratified analysis for MCP [80], five loci in males (n=178556) and ten loci in females (n=209093) were identified, respectively. Even though Rahman *et al.* [78] and Johnston *et al.* [79] used the same cohort (UKB) for their studies, the results differ as they used a slightly different phenotype definition for cases and controls. Johnston *et al.* [79] also showed that the genetic correlation between CWP and MCP was high ($rg = 0.83$, $p = 2.45 \times 10^{-54}$), and most SNPs showed consistent effect size and directions of effect between MCP and CWP.

Tsepilov *et al.* [81] investigated genetic factors underlying MCP at four locations (back, neck/shoulder, hip, and knee) by using a principal component analysis to reduce the heterogeneity in phenotypes (see table S4 for more details concerning the phenotype definition). They identified nine genome-wide significant loci, and six were replicated in the replication phase of this study (a variant in the 5'-UTR region of GDF5, two intronic variants in *EXD3* and *FOXP2,* respectively; two exonic variants in *SLC39A8* and *ECM1,* respectively; a variant in the 3'-UTR region of *AMIGO3/GMPPB*).

Complex Regional Pain Syndrome

"Complex regional pain syndrome (CRPS) is a type of chronic primary pain characterized by pain in a regional distribution that usually starts in an extremity after trauma, and further characterized by signs indicating autonomic and inflammatory changes" [76].

The etiology of CRPS remains largely unknown, but evidence suggests genetic predispositions in the HLA region [82, 83].

In a GWAS performed by Janicki *et al.* [84], no genome-wide significant variants were identified, and none of the previously reported SNPs in the HLA region remained significant after multiple-testing correction. The top associated SNP was an intronic variant in *NAV3* (P = 0.0003) in the discovery phase (n= 460). Although this locus failed to pass the suggestively significant threshold, it is reported as part of this review as it overlaps with the top locus in a GWAS on postoperative pain [85] (See paragraph 3.7).

Chronic pain mixed phenotypes

Nishizawa *et al.* [86] conducted a GWAS on chronic pain with mixed phenotypes (n= 473), including postherpetic neuralgia, lower back pain, hernia of intervertebral disk, spinal canal stenosis, postoperative pain, neck pain, and others. They were unable to identify any (suggestively) significant hits.

Overlap between studies on musculoskeletal pain phenotypes

Genes reported more than once in GWASes on back pain are *SOX5*, *C8orf34*, *SPOCK2*, *CCDC26/GSDMC*, and *DCC*. These genes functionally link to chondrogenesis (*SOX* genes family) [87]; cartilage [88], osteoarthritis [89], and lumbar disc degeneration (*CCDC26/GSDMC*) [90]; and nociceptive pathways (*DCC*) [91]. However, the function of some genes/loci (e.g., *C8orf34*) and how they are involved in back pain are still unexplained.

Several genes/loci have been reported more than once in CWP, fibromyalgia, and MCP, including *EXD3*, *SLC39A8*, *AMIGO3/GMPPB/RNF123*, *C6orf106*, *FAF1*, *SLC24A3*, and *LINC01065/LINC00558*. In addition, two SNPs associated with MCP from different studies were in LD (r^2 = 0.958), rs3737240 (in the exon of *ECM1*) [81] and rs59898460 (in the intergenic region near *FALEC/ADAMTSL4*) [79, 80]. The functions of the reported genes include cell-cycle progression (*EXD3*, *RNF123*), onset of inflammation (*SLC39A8*), brain development (*AMIGO3*), apoptosis (*FAF1*), and intracellular calcium homeostasis and electrical conduction (*SLC24A3*).

The following genes were reported for more than one musculoskeletal pain phenotype: *DCC*, *FALEC/ADAMTSL4*, *CA10/LINC01982*, *FOXP2*, and *GDF5*. Relevant functions include patterning of the developing nervous system (*ADAMTSL4*), development and maintenance of synapses (*CA10*) [92], brain development, neurogenesis, signal transmission and synaptic plasticity (*FOXP2*) [93], and overlap with genes associated with osteoarthritis (*GDF5*).

The overlap within musculoskeletal pain should be interpreted carefully as many musculoskeletal GWASes included UK Biobank (UKB) samples as (part of the) discovery cohort. Therefore, the overlapping genes/loci might be due to the sample overlap. For instance, multisite chronic pain GWASes include UKB participants reporting all kinds of chronic pain, including back pain. However, back pain was also investigated as an individual phenotype in another GWAS. Besides overlap in cohorts, the overlapping finding of sex-stratified and sex-unstratified analyses on the same phenotype are also reported in this review.

Neuropathic pain

Neuropathic pain is defined as "*pain caused by a lesion or disease of the somatosensory system*" [94]. The etiology of neuropathic pain can be diverse, including metabolic disease (e.g., diabetic neuropathy), surgery or trauma, infections (e.g., shingles and HIV), exposure to chemotherapy, or unknown etiology (e.g., idiopathic neuropathies).

Diabetic neuropathic pain

Diabetic peripheral neuropathy is the most common cause of neuropathy [95], with 21% of diabetic patients suffering from it [96]. The first GWAS on diabetic peripheral neuropathy was conducted in the GoDARTS cohort by Meng *et al.* [97]. Cases were defined as type 2 diabetic individuals with at least one prescription history of any of the following medicines for diabetic peripheral neuropathy: duloxetine, gabapentin, pregabalin, capsaicin cream/patch, and lidocaine patch (see Table S4 for more details concerning the phenotype definition). One suggestively significant intergenic region near *GFRA2/DOK2* was found to be associated with diabetic peripheral neuropathy (n=3063).

In the same year, Meng *et al.* [98] conducted a GWAS in the same cohort with a more stringent case definition: cases were defined as type 2 diabetic patients who have a minimum of two prescriptions of the five medicines that were also included in the previous study (see above). This analysis led to the identification of one suggestively significant locus in the intronic region of *ZSCAN20*. In the sex-stratified analysis, this locus remained suggestively significant in females (n=1730), and another intergenic region near *ABRA/ANGPT1* passed the suggestively significant threshold in males (n=2491).

Tang *et al.* [99] conducted a GWAS on diabetic peripheral neuropathy in two diabetic trials (n= 5168 in total). An intergenic region near *SCN7A/XIRP2* passed the genome-wide significant threshold and was successfully validated. The minor allele at this locus was associated with a higher expression of the adjacent gene *SCN2A*

in the tibial nerve. Two additional intronic variants in *NTRK3* and *THEG5* passed the suggestively significant threshold in the discovery phase and almost reached genome-wide significance in the meta-analysis.

Neuropathy/Neuropathic pain

As various types of peripheral neuropathy can cause neuropathic pain, we also included GWASes on neuropathy in this review, although not all neuropathy patients have pain experience. Therefore, the genetic association findings in neuropathy might not directly link to pain. Reyes-Gibby *et al.* [100] conducted a GWAS on neuropathy in untreated head and neck cancer patients (n=1043) defined by ICD codes. They identified four loci passing the genome-wide significance threshold (an intergenic region in *KNG1/EIF4A2*, an upstream region in *PCP2*, and two intronic variants in *RORA* and *SNX8*, respectively), but no validation was done as part of this study. Veluchamy *et al.* [101] conducted a two-stage analysis for neuropathic pain. The first stage consisted of a meta-analysis of the GoDARTS (n=803) and GS:SFHS cohorts (n=3273). Both cohorts used the Brief Pain Inventory questionnaire and Douleur Neuropathique 4 Questions to define neuropathy. In stage two, they combined the results of stage one with UKB data (n=428925). For these participants, a proxy phenotype was used to define neuropathy. In stage one, one intergenic region near the *EPHA3* gene showed genome-wide significance. In stage two, a locus near *SLC9A7P1* showed genome-wide significance. The *EPHA3* locus identified in stage one and another intronic variant in the *CAB39L* gene were close to genome-wide significance.

Winsvold *et al.* [102] conducted a GWAS on idiopathic polyneuropathy in the HUNT and UKB cohorts (n= 63351 in total). Only in the meta-analysis, two genome-wide significant loci were identified (one intronic variant in *B4GALNT3 and* one intergenic variant near *NR5A2/LINC01221*). They aimed to validate five previously identified variants in five genes that are reported to be associated with related (polyneuropathy) phenotypes; *PRPH* associated with nerve conduction [103], *CEP72* and *VAC14* associated with CIPN [44, 51], *IL2RA* associated with drug-induced peripheral neuropathy [104], *XIRP2* associated with diabetic peripheral neuropathy [99]. Unfortunately, none of these five variants were successfully validated in this study (FDR-corrected P-value < 0.05 for five tests). They also selected 69887 variants near 175 genes related to monogenic forms of polyneuropathy (e.g., hereditary neuropathy, familial dysautonomia) to validate. None of these variants remained significant after multiple testing correction (FDR-corrected P-value < 0.05 for 69887 tests).

Sciatica

The typical symptom of sciatica is sciatic pain or lumbar radicular pain, and it is usually caused by a common low back disorder, e.g., lumbar disc herniation [105]. Lemmela *et al.* [106] conducted a GWAS on sciatica using a meta-analysis of two discovery cohorts (n= 3962 in total). In the discovery phase, they identified two genome-wide significant variants (in the intronic region of *MYO5A* and *NFIB*, respectively). Only the variant in *MYO5A* was replicated in an independent cohort (n=19265).

Postherpetic neuralgia

Nishizawa *et al.* [86] conducted a GWAS on postherpetic neuralgia (n= 371). One intronic SNP in the *ABCC4* gene showed a genome-wide significant association with postherpetic neuralgia using an additive model.

Drug-induced peripheral neuropathy

Leger, 2014 [104] identified five suggestively significant loci associated with stavudine and didanosine-induced peripheral neuropathy (n= 254), which were an intronic variant in *ADAMTS2*, an exonic variant in *KRR1*, an intergenic variant near *MIR8054/LUZP2*, a variant in the 3'-UTR region of *SASH1*, and an intergenic variant near *SLCO3A1/ST8SIA2*.

Overlap between studies on neuropathic pain phenotypes

Based on the p-value thresholds used in the papers, we could not identify overlapping genes/loci between the GWAS studies on neuropathic pain phenotypes.

Visceral pain

Chronic visceral pain is chronic pain originating from internal organs of the head or neck region or of the thoracic, abdominal, or pelvic region [7].

Dysmenorrhea pain

Dysmenorrhea pain is an intense and often disabling abdominal or pelvic pain during every menstrual cycle, and it can be primary or secondary (e.g., to endometriosis). Three GWASes have been conducted on dysmenorrhea pain, and each study included participants from different populations: European (n=11891) [107], Chinese (n=5324) [108], and Japanese (n=11348) [109]. One shared intergenic region was identified in these three studies: *TSPAN2/NGF*. An intronic variant in *ZMIZ1* was only identified in the Chinese study, and an intergenic region near *IL1A/IL1B* was only identified in the Japanese study. The *IL1A* signal might reflect endometriosis as this locus was previously reported to be associated with endometriosis [110].

Constant-severe pain in chronic pancreatitis

Dunbar *et al.* [111] conducted a GWAS on constant-severe pain in chronic pancreatitis (n= 1357) [112]. One suggestively significant locus was identified in the intronic region of *SGCZ* without replication.

Postoperative pain

Postoperative pain can be acute or chronic. "*Chronic Postoperative pain develops or increases in intensity after a surgical procedure and persists beyond the healing process, i.e., at least three months after the surgery*" [113].

Kim *et al.* [114] conducted the first GWAS on acute postoperative pain using two phenotypes separately, i.e., the maximum postoperative pain rating and postoperative pain onset time (n=112 for both phenotypes). However, no significant loci were identified after correcting for multiple comparations. In another study, Cook-Sather *et al.* [115] identified two suggestively significant loci (an intergenic variant near *CDC5L/LOC105375075* and an upstream variant of *LOC105375075*) associated with acute postoperative pain scores (n=277).

Two additional studies investigated chronic post-postoperative pain. Warner *et al.* [116] identified four suggestively significant loci associated with postoperative neuropathic pain in post-total joint replacement patients (n=613). To define neuropathic pain cases, a seven-item questionnaire was used to describe the nature of pain. The top hit in the meta-analysis was an intronic variant in the *PRKCA* gene ($P = 1.65 \times 10^{-5}$). The study of van Reij *et al.* [85] found 11 loci suggestively significantly associated with chronic postoperative pain measured by the numeric rating scale (NRS) (n=330). Only one intronic variant in *NAV3* was replicated in an independent cohort ($P = 0.009$).

No overlap was found between the studies that investigated postoperative pain.

Orofacial pain

The most investigated orofacial pain is temporomandibular disorder (TMD), which can be primary or secondary to persistent inflammation, structural changes (such as osteoarthritis or spondylosis), injury, or nervous system diseases. Sanders *et al.* [117] conducted the first GWAS on TMD in participants of Hispanic/Latino ancestry (n=10153). A stringent case definition was applied, i.e., reporting pain in both face and jaw joint, but information on symptoms duration was not available. One genome-wide significant locus in the intronic region of the *DMD* gene was identified. Unfortunately, this SNP was not genotyped in the replication cohorts. Another suggestively associated intergenic variant near *PPP1R9B/SGCA* was

replicated in one replication cohort. This study also performed a sex-stratified analysis and identified two genome-wide significant loci in females (an intergenic variant near *B3GLCT/RXFP2* and an intronic variant in *BAHCC1*); only the variant in *BAHCC1* was replicated among females in the meta-analysis of this study. This paper does not mention whether they conducted the analysis in males only.

Smith *et al.* [118] investigated genetic variants associated with examiner-verified chronic TMD (see Table S4 for more details concerning the phenotype definition) in the OPPERA cohort (n=3030), and this cohort was one of the replication cohorts in the study described above [117]. They identified one genome-wide significant variant (in the intergenic region near *OTUD4 /LINC02266*) in the analysis including males and females. A sex-stratified analysis identified two loci in females (an intronic variant in *SFRP1* and the same intergenic variant as in the analysis including all subjects) and one in males (an intergenic region near *LINC01210/CLDN18*). However, no SNPs were replicated in the meta-analysis of seven independent cohorts after applying Bonferroni correction.

Also for orofacial pain, no overlap in the reported genes was found.

Pain sensitivity

Fontanillas *et al.* [25] conducted the first GWAS on pain sensitivity. Two phenotypes were used to measure pain sensitivity: a pain questionnaire (n=25321) and a cold pressor test (n=6853). In the GWAS using the first phenotype, they identified one genome-wide significant locus in the intronic region of *EIPR1* and two suggestively associated loci (an intergenic region near *VAPA/LINC01254* and one intronic variant in *NALCN*). The GWAS using the cold pressor test as phenotype led to the identification of one suggestively significant locus in the intronic region of *PITPNC1*. The reported loci for each of the two phenotypes were not associated with the other phenotype. In addition, Fontanillas *et al*. [25] also validated the previously reported *MC1R* variants in their study. Variants in this gene were associated with red hair and modulate pain sensitivity, especially in women [119-121]. Three *MC1R* variants were tested for association with increased self-perceived pain sensitivity, and the most statistically significant variant was rs1805007 (P-value = 5.10E-03).

Pain treatment responses

Non-steroidal anti-inflammatory drugs

Kim *et al.* [114] conducted a GWAS on analgesic onset time after ketorolac administration (n=112). They identified one genome-wide significant locus in the upstream region of *ZNF429*.

Opioids

Galvan *et al.* [122] conducted a GWAS on pain relief measured by an 11-point numerical rating scale in patients receiving opioid treatment (morphine, oxycodone, and fentanyl) (n=438). They split the cohort into two groups and applied a two-stage analysis. Eight suggestively significant loci were identified in stage one, but only one intergenic region near *RHBDF2/CYGB* showed significance in the combined analysis of stages one and two.

Cook-Sather *et al.* [115] performed a GWAS using total postoperative morphine requirement as phenotype. They identified three suggestively significant loci in Europeans (n=277) and nine in African Americans (n=241). The top SNP from the analysis including Europeans, rs795484 in the intronic region of *TAOK3*, was replicated in a small replication cohort (n=75). This variant was also associated with postoperative pain scores in the same study (see above under 3.7 postoperative pain) in both Europeans ($P < 5E-5$) and African Americans ($P < 0.01$).

Nishizawa *et al.* [123] conducted a GWAS on opioid analgesic (fentanyl) requirements during the 24-h postoperative period (n=355). They divided one cohort into three groups for a three-stage analysis: SNPs that showed P-values < 0.05 in one stage were selected as candidate SNPs for the next stage. In the final stage, SNPs with $Q < 0.05$ (the Q-values of false discovery rate for multiple testing correction) were considered significant. This study identified one significant intergenic variant near *METTL21A/LINC01857*. Three additional studies applied the same method to investigate genetic variants associated with opioid analgesia. These studies identified one exonic variant in *LAMB3* [124], an intronic variant in *SLC9A9*, a variant in the 5'-UTR region of *TMEM8A* [125], and two intergenic regions near *C3orf38/EPHA3* and *LOC389602/LOC285889* [126]. In addition, Yokoshima *et al.* [127] identified two genome-wide significant loci (one intronic variant in *ABAT*, an intergenic region near *DAZL*/*PLCL2*), which were associated with pain decrease corresponding to opioid analgesics but without replication (n=71).

No overlap was found between the genes identified for pharmacological pain treatment outcomes.

Follow-up research

Overlap between different pain phenotypes

Besides checking the overlap genes/loci in the same pain phenotype or category (as described above), we also investigated the overlap between the reported loci in

different pain categories (see Table 2 for a summary). Thirty loci were reported in at least two studies and covered a wide range of phenotypes. *DCC* is the most reported gene, with four studies on musculoskeletal pain and one on CIPN. Two gene families were reported frequently, i.e., the ephrin receptor subfamily of the protein-tyrosine kinase family (*EPHA3* and *EPHA4*) and the *SOX* (SRY-related HMG-box) family of transcription factors (*SOX5* and *SOX6*). *EPHA3* was reported in one opioid analgesia study and one neuropathic pain study, and *EPHA4* was reported in one CIPN and one chronic postoperative pain study. *SOX5* was reported in three chronic back pain studies, *SOX6* was reported in one CIPN study, and one multisite chronic pain study. Many genes are implicated in neurological functions (see Table 2).

SNPs in linkage disequilibrium were summarized in Table S8. Except for two additional SNPs associated with MCP (see 3.4.7), all SNPs in linkage disequilibrium were identified by checking overlapping gene symbols.

Table 2. Loci reported more than once from all included papers. CIPN: chemotherapy-induced peripheral neuropathy.

Mapped genes/ loci region	Gene functions	SNP	Outcome	PMID	Comments
DCC	Nociceptive pathways	rs4384683	Chronic back pain	30261039	*
		rs62098013	Multisite Chronic Pain	31194737	*
		rs72922230	Chronic back pain	33021770	*#
		rs17748074	CIPN	28317148	
		18:50442591_TTTC_T	Multisite Chronic Pain	33830993	*
FALEC;ADAMTSL4	Nervous system development (*ADAMTSL4*)	rs59898460	Multisite Chronic Pain	31194737	*
		rs367563576	Chronic back pain	33021770	*#
		rs59898460	Multisite Chronic Pain	33830993	*#
CA10;LINC01982	brain development (*CA10*)	rs12453010	Shoulder And Neck Pain	32246137	*
		rs11079993	Multisite Chronic Pain	33830993	*#
		rs11079993	Multisite Chronic Pain	31194737	*
EXD3	Cell-cycle progression	rs73581580	Multisite Chronic Pain	31194737	*
		rs73581580	Genetic components of chronic musculoskeletal pain	32587327	*
		rs73581580	Multisite Chronic Pain	33830993	*#
FOXP2	Brain development	rs12537376	Multisite Chronic Pain	31194737	*
		rs2049604	Shoulder And Neck Pain	32246137	*
		rs12705966	Genetic components of chronic musculoskeletal pain	32587327	*
LRP12;ZFPM2	Internalization of lipophilic molecules (*LRP12*)	rs2941627	CIPN	22843789	
		rs3110366	CIPN	32562552	
		rs3110290	CIPN	28611204	

Table 2. Continued

Mapped genes/ loci region	Gene functions	SNP	Outcome	PMID	Comments
TSPAN2;NGF	regulation of cell development, activation, growth and motility (*TSPAN2*), sensory neurons growth and differentiation (*NGF*)	rs7523831	Dysmenorrhoea pain	28447608	
		rs12030576	Dysmenorrhoea pain	29855537	
		rs7523086	Dysmenorrhoea pain	27454463	
SLC39A8	Inflammation	rs13135092	Multisite Chronic Pain	31194737	*
		rs13107325	Genetic components of chronic musculoskeletal pain	32587327	*
		rs13135092	Multisite Chronic Pain	33830993	*#
SOX5	Chondrogenesis	rs12310519	Chronic back pain	30261039	*
		rs12310519	Chronic back pain	30747904	*
		rs12308843	Chronic back pain	33021770	*#
ABCC4	Prostaglandins transportation	rs4584690	Acute post-radiation therapy pain	31196165	
		rs4773840	Postherpetic neuralgia	33685280	
LINC01065;LINC00558	Not known	rs1443914	Multisite Chronic Pain	31194737	*
		rs34003284	Multisite Chronic Pain	33830993	*#
RNF123;AMIGO3;GMPPB	Brain development, synapse assembly (*AMIGO3*), cell-cycle progression (*RNF123*)	rs7628207	Genetic components of chronic musculoskeletal pain	32587327	*
		rs1491985	Chronic Widespread Pain	33926923	*§
		rs7628207	Multisite Chronic Pain	31194737	*
C6orf106	Inflammation	rs6907508	Multisite Chronic Pain	31194737	*
		rs151060048	Multisite Chronic Pain	33830993	*#

Table 2. Continued

Mapped genes/ loci region	Gene functions	SNP	Outcome	PMID	Comments
C8orf34	Not known	rs1865442	Chronic back pain	30747904	*
		rs7834973	Chronic back pain	33021770	*#
CCDC26;GSDMC	Lumbar disc degeneration	rs7814941	Chronic back pain	30747904	*
		rs7833174	Chronic back pain	30261039	*
EPHA3	Nervous system development	rs13093031	Opioid analgesia	29502940	
		rs112990863	Neuropathic pain	34854908	
MIR4268;EPHA4	Nervous system development (*EPHA4*)	rs17348202	CIPN	23776197	
		rs10194315	Chronic postoperative pain	31903573	
FAF1	Apoptosis	rs10888692	Multisite Chronic Pain	31194737	*
		rs35072907	Multisite Chronic Pain	33830993	*#
FGD4	Peripheral nerve pathophysiology	rs10771973	CIPN	22843789	
		rs10771973	CIPN	32562552	
GDF5	Osteoarthritis	rs143384	Chronic knee pain	31482140	*
		rs143384	Genetic components of chronic musculoskeletal pain	32587327	*
LINC00290	Not known	rs12501594	CIPN	34391895	
		rs6552496	CIPN	28317148	
NAV3	Predominantly expressed in the nervous system	rs300501	CRPS	28025368	
		rs118184265	Chronic postoperative pain	31903573	
SLC24A3	Electrical conduction	rs2424248	Multisite Chronic Pain	31194737	*
		20:19709268_AAAAT_A	Multisite Chronic Pain	33830993	*#

Table 2. Continued

Mapped genes/ loci region	Gene functions	SNP	Outcome	PMID	Comments
SOX6	Chondrogenesis	rs4757366	CIPN	28611204	
		rs61883178	Multisite Chronic Pain	31194737	*
SPOCK2	Neurogenesis	rs1678626	Chronic back pain	33021770	*#
		rs3180	Chronic back pain	30747904	*
FCGBP	Maintenance of the mucosal structure	rs234348	Opioid analgesia	24909733	
		rs17796312	Chronic Widespread Pain	22956598	
LOC102546299;LINC01947	Not known	rs7734804	Post-operation neuropathic pain	28051079	
		rs10515902	Opioid analgesia	24909733	
MIR4422HG;LINC01753	Not known	rs1165472	CIPN	23776197	
		rs12566055	Opioid analgesia	24909733	
GPD2	Calcium ion binding and glycerol-3-phosphate dehydrogenase activity	rs298235	Post-operation neuropathic pain	28051079	
		rs13421094	Opioid analgesia	21622719	
SP4	DNA-binding transcription factor activity	rs73271865	Temporomandibular Disorder	28081371	
		rs7798894	Multisite Chronic Pain	31194737	*

CPRS: Complex regional pain syndrome. * Studies included the UK Biobank (UKB) as their study cohort, and the phenotype definition is based on the pain questionnaires in the UKB (Category 100048). # indicates sex-stratified analysis. § This variant was identified by checking linkage disequilibrium rather than checking gene symbol overlap.

Table 3. Overlapping genes between genes/loci from all included papers in this review and Human Pain Genetics Database.

Overlapping genes	GWAS Phenotype [PMID]	Phenotypes in HPGDB	Number of papers in HPGDB
COMT *	Chronic Widespread Pain [33926923]	Analgesia; cancer pain; fibromyalgia; migraine; musculoskeletal pain; neuraxial pain; neuropathic pain; nociception; other clinical pain; Postoperative pain; temporomandibular disorder	90
SUGCT	CIPN [32562552]	Migraine	9
TSPAN2; NGF	Dysmenorrhoea pain [28447608], [29855537], [27454463]	Temporomandibular disorder, migraine	9
ASTN2	Multisite Chronic Pain [31194737]	Migraine	7
IL1B	Dysmenorrhoea pain [29855537]	Analgesia; cancer pain; migraine; musculoskeletal pain; neuraxial pain	7
IL1A	Dysmenorrhoea pain [29855537]	Migraine; neuraxial pain; nociception	5
YTHDF2; OPRD1	CIPN [28611204]	Postoperative pain for both genes; These phenotypes only reported in OPRD1: analgesia; nociception; temporomandibular disorder	5
FALEC; ADAMTSL4	Multisite Chronic Pain [31194737][33830993], Chronic back pain [33021770]	Migraine	3
PLCE1	Fibromyalgia [24582949]	Migraine	3
SLC24A3	Multisite Chronic Pain [31194737] [33830993]	Migraine	3
GDF5	Chronic knee pain [31482140],Genetic components of chronic musculoskeletal pain [32587327]	Neuraxial pain; temporomandibular disorder	2
GPATCH2L; ESRRB	Diabetic neuropathic pain [31127053]	Temporomandibular disorder	2
LINC01777	Opioid analgesia [24909733]	Migraine	2
TAC1	CIPN [22020760]	Temporomandibular disorder	2
ABCC4	Acute post-radiation therapy pain [31196165], Postherpetic neuralgia [33685280]	Cancer pain	1

Table 3. Continued

Overlapping genes	GWAS Phenotype [PMID]	Phenotypes in HPGDB	Number of papers in HPGDB
CACNB2	CIPN [22843789]	Migraine	1
CDC5L; LOC105375075	Acute postoperative pain [24909733]	Migraine	1
CX3CL1	CIPN [32562552]	Postoperative pain	1
GABRB1	CIPN [34391895]	Neuraxial pain	1
IL1RN	Dysmenorrhoea pain [27454463]	Neuraxial pain	1
LINC00290	CIPN [34391895], CIPN [28317148]	Migraine	1
METTL21A; LINC01857	Opioid analgesia [23183491]	Analgesia	1
RSU1	Chronic postoperative pain [31903573]	Migraine	1
TAOK3	Opioid analgesia [24909733]	Postoperative pain	1
TPH1	Chronic Widespread Pain [22956598]	Migraine	1

CIPN: chemotherapy-induced peripheral neuropathy. HPGDB: human pain genetic database; PMID, publication PubMed ID. * Intergenic variants between *COMT* and *TXNRD2* in Human Pain Genetics database were also included.

Overlap between GWAS-identified pain genes and pain genetic databases

To check whether genes described in this review are associated with (other) pain phenotypes, we searched two pain genetic databases. The first is the Human Pain Genetics Database (HPGDB). Twenty-five genes/loci reported in pain GWASes were also reported in the HPGDB (see Table 3). *COMT* is the most investigated candidate gene with 90 published papers, followed by *SUGCT* (n=9) and *TSPAN2/NGF* (n=9) (see Data S2 for details of the genes identified in HPGDB). Of the 25 overlapping genes/loci, six genes/loci were associated with more than two phenotypes in HPGDB (*COMT*, *OPRD1*, *IL1A*, *IL1B, TSPAN2/NGF*, *GDF5*), and 15 genes were associated with migraine.

Besides the HPGDB, we also searched the mouse pain genetics database, which provides a repository of investigated genes in nociception, hypersensitivity, and analgesia in mice. Table 4 summarizes the overlapping genes between genes reported in this review and the mouse pain genetics database. Fourteen genes/loci could be found in the mouse pain genetics database. The functions of these genes are diverse (see Data S3 for details), but many overlapping genes are involved in neurological function, such as neurotransmitters (*COMT1*), neuromodulators (*TAC1*), neurotrophins (*EFNB2*, *GFRA2*, *NGF*), synaptic scaffolding/vesicles (*DTNBP1*, *PPP1R9B*). An overview of the number of (overlapping) genes identified using different approaches/sources (GWAS findings, HPGDB, and the mouse pain genetics database) is depicted in Figure 4.

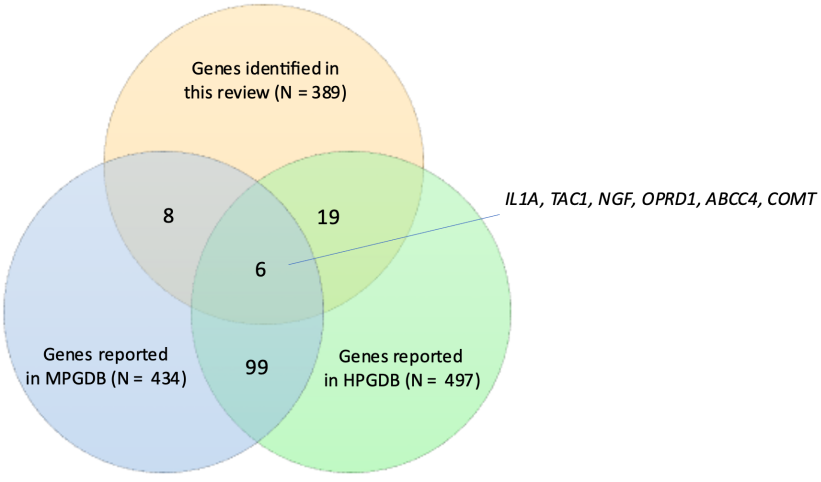

Figure 4. The number of (overlapping) genes reported from genome-wide association studies on pain included in this review, human pain genetic database (HPGDB), and mouse pain genetic database (MPGDB).

Table 4. Overlapping genes between genes/loci from all included papers in this review and mouse pain genetics database. PMID, publication PubMed ID.

Genes	GWAS Phenotype [PMID]	Nociception in Mouse	Hypersensitivity in Mouse	Analgesia in Mouse
ABCC4	Acute post-radiation therapy pain [31196165], Postherpetic neuralgia [33685280]	Not tested	Mutant less sensitive	Not tested
COMT	Chronic Widespread Pain [33926923]	Mutant more sensitive	Not tested	Contradictory data
DTNBP1	CIPN [34391895]	Mutant less sensitive	Not tested	Not tested
EFNB2	Chronic back pain [33021770]	No difference	Mutant less sensitive	Not tested
GFRA2	Diabetic neuropathic pain [24974787]	Mutant more sensitive	Not tested	Not tested
HDAC4	Diabetic neuropathic pain [31127053]	Mutant less sensitive	Not tested	Not tested
IL1 (IL1A,IL1B)	Dysmenorrhoea pain [29855537]	Mutant less sensitive	Mutant less sensitive	Mutant less sensitive
MAPK9	CIPN [28611204]	No difference	Mutant less sensitive	Not tested
NGF	Dysmenorrhoea pain [28447608], [29855537], [27454463]	Mutant less sensitive	Not tested	Not tested
OPRD1	CIPN [28611204]	No difference	Mutant more sensitive	Contradictory data
PMP22	Chronic Widespread Pain [22956598]	Mutant less sensitive	No difference	Not tested
PPP1R9B	Temporomandibular Disorder [28081371]	No difference	Not tested	Contradictory data
PRKCA	Post-operation neuropathic pain [28051079]	No difference	Mutant more sensitive	Not tested
TAC1	CIPN [22020760]	Mutant less sensitive	Contradictory data	Contradictory data

Discussion

This review summarizes the findings from GWAS on pain and related phenotypes (nociception, neuropathy, and pain treatment response). In all GWAS studies included in this review, 32 overlapped loci were found between studies, and some loci reported in GWAS also overlapped with candidate gene studies in humans and mice. Our study provides an overview of the identified and potential genetic

risk factors for pain from GWAS findings. Our results suggest multiple genetic risk factors involved in different functions can influence susceptibility to pain. Especially, many GWAS-identified genes and overlapping genes between included studies are implicated in neurological functions and inflammations. These functions are critical for pain development as chronic pain mainly arises from inflammation and nerve injury at the peripheral level and neuroplasticity at the central level [128, 129].

The involvement of genes related to neurological functioning meets our expectations as pain is mediated by processes in the nervous system regardless of the nature of pain [130]. The most-reported locus is the *DCC* gene region. *DCC* plays several key roles in both central nervous system development [131] and mature neuron survival and death [132]. *DCC* might also be important for pain development as this gene is necessary for noxious stimuli localization in both mice and humans [133], and it is known to contribute to neuropathic pain [91, 134] and maladaptive responses (tolerance, dependence, and opioid-induced hyperalgesia) to opioids in mouse [135]. Interestingly, several other repeatedly reported genes can also be linked to neurological mechanisms, e.g., brain development and neuron functioning and development. This all stresses that the nervous system is indeed an important player in pain.

Besides neurological functions, the overlapping genes also suggest other possible mechanisms involved in pain, including inflammation. Inflammation events are highly relevant to pain as it plays a central role in the pathogenesis of chronic pain [136]. In addition, some overlapping genes were implicated in diseases with pain as one of the symptoms. For instance, the genes found in chronic back pain are involved in chondrogenesis (*SOX5* and *SOX6* [87]), or lumbar disc degeneration (*CCDC26/GSDMC* [90]). Similarly, the *GDF5* locus found in knee pain is also involved in osteoarthritis [137].

However, the function of some loci remains unclear as they were mapped to an intergenic region or non-protein coding genes with unknown functions (such as *LINC01065* and *C8orf34*). Rather than influencing protein coding, these variants might regulate gene expression levels [138]. However, this warrants future research using gene expressions mapping methods, such as eQTL mapping or chromatin interaction mapping [139].

The overlapping genes across studies should be interpreted cautiously. One reason is that many studies only reported variants that passed the suggestively significant threshold but not the genome-wide significant P-value. In addition, many studies

lacked replication. Secondly, the heterogeneity of study designs exists in included studies, such as variability of participant characteristics (e.g., age, ethnicity), the disease that led to pain (e.g., osteoarthritis, diabetes), the nature of pain (e.g., nociceptive, neuropathic, and nociplastic), differences in pain measurements (pain measured by pain questionnaire or ICD codes), and different genotyping platforms. Moreover, the overlapping findings on musculoskeletal pain phenotypes might be due to sample overlaps and should be further validated (see also paragraph 3.4.7).

Of the genes identified in candidate gene studies, only a few were replicated in GWASes. For instance, *COMT* and *OPRM1* are the two most investigated genes in various pain phenotypes in humans and mice. However, *COMT* was only reported once in all included GWAS papers, and *OPRM1* was not reported at all, which could be explained by the small effect size of these variants in the multifactorial pain phenotypes or insufficient statistical power of the candidate gene studies due to small sample size. Variants in these genes could still be identified for certain pain phenotypes when statistical power increases. On the other hand, these results also suggest that the choice of gene/pathway in the hypothesis-driven approach might be biased. Therefore, hypothesis-free methods are needed to uncover (novel) biological mechanisms of pain.

We excluded migraine and headache for this review, considering that central nervous system disorders might have different mechanisms compared to the peripheral types of pain. Surprisingly, 15 out of 26 genes that overlap with the Human Pain Genetic Database are previously associated with migraine. This overlap might be explained by the phenotypic correlations between migraine and other types of pain, such as fibromyalgia [140], and the possible link with dysmenorrhoea pain [141]. However, we also identified genes linked to migraine and phenotypes without a direct correlation with migraines, such as CIPN, opioid analgesia, and postoperative pain. These results indicate that investigation of the shared genetic background of pain might be worth pursuing, which can be done by cutting-edged methods, such as linkage disequilibrium score regression.

GWAS findings on pain will facilitate the understanding of pain development and clinical management of pain. To fully interpret how the non-coding variants identified by GWASes are involved in pain development, we need comprehensive biological annotation tools from different transcription levels, such as epigenetic regulation, non-coding RNA function, and gene expression profiles [142]. Although fully understanding the functions of these variants might be challenging at this moment, it does not withhold the introduction of these variants in a clinical setting.

One successful example is applying a polygenic risk score (PRS) based on GWAS results for breast cancer prediction [143]. However, we are still far from the clinical application of genetic factors for pain development prediction and personalized pain management. As this area is still under investigation, no unequivocal genetic predictors have been found yet [144, 145].

To the best of our knowledge, this review is the first systematic overview of GWASes on pain and related phenotypes in humans to date. Other strengths of this study are that we included articles reporting genetic association studies of pain-related phenotypes (such as neuropathy and pain treatment response), followed the standards of PRISMA guidelines, and checked the reporting quality according to STREGA for genetic association studies. In addition, a systematic examination of the overlap between different studies was performed.

However, our review also has some limitations. Concerning paper selection steps, we had to exclude two letters because no information on methods was provided to determine the reporting quality. However, these two papers might include important findings. One paper reported the genetic associations between a variant in *TRPM8* and pain in Parkinson's disease [146], and the other paper reported an association between an intergenic variant rs3115229 and acute severe vaso-occlusive pain [147]. In addition, papers investigating other markers than genetic markers (such as epigenetic markers) were excluded as this was not in line with our goal. Furthermore, the function of identified loci was not further annotated (e.g., expression quantitative trait locus). Moreover, this review only focuses on GWAS findings, which might neglect important findings from candidate gene studies. To overcome this, we checked recent findings in candidate gene studies by comparing overlap between genes reported in GWASes and two comprehensive pain genetic databases.

Our study provides an overview of the identified and potential genetic risk factors for pain from GWAS findings, suggesting that multiple genetic risk factors involved in different functions can influence susceptibility to pain. For further studies, the overlapping genes (such as the six overlapping genes reported from GWASes, HPGDB, and the mouse pain genetics database) might be worth validation with careful experiment design, sufficient statistical power, and robust statistical methods to minimize incidental findings and yield validated results [148]. Especially, genes implicated in neurological functions and inflammations might be prioritized for validation and further investigation. In addition, more efforts should be made to characterize the multi-omics biomarker signatures of pain, such as gene expression,

epigenetics, and metabolic profiles. Besides, to empower accurate replication, meta-analysis, and international collaborations, it is highly recommended that future studies use clear, consistent phenotype definitions aligned with the current diagnosis definition/system, such as ICD-11 classification for chronic pain [149]. A comprehensive understanding of the biological mechanisms of pain will finally benefit patients by improving the clinical management of pain.

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Competing interests statement

The authors have no relevant financial or non-financial interests to disclose. All authors' conflict of interest in the manuscript is in agreement with the COI statement on the ICJME forms submitted in the submission system.

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Supplementary materials

Table S1. Search strategy.

Search date	2022-02-21
PubMed/ MEDLINE	Element 1 (#1): "Pain Perception"[Mesh] OR "Pain Threshold"[Mesh] OR "Pain Measurement"[Mesh] OR "Pain"[Mesh] OR Pain[tiab] Element 2 (#2): "Nociceptors"[Mesh] OR nocicept*[tiab] Element 3 (#3): "Neuralgia"[Mesh] OR "Peripheral Nervous System Diseases"[Mesh] OR Neuropathy[tiab] Element 4 (#4): "Genome-Wide Association Study"[Mesh] OR GWAS[tiab] OR GWA Stud*[tiab] OR Genome Wide Association Stud*[tiab] OR Genome Wide Association Analys*[tiab] OR Whole Genome Association Stud*[tiab] OR Whole Genome Association Analys*[tiab] OR Genome-wide scan*[tiab] OR Genome Wide Association Scan*[tiab] OR Whole Genome association scan*[tiab] OR genome wide association meta-analys*[tiab] Search strategy: (#1 OR #2 OR #3) AND #4 AND ("2000/01/01"[Date - Publication]: "2021/12/31"[Date - Publication])
Embase	#1: exp pain/ or exp pain threshold/ or pain intensity/ or pain assessment/ or pain measurement/ or pain receptor/ or (Pain or pains).ti,ab,kf. #2: nociception/ or nocicept*.ti,ab,kf. #3: neuropathic pain/ or neuropathy/ #4: (GWAS or GWA Stud* or Genome Wide Association Stud* or Genome Wide Association Analys* or Whole Genome Association Stud* or Whole Genome Association Analys* or Genome-wide scan* or Genome Wide Association Scan* or Whole Genome association scan* or genome wide association meta-analys*). ti,ab,kf. or genome-wide association study/ #5: (1 or 2 or 3) and 4 #6: limit 5 to (full text and human and English language and "remove preprint records" and yr="2000 - 2021")

Table S2. Inclusion and exclusion criteria for paper selection process.

Inclusion criteria:	**Exclusion criteria:**
Studies focusing on pain, nociception, or neuropathy. Genome wide association studies (no other association studies). Studies performed in humans. Studies published in English and in peer-reviewed journals.	Wrong outcome – Studies focusing on headache or migraine. – Studies on the background of a disease or condition (such as osteoarthritis) rather than pain itself. Wrong study design: – Candidate gene study, not genome-wide, replication study, Mendelian randomization study, genetic correlation study, family study – Studies investigating gene expression, metabolic, methylation profiles rather than genetic markers. Non-human study: e.g., cell lines, animal, *in silico* studies Wrong publication type: – Review, case repot, commentary, abstract, study protocol, and letters.

Table S3. Quality assessment form according to the "STrengthening the REporting of Genetic Association studies" (STREGA) guidelines.

Item	Item number	STROBE Guideline
Title and Abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract. (b) Provide in the abstract an informative and balanced summary of what was done and what was found.
Introduction		
Background rationale	2	Explain the scientific background and rationale for the investigation being reported.
Objectives	3	State specific objectives, including any pre-specified hypotheses.
Methods		
Study design	4	Present key elements of study design early in the paper.
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.
Participants	6	**Cohort study –** Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **Case-control study –** Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. **Cross-sectional study –** Give the eligibility criteria, and the sources and methods of selection of participants. **Cohort study –** For matched studies, give matching criteria and number of exposed and unexposed. **Case-control study –** For matched studies, give matching criteria and the number of controls per case.
Variables	7	*(a)* Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.
Data sources measurement	8*	*(a)* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.
Bias	9	*(a)* Describe any efforts to address potential sources of bias.
Study size	10	Explain how the study size was arrived at.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why.

	Extension for Genetic Association Studies (STREGA)	Score
		1
		1
	State if the study is the first report of a genetic association, a replication effort, or both.	1
		1
		1
	Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	1
	(b) Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	1
	(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory/centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	1
	(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal with this.	1
	State power calculation.	1
	If applicable, describe how effects of treatment were dealt with. ***If applicable, describe how covariates measurement were dealt with.***	1

Table S3. Continued

Item	Item number	STROBE Guideline
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding. (b) Describe any methods used to examine subgroups and interactions. (c) Explain how missing data were addressed. **Cohort study –** If applicable, explain how loss to follow-up was addressed. **Case-control study –** If applicable, explain how matching of cases and controls was addressed. **Cross-sectional study –** If applicable, describe analytical methods taking account of sampling strategy. (e) Describe any sensitivity analyses.
Results		
Participants	13*	Report the numbers of individuals at each stage of the study – e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram.
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. (b) Indicate the number of participants with missing data for each variable of interest. **Cohort study –** Summarize follow-up time, e.g., average and total amount.
Outcome data	15 *	**Cohort study –** Report numbers of outcome events or summary measures over time. **Case-control study –** Report numbers in each exposure category, or summary measures of exposure. **Cross-sectional study –** Report numbers of outcome events or summary measures.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence intervals). Make clear which confounders were adjusted for and why they were included. (b) Report category boundaries when continuous variables were categorized. (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.

Extension for Genetic Association Studies (STREGA)	Score
State software version used and options (or settings) chosen.	1
(f) State whether Hardy-Weinberg equilibrium was considered and, if so, how.	1
(g) Describe any methods used for inferring genotypes or haplotypes.	1
(h) Describe any methods used to assess or address population stratification.	1
(i) Describe any methods used to address multiple comparisons or to control risk of false positive findings.	1
(j) Describe any methods used to address and correct for relatedness among subjects	1
Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	1
Consider giving information by genotype.	1
Report outcomes (phenotypes) for each genotype category over time	1
Report numbers in each genotype category	
Report outcomes (phenotypes) for each genotype category	
Report effect size/odds ratio, allele frequency, confidence intervals and P-value	
	1

Table S3. Continued

Item	Item number	STROBE Guideline
Other analyses	17	Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses.
Discussion		
Key results	18	Summarize key results with reference to study objectives.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.
Generalizability	21	Discuss the generalizability (external validity) of the study results.
Other Information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.

	Extension for Genetic Association Studies (STREGA)	Score
	(d) Report results of any adjustments for multiple comparisons.	1
		1
	(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken.	1
	(c) If detailed results (e.g., summary statistics) are available elsewhere, state how they can be accessed.	1
		1
		1
		1
		1
		1

Table S4. Outcome phenotype definitions of included papers.

Author, year	Phenotype definition
Adjei, 2021	Chemotherapy-induced peripheral neuropathy (CIPN) was measured using the QLQ-CIPN20 questionnaire. The CIPN20 scores were re-scaled in both populations so that 0 represented the most severe symptoms and 100 represented no symptoms. Hence, a negative change from baseline in N08Cx cohort corresponded to worsening of symptoms, and a lower score corresponded to worse symptoms in both N08Cx and the MCBDR cohorts.
Baldwin, 2012	The adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 2.0. The analyses were carried out using 2 complementary endpoints: (i) the cumulative dose level triggering the first grade II or higher treatment related sensory peripheral neuropathy episode and (ii) the maximum observed treatment-related sensory peripheral neuropathy grade.
Campo, 2017	Adverse Events, defining the range of severity of neuropathy cases as grade 0–4. Comparisons were made between peripheral neuropathy (PN) (grade 2-4) and no or subclinical PN (grade 0-1).
Chua, 2020	Chemotherapy-induced peripheral neuropathy (CIPN) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, defining the range of severity of neuropathy cases as grade 0–4. Because the incidence of the toxicity is dependent on cumulative drug exposure, sensory peripheral neuropathy (PN) was assessed with a dose-to-event phenotype. A microtubule targeting agents (MTA)-induced sensory PN event was defined as the cumulative MTA dose (mg/m2) to first instance of grade 2 or higher sensory PN.
Cook-Sather, 2014	Children's Hospital of Eastern Ontario Pain Scale scores ranging from 4 to 13 were used, they were normalized to a 0–10 scale. Maximum and minimum postoperative pain scores (0–10), and postoperative and total (intraoperative plus postoperative) morphine in µg/kg were calculated.
Diouf, 2015	Children in the St Jude Total XIIIB study were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 1.0 and those in the COG cohort according to a modified NCI CTCAE version 2.0. Neuropathy events were assessed as mild (grade 1), moderate (grade 2), serious/disabling (grade 3), or life threatening (grade 4). Those with grades 2, 3, or 4 motor and/or sensory neuropathy were considered neuropathy cases. There were no neuropathy-related deaths (grade 5).
Docampo, 2014	All patients fulfilled the 1990s American College of Rheumatology (ACR) criteria for fibromyalgia (FM) and were selected by the rheumatologists of the units participating in the study. Patients were then evaluated by another group of physicians trained in the assessment of FM patients. Diagnosis of FM was based on questionnaires and physical examination, and detailed descriptions of these information can be found in this paper [PMID: 24098674]. Three different control cohorts were used for this study: Gabriel consortium (ECHRS), National DNA Bank of Salamanca, and Spanish blood donor samples.
Dolan, 2017	The frequency of sensory neuropathy was evaluated using nine items in the EORTC-CIPN20. Four ordinal groups were derived reflecting the average severity across symptoms: none (0; mean = 0), a little (1; 0 < mean ≤ 1), quite a bit (2; 1 < mean ≤ 2), very much (3; 2 < mean ≤ 3). Groups 2 and 3 were combined due to low frequency.
Dunbar, 2020	Patterns of pain were defined using a 6-category severity–frequency classification system with O = no pain; A = episodes of mild pain; B = constant mild to moderate pain; C = episodes of severe pain; D = constant mild and episodes of severe pain; E = constant-severe pain. For this study, subjects responding with D or E were classified as constant-severe pain, while subjects responding with O, A, B, or C were classified as not constant- severe pain.

Table S4. Continued

Author, year	Phenotype definition
Fontanillas, 2021	The pain sensitivity questionnaire (PSQ) contains 14 questions in which participants should imagine themselves in certain situations. Participants should then grade how painful they would be, from 0 that stands for no pain to 10, the most severe pain that participants can imagine or consider possible. The total PSQ score is the mean of the 14 responses. For the cold pressor test (CPT), participants were asked to prepare their own bath of ice water at home, and to keep their non-dominant hand submerged to the wrist for no more than 150 seconds. Two primary outcomes were assessed: cold pain threshold and cold pain tolerance. Cold pain threshold was the time to the first report of pain and cold tolerance the time to removal of the hand from the water.
Freidin, 2019	For the UK Biobank, cases of back pain (BP) were defined as those who reported "Back pain" in the response to the question: "Pain type(s) experienced in the past month." Controls were defined as those who did not report BP in response to this question. Individuals who did not reply or replied: "Prefer not to answer" or "Pain all over the body" were excluded. For the CHARGE Consortium, cases were defined as those reporting BP present for at least 3 months, whereas the controls were defined as those who reported no BP or BP with shorter duration. Thus, the definition of BP in these cohorts corresponded to chronic BP.
Freidin, 2021	For the UK Biobank: Those who indicated "Back pain" in response to the data-field 6159 (Pain types) question and also replied "Yes" to the data-field 3571 (Back pain for 3 months) question were classified as cases. Those who did not indicate "Back pain" in response to the data field 6159 or replied "No" to the data field 3571 question were classified as controls. For the Generation Scotland (GS): The definition of chronic back pain (cBP) cases is those who selected BP option for more than 3 months, while the controls are all other participants. For the orofacial Pain cohort: The phenotype of cBP was defined as participants that reported having more than 5 episodes of BP in the past year and those that reported between 2 to 4 episodes last year and that the episode lasted more than 2 hours. Participants reporting chronic widespread pain and fibromyalgia were excluded. For the HUNT study: The questionnaire data were used, with the participants who at least 3 consecutive months of pain and/or stiffness in muscles and listed lower BP or upper BP as complaint regions. Fibromyalgia participants were excluded both from cases and controls. For ELSA cohort: Participants who positively responded to the questions "Whether often troubled with pain" and "Whether feel pain in back" were considered to have BP during a particular wave, whereas those who replied negatively to the first and/or second question were considered not to have BP. After obtaining these data in each wave separately, those who were cases in at least 2 waves were defined as cBP cases, whereas the rest were defined as controls.
Galvan, 2011	The pain relief phenotype under study is semi-quantitative and determined based on the BPI, a robust and psychometric validated method to assess the subjective severity of pain. Pain relief is measured using an 11-point numerical rating scale, from 0%, representing "no pain relief" to 100% or "complete pain relief," that is, 0%, 10%, 20%, etc.. For genome-wide association analysis, the cancer patients were first defined as "good" or "poor" responders to opioid therapy, based on their pain relief phenotype score of 90% or more or 40% or less, respectively.
García-Sanz, 2017	Patients who developed neuropathy (grade ≥ 2, NCI-CTCAE) were compared with those who did not.
Hertz, 2016	The primary endpoint for GWAS analysis was the cumulative docetaxel dose (mg/m2) at first report of treatment-related grade 3+ sensory peripheral neuropathy defined by National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0.

Table S4. Continued

Author, year	Phenotype definition
Hirata, 2018	Dysmenorrhea pain severity was originally queried in Japanese using a five-level word-association scale with 1 = not at all painful, 2 = not very painful, 3 = neither painful or unpainful, 4 = slightly painful, and 5 = very painful; (Closest English translations). Then, the integer values were transformed into an 11-point Numeric Rating Scale (NRS) which can be sub-divided into ranges with 0 = No pain, 1–3 = Mild Pain, 4–6 = Moderate Pain, and 7–10 = Severe Pain. Five-levels of pain severity were mapped to the NRS as 1-> 0 (No pain), 2-> 1 (Mild pain), 3-> 2 (Mild pain), 4-> 5 (Moderate pain), and 5-> 10 (Severe pain) for the genome-wide association analysis.
Janicki, 2016	Diagnosis of complex regional pain syndrome (CRPS) confirmed by motor/trophic changes that fulfill the International Association of the Study of Pain criteria for CRPS, duration of CRPS symptoms>1 year, and currently under follow-up treatment in the Pain Clinic at either PSHMC or Drexel. CRPS was diagnosed by pain specialists of the participating centers experienced in diagnosis and treatment of CRPS patients. Controls were subjects without CRPS.
Johnston, 2019	A genome-wide association was conducted for multisite chronic pain (MCP), which was defined as the sum of body sites (category ID 100048) at which chronic pain (at least 3 months duration) was recorded: 0 to 7 sites. Those who answered that they had chronic pain 'all over the body' were excluded from the GWAS as there is some evidence that this phenotype relating to widespread pain can be substantially different from more localised chronic pain and should not, therefore, be considered a logical extension of the multisite scale.
Johnston, 2021	Multisite Chronic Pain (MCP) was a quasi-quantitative variable defined as previously reported [PMID: 31194737]; briefly, this variable captures the number of body sites at which chronic pain (at least 3 months duration) was recorded: phenotypic values therefore ranged from 0 to 7. (excluding those with chronic widespread pain).
Jones, 2016	The dysmenorrhea pain severity phenotype was captured as an ordered variable with the following possible values scored as 0, 1, 2, 3 ("not painful". "little painful". "moderately painful". "extremely painful"), respectively, excluding responders selecting "I'm not sure."
Kanai, 2021	Patients with grade 0/1 peripheral sensory neuropathy (PSN) were compared to patients with grade 2/3 PSN. For extreme phenotypes of PSN, patients who discontinued oxaliplatin early due to grade 2/3 PSN were selected and compared to those who maintained the status of grade 0 PSN after the completion of preplanned 6-month treatment without any dose reduction or delay of L-OHP.
Kim, 2009	Clinically induced pain was recorded with a paper and pencil form of a 100 mm visual analog scale (VAS). After the extraction of the impacted third molars, pain was recorded every 20 min by VAS until subjects requested analgesic medication as the local anesthesia was eliminated and post-operative pain onset occurred. The maximum post-operative pain rating, post-operative pain onset time and the analgesic onset time after ketorolac administration were used as measures of clinical pain and the onset of nonsteroidal anti-inflammatory drug (NSAID) analgesia.
Komatsu, 2015	Patients who developed ≥ grade 2 paclitaxel-induced sensory peripheral neuropathy (cases) were compared with patients who did not show neuropathy (controls). The grade of toxicity was classified in accordance with the US National Cancer Institute's Common Toxicity Criteria version 2.0.
Leandro-García, 2013	Neuropathy symptoms at baseline and cumulative paclitaxel dose at first neuropathy event, at grade 2 sensory neuropathy, and at maximum neuropathy grade were also collected from all patients. Patients with no or minimal adverse reaction (grade 0/1) were censored at total paclitaxel cumulative dose (mg). The grading was based on a common questionnaire modified on the National Cancer Institute Common Toxicity Criteria V.2.

Table S4. Continued

Author, year	Phenotype definition
Lee, 2019	A pain score was determined as the mean of four pain severity items (from 0 = no pain to 10 = the worst imaginable pain) as suggested by the Brief Pain Inventory developers, and moderate to severe pain (pain score ≥ 4) was considered clinically relevant. Therefore, cases were defined as those that had a pain score ≥ 4 at post-radiotherapy and the reference group included those with a pain score < 4 at post- radiotherapy.
Leger, 2014	Peripheral neuropathy was assessed at each study visit and was categorized as grade 1 (asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social and functional activities), grade 2 (sensory alteration or paresthesia causing greater than minimal interference with usual social and functional activities), and grade 3 (sensory alteration or paresthesia causing inability to perform usual social and functional activities). Analyses were performed separately for grade ≥1, grade ≥2, and grade 3 peripheral neuropathies. Controls were patients who did not develop such signs or symptoms within 96 weeks of initiating didanosine- or zidovudine/lamivudine-containing regimens/Stavudine.
Lemmela, 2016	In the YFS study, information on physician-diagnosed sciatica was inquired during on-site examinations using a self-administered questionnaire ("Do you currently have or have you had a long-term disease diagnosed by a physician, such as sciatica?"). In the H2000, the diagnosis of sciatica was based on the presence of chronic (>3 months) low back pain radiating down to the leg and either clinical findings of lumbar nerve root compression or a history of lumbar disc herniation that had been previously verified by imaging or required surgery. For the replication study (The FINRISK Study), those diagnosed with one of the ICD-codes selected a priori by two experts on musculoskeletal diseases (EVJ and MH) as relevant for sciatica or sciatic syndrome (ICD8 353, 728.8; ICD9 724.3, 722.1, 722.10, 722.5, 722.52, 355.0; ICD10 M54.3, M51.1, M54.1, M54.4) were included as cases.
Li, 2017	A visual analogue scale has previously been used to rate menstrual pain. In this study, a horizontal 10-cm visual analogue scale with endpoints were adopted, spanning from 'no pain at all' (score=0) on the far left to 'the worst pain' (score=10) on the far right. Scores of less than 1 were assigned to a control group, and scores higher than 4 (moderate intensity) were assigned to the case group. The participants with possible causes of secondary dysmenorrhoea, such as endometriosis and other gynecological problems, were excluded.
Li, 2019	POG studies 9904 and 9905. Based on National Cancer Institute Common Toxicity Criteria for Adverse Events version 2.0, the vincristine induced peripheral neuropathy (VIPN) events are defined when patients experienced symptomatic neurotoxicity with neuropathy grade ≥ 3 in either motor or sensory neurons. ADVANCE trial. A 5-item total neuropathy score (TNS-PV) was used as phenotype to summarize the VIPN in this genetic association data analysis. The 5-item score includes sensory symptoms (i.e., numbness, tingling, and neuropathic pain), temperature sensibility, vibration sensibility, strength, and tendon reflexes.
Magrangeas, 2016	Adverse events including peripheral neuropathy were graded by NCI Common Toxicity Criteria Version 3.0. Grade ≥ 2 bortezomib-induced peripheral neuropathy (BiPN) patients were compared with control patients defined as grade 1 or no BiPN.

Table S4. Continued

Author, year	Phenotype definition
Meng, 2015 #1	A neuropathic pain case was defined in this study as a type 2 diabetic individual with a history of at least one prescription of any of the following five medicines, which are effective and recommended in diabetic peripheral neuropathy (Attal et al., 2010; Finnerup et al., 2010; NICE, 2013) and used less frequently for other indications: duloxetine, gabapentin, pregabalin, capsaicin cream/patch and lidocaine patch. The cases also had positive monofilament tests in at least one foot, indicating the likely presence of sensory neuropathy. A control was defined as a type 2 diabetic individual with no prescription history of these five drugs, nor of the following 16 opioid analgesics (buprenorphine, codeine phosphate, diamorphine, dihydrocodeine, dipipanone, fentanyl, hydromorphone, meptazinol, methadone, morphine, oxycodone, papaveretum, pentazocine, pethidine, tapentadol and tramadol). Individuals with a prescription history of amitriptyline, carbamazepine or nortriptyline were excluded from controls since these are also frequently used to treat other disorders (although these drugs are effective in neuropathic pain).
Meng, 2015 #2	A neuropathic pain case was defined as a type 2 diabetic patient who has a history of multiple usages (minimum twice) of at least one of the following five medicines which are recommended and effective in diabetic peripheral neuropathy and prescribed uncommonly for other disorders: duloxetine, gabapentin, pregabalin, capsaicin cream (or patch) and lidocaine patch (Attal et al., 2010; National Institute for Health & Care Excellence NICE (UK), 2013; Finnerup et al., 2010). A control was defined as a type 2 diabetic patient who has not been prescribed any of these five drugs before. Individuals who had a prescription history of amitriptyline, carbamazepine, or nortriptyline were not included as controls because these drugs are often used for the treatment of other medical conditions, as well as neuropathic pain. Individuals with a history of only one single prescription for any of these five drugs were excluded from both cases and controls.
Meng, 2019	For the UK Biobank: The knee pain cases were those who selected the 'knee pain' option for the UK Biobank Questionnaire field ID 6159, regardless of whether they had selected other options. The controls in this study were those who selected the 'None of the above' option. Independent cohort 1—23andMe, Inc: Cases were defined as those self-reported having been diagnosed or treated for osteoarthritis. Controls were defined as those self-reporting as having not been diagnosed or treated for osteoarthritis. Independent cohorts 2—OAI and JoCo: Cases were those with definitive knee osteoarthritis, defined as radiographic evidence of the presence of definite osteophytes and possible joint space narrowing (Kellgren-Lawrence grade ≥ 2) or total joint replacement in one or both knees. Controls were those having no or doubtful evidence of OA (Kellgren-Lawrence grade = 0 or 1) in both knees at all available time points.

Table S4. Continued

Author, year	Phenotype definition
Meng, 2020	For the UK Biobank: Cases were defined as participants who reported having activity limiting pain in the neck or shoulder in the past month (the UK Biobank Questionnaire field ID 6159), regardless of whether they reported pain in other regions. The controls were defined as participants who chose the 'None of the above' option. Replication cohort 1—GS:SFHS: Participants were asked a few question, if a participant selected both 'Neck or shoulder pain' and 'Have you had this pain or discomfort for more than 3 months?', then he/she was defined as a case. All other subjects were defined as controls. Replication cohort 2: TwinsUK: participants were asked 'In the past three months, have you had pain in your neck or shoulders?' Those who answered 'Yes' were defined as cases. Those who answered 'No' were defined as controls. Those with missing answers were not included in the study.
Mieda, 2016	24-h patient-controlled analgesia (PCA) fentanyl consumption, defined as the cumulative doses of fentanyl that were actually administered to the patients via the PCA pump during the first 24-h postoperative period, was used as the primary endpoint among various quantitative phenotypic traits.
Nishizawa, 2014	Requirements for an opioid analgesic as a continuous variable, fentanyl (μg kg-1), during the 24-h postoperative period.
Nishizawa, 2018	The average remifentanil infusion rate (in μg/kg/min) during surgery was calculated by dividing the total dose of remifentanil that was required during surgery by the duration of surgery and body weight. Prior to the analyses, the quantitative values of the average remifentanil infusion rate (μg/kg/min) were natural-log-transformed for approximation to the normal distribution according to the following formula: value for analyses = Ln (1 + average remifentanil infusion rate [μg/kg/min]).
Nishizawa, 2021	Chronic pain cases include: postherpetic neuralgia (PHN), lower back pain (LBP), hernia of intervertebral disk, spinal canal stenosis, postoperative pain, neck pain, others. 282 healthy adult volunteers were enrolled as controls who were disease-free, did not experience chronic pain, and who lived in or near the Kanto area in Japan.
Peters, 2012	Chronic widespread pain (CWP) was defined as subjects having pain in the left side of the body, in the right side of the body, above the waist, below the waist, and in the axial skeleton (following the Fibromyalgia Criteria of the American College of Rheumatology). Controls were defined as subjects not having CWP. Subjects using analgesics (ATC code: N0231) were excluded from the control group.
Rahman, 2021	In UKB, chronic widespread pain (CWP) cases were defined by combining self-reported diagnosis of pain all over the body lasting for >3 months; simultaneous pain in the knee, shoulder, hip and back lasting 3+ months and fibromyalgia. Controls comprised those who reported no pain in the last month or reported pain all over the body in the previous month that did not last for 3 months or reported only ≥3 months of non-musculoskeletal pain (headache, facial and abdominal pain). Those reporting a self-reported diagnosis of rheumatoid arthritis, polymyalgia rheumatica, arthritis not otherwise specified, systemic lupus erythematosus, ankylosing spondylitis and myopathy were excluded from the study.
Reyes-Gibby, 2016	Pain "during the past week" was rated using a standardized 11-point numeric scale (0 = "no pain" and 10 = "pain as bad as you can imagine"). A binary pain phenotype was adopted, where cases were individuals with severe pre-treatment pain (score ≥ 7) and controls were individuals with non-severe pre-treatment pain (score < 7), based on the National Comprehensive Cancer Network cutoff score for severe pain.

Table S4. Continued

Author, year	Phenotype definition
Reyes-Gibby, 2018	The following ICD-9 codes (053.13; 337.0; 337.09; 337.1; 356.4; 356.8; 356.9; 357.2; 357.3; 357.9; 377.41) and ICD-10 codes (G58.8; G58.9; G62.0; G62.2; G62.9; G63.0) were used to identify neuropathy in head and neck cancer (HNSCC) patient's cohort. HNSCC patients without these ICD codes were defined as controls.
Sanders, 2017	The HCHS/SOL cohort: To be classified as temporomandibular disorder (TMD) case, participants had to report having had pain in both their face and in their jaw joint. OPPERA cohort: Examiners determined classification of TMD cases who met all 3 of the following criteria: 1) pain reported with sufficient frequency in the cheeks, jaw muscles, temples, or jaw joints during the preceding 6 months; 2) pain reported in the examiner-defined orofacial region for at least 5 days out of the prior 30 days; and 3) pain reported in at least 3 masticatory muscles or at least 1 temporomandibular joint in response to palpation of the orofacial muscles or maneuver of the jaw. SHIP cohort: Participants reported symptoms by questionnaire regarding pain in the temporomandibular joint and facial muscles; presence and frequency of pain were assessed. During a clinical exam, the examiner inquired about pain or discomfort upon palpation of masticatory tissues, including temporomandibular joints (dorsocranial and lateral) at 2 kg/cm2 and masseter, temporalis, and medial pterygoid at 1 kg/cm2. NFBC: Participants (52% female) reported symptoms by responding to a questionnaire with the following questions: 1) "Do you experience temple, temporomandibular joint, face, or jaw pain once a week or more often?" 2) "Do you experience pain once a week or more often while opening your mouth wide?" A clinical exam determined the presence of examiner-evoked pain in 3 or more temporomandibular muscles and/or joints. Brazilian Cohort: Pain history was determined by asking participants the following question: "Have you had pain in your head, face, jaw, or in front of the ears in the last 30 days?" The examiner manually palpated lateral and posterior temporomandibular joints (0.45 kg) and asked participants to report yes or no responses to the presence of pain. Subjects did not meet those criteria in the cohort were defined as controls.
Schneider, 2015	Cases. Cases were defined as those experiencing grade 2–4 taxane-induced peripheral neuropathy (TIPN) as assessed by the Common Toxicity Criteria Adverse Events (CTCAE) version 3.0. Cases included patients who received at least one dose of paclitaxel and the neuropathy event occurred during treatment or within 3 months of the last dose of therapy. Controls. Controls included patients who met all the following: (i) received all planned doses of paclitaxel; (ii) had follow-up for at least 3 months after the last dose of drug; (iii) did not meet any of the case definitions as outlined above; and (iv) had either paclitaxel or bevacizumab held or modified for any reason (i.e., disease progression or other toxicity) were excluded.
Smith, 2019	Temporomandibular disorder (TMD) cases met all 3 of the following criteria: (1) pain in the cheeks, jaw muscles, temples, or jaw joints that occurred for at least 5 days per month during the preceding 6 months, including at least 15 days in the month before enrollment; (2) pain reported in the examiner-defined orofacial region for at least 5 days out of the prior 30 days; and (c) pain evoked by palpation of the orofacial muscles or maneuver of the jaw that occurred in at least 3 masticatory muscles or at least 1 temporomandibular joint or both. Subjects did not meet those criteria in the cohort were defined as controls.

Table S4. Continued

Author, year	Phenotype definition
Sucheston-Campbell, 2018	Taxane-induced peripheral neuropathy were monitored and reported using the CTCAE, version 3.0, which contains descriptive terminology to be used for adverse event reporting. Grade 3 toxicities interfere with activities of daily living, and grade 4 adverse events are life-threatening and often require hospitalization. Logistic regression was performed between <grade 3 and >=grade 3.
Suri, 2018	Chronic back pain (CBP) cases were defined in this study using one of 3 definitions depending on the cohort: 1) ≥ 3 months of back pain, 2) ≥ 6 months of back pain, and 3) ≥ 1 month of back pain in consecutive years (reflecting ≥ 12 months of back pain). For each cohort, the comparison group ("controls") was comprised of those who reported not having back pain or reported back pain of insufficient duration to be included as a case.
Suri, 2021	For the low back pain requiring healthcare utilization (LBP-HC) phenotype: cases were defined as adults with 2 or more ICD-9 or ICD-10 codes indicating a phenotype, and controls were defined as adults with no codes indicating a phenotype (all codes included, available at http://links.lww.com/PAIN/B304). Adults with only 1 diagnostic code indicating a phenotype were omitted from the analysis (i.e., not included as cases or controls).
Takahashi, 2018	The baseline latency to pain perception, defined as the time of immersion of the hand in the ice water, before the i.v. injection of fentanyl (PPLpre) was recorded. A cut-off point was set at 150s. The hand was warmed with a hair dryer as soon as it was withdrawn from the ice water until the sensation of cold was completely abolished, then Fentanyl, 2 mg/kg was injected i.v. Three minutes after the injection, the pain perception latency of the dominant hand (PPLpost) was measured again. The analgesic effect of fentanyl in the preoperative cold pressor-induced pain test was evaluated simply as the difference between PPLpost and PPLpre (PPLpost - PPLpre).
Tang, 2019	The ACCORD and BARI 2D trials both defined neuropathy based on a Michigan Neuropathy Screening Instrument (MNSI) clinical examination that includes a focused examination of the feet to assess skin and structural abnormalities, along with assessment of distal vibration perception with a 128-Hz tuning fork and ankle reflexes. Diabetic peripheral neuropathy (DPN) case subjects were defined as participants having an MNSI > 2.0 at study entry and/or at any time during follow-up, whereas DPN control subjects were defined as participants having an MNSI < 2.0 at study entry and for the entire duration of follow-up.
Tsepilov, 2020	For the UK Biobank, those who reported back, neck or shoulder, hip, or knee pain lasting more than 3 months were considered chronic back, neck/shoulder, hip, and knee pain cases, respectively. Participants reporting no such pain lasting longer than 3 months were considered controls (regardless of whether they had another regional chronic pain, such as abdominal pain, or not). Individuals who preferred not to answer, reported more than 3 months of pain all over the body were excluded from the study. To obtain genetic components explaining four chronic musculoskeletal pain phenotypes (chronic back, neck/shoulder, hip, and knee pain), a modified principal component analysis (PCA) technique was used to combine multiple correlated variables into a set of uncorrelated principal components (PCs). PCs are linear combinations of variables constructed such that the first PC explains the maximum proportion of the total variance of the set of traits, the second PC accounts for the largest proportion of the remaining variance, and so on.
van Reij, 2020	The primary outcome measured in this cohort was the highest surgery-related pain score at rest during the last week at 3 months after surgery measured by the numeric rating scale (NRS). Based on the primary outcome measure, patients were divided into a non-pain (NRS = 0) and a chronic postoperative pain (NRS > 3) group to perform an extreme phenotype analysis to increase the power. Patients with mild pain (NRS between 1 and 3) score were not included in the genetic analysis.

Table S4. Continued

Author, year	Phenotype definition
Veluchamy, 2021	GoDARTS and GS:SFHS: Individuals with of possible Neuropathic pain (NP) (ie, case participants) were identified based on current reported pain and/or currently taking pain medications, pain duration of at least 3 months, and Douleur Neuropathique en 4 Questions (DN4) score greater than or equal to 3 of 7. Control participants were defined as those reporting no pain or not taking any pain medications at the time of completing the questionnaire. Participants who reported pain of less than 3 months' duration or who scored less than 3 on the DN4 were excluded. For the UK Biobank: Self-reported prescribed medication linked to routine hospital admissions records were used as a proxy phenotype for NP. Briefly, case participants were defined as individuals with a record of the most commonly prescribed anti-neuropathic medicines, based on the NeuPSIG guidelines (ie, gabapentin, pregabalin, duloxetine). Control participants were those with no such reported prescriptions. Individuals reporting receipt of amitriptyline, other tricyclic antidepressants, and/or tramadol were excluded from the control and case groups, despite the potential role of these medicines in treating NP because of their frequent use to treat other conditions and consequent nonspecificity for NP. Individuals who self-reported an epilepsy diagnosis and/or any anti-epileptic medication concomitantly with a gabapentinoid alone were excluded.
Warner, 2017	Individuals were assigned a phenotype by classifying them according to their scores on the painDETECT questionnaire. This is a seven-item questionnaire scored from 0 to 39 that uses a Likert scale for participants to describe the nature of their pain, in order to distinguish it from nociceptive pain. Questions are included on qualities such as burning pain, tingling, sudden pain and sensitivity to heat and cold. In all cohorts, scores of > 12 were classified as 'possible neuropathic pain' according to the validated cut-offs for diagnosis by Freynhagen *et al* [PMID: 17022849]. All others in the cohort were defined as controls.
Winsvold, 2021	Identical criteria were used to define cases and controls in the HUNT and UK Biobank studies. Cases were defined by 1) the presence of at least one hospital contact with a registered diagnosis of idiopathic progressive neuropathy (ICD-10 G60.3, ICD-9 356.4), other specified idiopathic peripheral neuropathy (ICD-9 356.8), unspecified hereditary and idiopathic neuropathy (ICD-10 G60.9), or unspecified polyneuropathy (ICD-10 G62.9, ICD-9 356.9); 2) no hospital contact with a registered diagnosis of diabetes (ICD-10 E10–E14, ICD-9 250). Controls included all participants who had no hospital contacts with a registered diagnosis of hereditary or idiopathic polyneuropathy (ICD-10 G60, ICD-9 356), other inflammatory polyneuropathy (ICD-10 G61.8, ICD-9 357), unspecified inflammatory polyneuropathy (ICD-10 G61.9), other and unspecified polyneuropathies (ICD-10 G62), polyneuropathy in diseases classified elsewhere (ICD-10 G63), idiopathic peripheral autonomic neuropathy (ICD-10 G90.0), paraneoplastic neuropathy (ICD-10 G13.0), autonomic neuropathy in diseases classified elsewhere (ICD-10 G99.0), or diabetes (ICD-10 E10–E14, ICD-9 250).
Won, 2012	Neuropathy was evaluated and rated in accordance with National Cancer Institute (NCI) criteria. Cases were defined as prolonged (≥7 days) grade 2 or grade 3 events. All the others in the cohort were defined as controls.
Yokoshima, 2018	Opioid analgesic responsiveness were evaluated by pain intensity (NRS) on a 5-point Likert scale (responses were scored as 0 = absence of symptoms, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe) before and after prescribing firstly or increasing opioid analgesics. Opioid analgesic responsiveness was defined as pain decrease corresponding to increased opioid analgesics.

Table S5. List of excluded studies after full-text assessment.

Author, Year	Title	PubMed ID	Reason for exclusion
Bjornsdottir, 2019	A PRPH splice-donor variant associates with reduced sural nerve amplitude and risk of peripheral neuropathy	30992453	Outcome: nerve conduction
Bjornsdottir, 2017	Sequence variant at 8q24.21 associates with sciatica caused by lumbar disc herniation	28223688	Outcome: lumbar disc herniation
Chaturvedi, 2017	Genome-wide association study to identify variants associated with acute severe vaso-occlusive pain in sickle cell anemia	28584135	Publication type: letter
Cox, 2020	Genome-wide association study of opioid cessation	31936517	Outcome: opioid cessation
Freidin, 2021	An association between chronic widespread pain and the gut microbiome	33331911	Study design: not genetic marker of humans
Johnston, 2019	Identification of novel common variants associated with chronic pain using conditional false discovery rate analysis with major depressive disorder and assessment of pleiotropic effects of LRFN5	31748543	Study design: analysis using published GWAS summary statistics
Mahmoudpour, 2018	Chemotherapy-induced peripheral neuropathy: evidence from genome-wide association studies and replication within multiple myeloma patients	30111286	Study design: replication study
Meng, 2017	A genome-wide association study suggests that MAPK14 is associated with diabetic foot ulcers	28672053	Outcome: diabetic foot ulcer
Ruau, 2012	Integrative approach to pain genetics identifies pain sensitivity loci across diseases	22685391	Study design: candidate gene study not GWAS
Sanchez-Roige, 2021	Genome-wide association study of problematic opioid prescription use in 132,113 23andMe research participants of European ancestry	34728798	Outcome: opioid dependence/cession
Smith, 2017	Genome-wide association study of therapeutic opioid dosing identifies a novel locus upstream of OPRM1	28115739	Outcome: opioid dependence/cessation
Trendowski, 2020	Clinical and genome-wide analysis of multiple severe cisplatin-induced neurotoxicities in adult-onset cancer survivors	32998964	Study design: hearing loss was included for cases
Ustinova, 2021	Novel susceptibility loci identified in a genome-wide association study of type 2 diabetes complications in population of Latvia	33430853	Study design: peripheral circulatory complications were included for cases

Table S5. Continued

Author, Year	Title	PubMed ID	Reason for exclusion
Wheeler, 2013	Integration of cell line and clinical trial genome-wide analyses supports a polygenic architecture of Paclitaxel-induced sensory peripheral neuropathy	23204130	Study design: analysis using previous GWAS summary statistics
Williams, 2013	Novel genetic variants associated with lumbar disc degeneration in northern Europeans: a meta-analysis of 4600 subjects	22993228	Outcome: lumbar disc degeneration
Williams, 2020	Genome-wide association study of pain in Parkinson's disease implicates TRPM8 as a risk factor	32078185	Publication type: letter
Backman, 2021*	Exome sequencing and analysis of 454,787 UK Biobank participants.	34662886	Study design: not a focus on pain related phenotype
Jiang, 2021*	A generalized linear mixed model association tool for biobank-scale data.	34737426	Study design: not a focus on pain related phenotype
Dönertaş, 2021*	Common genetic associations between age-related diseases.	33959723	Study design: not a focus on pain related phenotype
Sakaue, 2021*	A cross-population atlas of genetic associations for 220 human phenotypes.	34594039	Study design: not a focus on pain related phenotype
Watts, 2021*	Genome-wide association studies of toxicity to oxaliplatin and fluoropyrimidine chemotherapy with or without cetuximab in 1800 patients with advanced colorectal cancer.	34270794	Outcome: No results on neuropathy were reported

* Asterisk indicates additional papers found by checking the GWAS catalog.

Table S6. Quality assessment score of included papers according to STREGA guidelines.

Study	Title & Abstract (Max. score =1)	Introduction (Max. score =2)	Method (Max. score =14)	Results (Max. score =8)	Discussion (Max. score =4)	Funding (Max. score =1)	Quality score (Max. score =30)
Adjei, 2021	1	2	11	7	4	1	26
Baldwin, 2012	1	2	10	5	4	1	23
Campo, 2017	1	2	10	5	2	1	21
Chua, 2020	1	2	12	7	3	1	26
Cook-Sather, 2014	1	2	12	4	4	1	24
Diouf, 2015	1	2	10	7	3	1	24
Docampo, 2014	1	2	9	4	4	0	20
Dolan, 2017	1	2	12	6	3	1	25
Dunbar, 2020	1	2	10	6	3	1	23
Fontanillas, 2021	1	2	11	6	3	1	24
Freidin, 2019	1	2	11	7	4	1	26
Freidin, 2021	1	2	11	7	4	1	26
Galvan, 2011	1	2	8	5	2	1	19
García-Sanz, 2017	1	2	8	4	2	1	18
Hertz, 2016	1	2	11	6	4	1	25
Hirata, 2018	1	2	11	6	2	0	22
Janicki, 2016	1	2	10	4	3	1	21
Johnston, 2019	1	2	13	8	3	1	28
Johnston, 2021	1	2	11	6	3	1	24
Jones, 2016	1	2	11	6	3	1	24
Kanai, 2021	1	2	11	6	2	1	23

Table S6. Continued

Study	Title & Abstract (Max. score =1)	Introduction (Max. score =2)	Method (Max. score =14)	Results (Max. score =8)	Discussion (Max. score =4)	Funding (Max. score =1)	Quality score (Max. score =30)
Kim, 2009	1	2	10	3	3	0	19
Komatsu, 2015	1	2	6	4	2	1	16
Leandro-García, 2013	1	2	11	6	2	1	23
Lee, 2019	1	2	11	7	3	1	25
Leger, 2014	1	2	7	5	3	1	19
Lemmela, 2016	1	2	11	7	3	1	25
Li, 2017	1	2	11	6	4	1	25
Li, 2019	1	2	11	6	4	1	25
Magrangeas, 2016	1	2	10	5	3	1	22
Meng, 2015 #1	1	2	12	4	3	1	23
Meng, 2015 #2	1	2	13	5	3	1	25
Meng, 2019	1	2	11	5	4	1	24
Meng, 2020	1	2	11	6	4	1	25
Mieda, 2016	1	2	8	6	2	1	20
Nishizawa, 2014	1	2	8	6	2	1	20
Nishizawa, 2018	1	2	8	6	3	1	21
Nishizawa, 2021	1	2	7	4	3	1	18
Peters, 2012	1	2	12	6	4	1	26
Rahman, 2021	1	1	14	8	4	1	29
Reyes-Gibby, 2016	1	2	12	5	3	1	24
Reyes-Gibby, 2018	1	2	12	7	3	1	26

Table S6. Continued

Study	Title & Abstract (Max. score =1)	Introduction (Max. score =2)	Method (Max. score =14)	Results (Max. score =8)	Discussion (Max. score =4)	Funding (Max. score =1)	Quality score (Max. score =30)
Sanders, 2017	1	2	12	5	4	1	25
Schneider, 2015	1	2	11	5	4	1	24
Smith, 2019	1	2	13	7	4	1	28
Sucheston-Campbell, 2018	1	2	11	6	3	1	24
Suri, 2018	1	2	12	7	4	1	27
Suri, 2021	1	2	13	7	4	1	28
Takahashi, 2018	1	2	10	6	2	1	22
Tang, 2019	1	2	12	7	4	1	27
Tsepilov, 2020	1	2	13	7	4	1	28
van Reij, 2020	1	2	14	7	4	1	29
Veluchamy, 2021	1	2	13	5	4	1	26
Warner, 2017	1	2	13	7	4	1	28
Winsvold, 2021	1	2	14	5	4	1	27
Won, 2012	1	2	7	6	3	1	20
Yokoshima, 2018	1	2	7	5	3	1	19

Table S7. Replication and meta-analysis information of included papers.

	Replication study				
Author, Year	**Replication analysis type**	**Sample size**	**Ethnicity**	**P-value threshold**	**No. of replicated locus in replication**
Adjei, 2021	Single independent replication cohort	381	NS	1.00E-06	4
Baldwin, 2012	Replication was split from the discovery cohort	271	EA; AA	NS	NS
Campo, 2017	No replication	NS	NS	NS	NS
Chua, 2020	Single independent replication cohort from previous published results: CALGB 40101	855	EA	NS	NS
Cook-Sather, 2014	Replication was split from the discovery cohort	145	EA; AA	NS	NS
Diouf, 2015	Single independent replication cohort	99	EA; AA; Asian; Hispanic; other	NS	NS
Docampo, 2014	Replication was split from the discovery cohort	1532	White Spanish	2.94E-03	0
Dolan, 2017	No replication	NS	NS	NS	NS
Dunbar, 2020	No replication	NS	NS	NS	NS
Fontanillas, 2021	Two phenotype cohorts replicated in each other	25321 for CPT phenotype replication, 6853 for PSQ phenotype replication	EA	NS	NS
Freidin, 2019	Meta-analysis of several independent cohorts	154970-157752	EA; AA; SA; Chinese	1.00E-02	3
Freidin, 2021	Meta-analysis of several independent cohorts	43740 males; 50092 females	EA	5.60E-03	2
Galvan, 2011	Replication was split from the discovery cohort	570	EA	NS	NS
García-Sanz, 2017	No replication	NS	NS	NS	NS
Hertz, 2016	Single independent replication cohort from previous published results: CALGB 40101	855	EA	5.00E-02	0
Hirata, 2018	No replication	NS	NS	NS	NS

	Joint meta-analysis			
	Available	**P-value threshold**	**No. of replicated locus in joint meta-analysis**	**Comments**
	No	NS	NS	The SNPs found in replication were different independent loci from discovery
	No	NS	NS	This study is CALGB 40101, which is used for many following studies as replication cohort.
	No	NS	NS	
	Yes	1.00E-05	15	
	No	NS	NS	Replicaiton was only conducted for total morphine dose phenotype
	Yes	5.00E-08	1	
	Yes	NS	NS	Three suggestive significant signals in joint meta-analysis were added to describe in the results section
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	Yes	5.00E-08	1	Meta-analysis in EA only
	No	NS	NS	Discovery study is sex-stratified analysis
	Yes	5.00E-08	1	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	

Table S7. Continued

	Replication study				
Author, Year	**Replication analysis type**	**Sample size**	**Ethnicity**	**P-value threshold**	**No. of replicated locus in replication**
Janicki, 2016	Replication was split from the discovery cohort	230	EA;AA;Hispanics	2.00E-03	0
Johnston, 2019	No replication	NS	NS	NS	NS
Johnston, 2021	No replication	NS	NS	NS	NS
Jones, 2016	No replication	NS	NS	NS	NS
Kanai, 2021	No replication	NS	NS	NS	NS
Kim, 2009	No replication	NS	NS	NS	NS
Komatsu, 2015	No replication	NS	NS	NS	NS
Leandro-García, 2013	No replication	NS	NS	NS	NS
Lee, 2019	No replication	NS	NS	NS	NS
Leger, 2014	No replication	NS	NS	NS	NS
Lemmela, 2016	Single independent replication cohort	19265	Finnish	5.00E-02	1
Li, 2017	Single independent replication cohort from previous published results: CALGB 40101	1446	Chinese	NS	NS
Li, 2019	Single independent replication cohort from previous published results: CALGB 40101	63	EA	NS	NS
Magrangeas, 2016	Single independent replication cohort	114	NS	NS	NS
Meng, 2015 #1	No replication	NS	NS	NS	NS
Meng, 2015 #2	No replication	NS	NS	NS	NS
Meng, 2019	Individual analysis of several replication cohorts	23andMe: 1540125; OAI and JoCo: 4448	EA	NS	NS

	Joint meta-analysis			
	Available	**P-value threshold**	**No. of replicated locus in joint meta-analysis**	**Comments**
	Yes	2.00E-03	0	One strong signal in joint meta-analysis was added to describe in the results section
	No	NS	NS	
	No	NS	NS	Discovery study is sex-stratified analysis
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	Discovery GWAS results were not reported
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	Yes	5.00E-08	2	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	This study included both sex-unstratified and sex-stratified GWASes; The genome-wide significant locus found in sex-unstratified analysis was overlapped with one locus found in females
	No	NS	NS	

Table S7. Continued

	Replication study				
Author, Year	**Replication analysis type**	**Sample size**	**Ethnicity**	**P-value threshold**	**No. of replicated locus in replication**
Meng, 2020	Individual analysis of several replication cohorts	GS:SHFS: 19598; TwinsUK: 3982	EA	5.00E-02	2
Mieda, 2016	Three-stage analysis: one cohort was divided into 3 sub-cohorts	NS	NS	NS	NS
Nishizawa, 2014	Three-stage analysis: one cohort was divided into 3 sub-cohorts	NS	NS	NS	NS
Nishizawa, 2018	Three-stage analysis: one cohort was divided into 3 sub-cohorts	NS	NS	NS	NS
Nishizawa, 2021	No replication	NS	NS	NS	NS
Peters, 2012	Meta-analysis of several independent cohorts	9469	EA	NS	NS
Rahman, 2021	Meta-analysis of several independent cohorts	57257	EA	1.70E-02	1
Reyes-Gibby, 2016	Replication was split from the discovery cohort	410	EA	NS	NS
Reyes-Gibby, 2018	No replication	NS	NS	NS	NS
Sanders, 2017	Meta-analysis of several independent cohorts	8814	Multiple cohorts: OPPERA; SHIP; NFBC; Brazilian Cohort	5.00E-02	1
Schneider, 2015	Single independent replication cohort	925	EA; AA; other	1.70E-03	1
Smith, 2019	Meta-analysis of several independent cohorts	157164	Multiple cohorts: SHIP; NFBC; SPB; OP2; CPPC; HCHS;SOL; UKB	1.70E-02	0
Sucheston-Campbell, 2018	Single independent replication cohort from previous published results: CALGB 40101	855	EA	NS	NS
Suri, 2018	Meta-analysis of several independent cohorts	283752	EA	1.25E-02	3
Suri, 2021	No replication	NS	NS	NS	NS

	Joint meta-analysis			
	Available	**P-value threshold**	**No. of replicated locus in joint meta-analysis**	**Comments**
	Yes	NS	NS	Two loci identified in discovery study were replicated only in one of the replication cohort
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	No	NS	NS	
	Yes	NS	NS	
	No	NS	NS	
	Yes	5.00E-08	1	
	No	NS	NS	
	No	NS	NS	Two suggestive significant signals identified in the discovery analysis were added to describe in the results section
	No	NS	NS	
	No	NS	NS	This study included both sex-unstratified and sex-stratified GWASes; The genome-wide significant locus found in sex-unstratified analysis was overlapped with one locus found in females
	Yes	1.00E-05	3	
	Yes	5.00E-08	3	
	No	NS	NS	

Table S7. Continued

	Replication study				
Author, Year	**Replication analysis type**	**Sample size**	**Ethnicity**	**P-value threshold**	**No. of replicated locus in replication**
Takahashi, 2018	Three-stage analysis: one cohort was divided into 3 sub-cohorts	NS	NS	NS	NS
Tang, 2019	Single independent replication cohort from previous published results: CALGB 40101	949	EA	4.00E-03	1
Tsepilov, 2020	Replication was split from the discovery cohort	191580	EA; AA; SA	5.60E-03	6
van Reij, 2020	Single independent replication cohort from previous published results: CALGB 40101	203	EA	9.00E-03	1
Veluchamy, 2021	Single independent replication cohort from previous published results: CALGB 40101	428925	EA	NS	NS
Warner, 2017	Individual analysis of several replication cohorts	Rotterdam: 212; Nottingham: 908	NS	NS	NS
Winsvold, 2021	Single independent replication cohort	383998	EA	NS	NS
Won, 2012	Replication was split from the discovery cohort	247	Korean	NS	NS
Yokoshima, 2018	No replication	NS	NS	NS	NS

EA, European ancestry; AA, African American. SA, south Asian. NS, not specified.

	Joint meta-analysis			
	Available	**P-value threshold**	**No. of replicated locus in joint meta-analysis**	**Comments**
	No	NS	NS	
	Yes	5.00E-08	1	
	Yes	5.00E-08	2	
	Yes	NS	NS	
	Yes	5.00E-08	1	The SNPs found in meta-analysis were different independent loci from discovery study; One suggestive significant signals in the joint meta-analysis was added to describe in the results section
	Yes	NS	NS	
	Yes	5.00E-08	2	
	Yes	1.00E-05	3	
	No	NS	NS	

Table S8. SNPs in linkage disequilibrium ($r^2 > 0.6$) from all included papers.

SNP1	Phenotype and PMID of SNP1	SNP2	Phenotype and PMID of SNP2	r^2
rs10888692	Multisite Chronic Pain [31194737]	rs35072907	Multisite Chronic Pain [33830993]	0.673
rs12030576	Dysmenorrhoea pain [29855537]	rs7523086	Dysmenorrhoea pain [27454463]	0.977
rs3737240	Genetic components of chronic musculoskeletal pain [32587327]	rs59898460	Multisite Chronic Pain [31194737, 33830993]	0.958
rs1491985	Chronic Widespread Pain [33926923]	rs7628207	Multisite Chronic Pain [31194737]	1
rs13107325	Genetic components of chronic musculoskeletal pain [32587327]	rs13135092	Multisite Chronic Pain [31194737, 33830993]	0.895
rs2049604	Shoulder and Neck Pain [32246137]	rs12537376	Multisite Chronic Pain [31194737]	0.636
rs12537376	Multisite Chronic Pain [31194737]	rs12705966	Genetic components of chronic musculoskeletal pain [32587327]	0.693
rs7833174	Chronic back pain [30261039]	rs7814941	Chronic back pain [30747904]	0.919
rs3180	Chronic back pain [30747904]	rs1678626	Chronic back pain [33021770]	0.979
rs12308843	Chronic back pain [33021770]	rs12310519	Chronic back pain [30261039,30747904]	0.616
rs11079993	Multisite Chronic Pain [33830993]	rs12453010	Multisite Chronic Pain [31194737]	0.959
rs4384683	Chronic back pain [30261039]	rs72922230	Chronic back pain [33021770]	0.85

PMID: PubMed Identifier

All Supplementary data can be found on https://cdn-links.lww.com/permalink/pain/b/pain_2023_03_06_coenen_pain-d-22-01181_sdc1.pdf

Supplementary data 1: full list of reported SNPs from all included papers. CHR: Chromosome. POS: Position, CHR_ band: Chromosome band, EA: Effect allele, EAF: Effect allele frequency, BETA (SE): Effect size (standard error).

Supplementary data 2: Publication list in the Human Pain Genetics Database (HPGDB) of overlapping genes between genes identified in this review and the HPGDB.

Supplementary data 3: Details for Mouse Pain Genetics Database for overlapped genes with genes identified in this review.

Chapter 4

Genome-wide association study on chronic postsurgical pain after abdominal surgeries in the UK Biobank

Song Li [1], Masja K. Toneman [2], Judith P.M. Mangnus [2], Stefano Strocchi [1], Regina L.M. van Boekel [3,4], Kris C. P. Vissers [3], Richard P.G. ten Broek [2], Marieke J.H. Coenen [5]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[2] Department of Surgery, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands

[3] Department of Anesthesiology, Pain and Palliative Medicine, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[4] Research Department Emergency and Critical Care, HAN University of Applied Sciences, School of Health Studies, Nijmegen, the Netherlands

[5] Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands.

Corresponding author

Marieke J.H. Coenen, Ph.D., Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands. M.Coenen@erasmusmc.nl

Abstract

Background. Chronic postsurgical pain is one of the most common and severe complications after surgery, affecting quality of life and overall well-being of patients. Although several risk factors were identified for chronic postsurgical pain, the mechanisms of chronic postsurgical pain development remain unclear. This study aims to identify single-nucleotide polymorphisms (single-nucleotide polymorphisms) associated with chronic postsurgical pain development after one of the most common types of surgery: abdominal surgery.

Methods. A genome-wide association study (genome-wide association study) was performed on 27,603 participants from the UK Biobank who underwent abdominal surgery. The robustness of identified loci were validated by split-half validation analysis. Functionally related top loci were selected for expression validation in clinical samples of adhesions from patients with and without pain.

Results. One locus (rs185545327) reached genome-wide significance for association with chronic postsurgical pain development, and ten loci surpassed the suggestively significant threshold ($P < 1 \times 10^{-6}$). In the robustness analysis, eight loci had at least nominal significance. The loci passing the suggestively significant threshold were mapped to 15 genes, of which two loci containing pain-related genes (*SRPK2*, *PDE4D*). Although marginally approaching statistical significance in the expression validation of clinical samples, the detection rate and expression level of PDE4D were modestly higher in patients with pain compared to the control group.

Conclusion. This study provides preliminary evidence for genetic risk factors implicated in chronic postsurgical pain following abdominal surgery, particularly the *PDE4D* gene, which is associated with pain in previous studies. The findings promise the development of a clinically applicable tool for personalised risk prediction, aiding clinicians in stratifying patients and enhancing clinical decision-making through individualised risk assessments.

Keywords
Chronic postsurgical pain, abdominal surgery, Genome-wide association study, UK Biobank, Genetics

Introduction

Chronic postsurgical pain is pain that develops after a surgical procedure or increases in intensity after a surgical procedure beyond the normal healing process, i.e., lasting three months or longer after surgery [1]. Severe chronic postsurgical pain can affect patients' physical and psychological well-being, leading to reduced quality of life, limitations in physical activities, emotional distress, and sleep disturbances [2, 3].

The prevalence of chronic postsurgical pain varies from 5% to 85% in different types of surgeries [4]. A surgical category of particular interest is abdominal surgery, one of the most common and painful surgeries. The lifetime risk of undergoing abdominal surgery in developed countries is over 50% [5, 6], and the estimated incidence of chronic postsurgical pain is anywhere from 10 to 50% depending on the type of surgery (e.g., cholecystectomy, herniorrhaphy, laparotomy) and surveying methods [7, 8]. Considering the large number of surgical procedures performed annually (estimated 310 million major surgeries performed per year worldwide) [9] and abdominal surgeries accounting for more than 50% of major surgeries [10], the burden of chronic postsurgical pain following abdominal surgery is considerable.

Despite the significant negative effects of chronic postsurgical pain on quality of life and physical well-being [11-13], chronic postsurgical pain is still widely underdiagnosed and often poorly treated [2]. Successful pain management necessitates that healthcare providers implement pre-operative interventions tailored to a patient's genetic risk profile and adapt their peri-operative care accordingly, thereby mitigating the risk of chronic postsurgical pain and enhancing overall patient outcomes. This approach depends on the identification of patients at high risk of developing chronic postsurgical pain by implementing high-quality clinical prediction models for risk stratification. Building high-quality risk prediction models requires a comprehensive understanding of the complex mechanisms underlying chronic postsurgical pain development [14]. Several risk factors before, during, and after surgery have been identified, including demographic characteristics (age and gender), and clinical factors (psychosocial factors, preceding pain). Incorporating genetic risk factors into prediction models can improve the accuracy of chronic postsurgical pain risk assessment [15]. However, the genetic variants contributing to chronic postsurgical pain development have yet to be fully elucidated.

Genetic studies might shed more light on the biological mechanisms underlying chronic postsurgical pain [4, 7, 16]. A recent systematic review included 21 full-text

articles reporting variants/haplotypes of 26 genes significantly associated with chronic postsurgical pain [17]. These gene functions involve neurotransmission, pain signaling, immune responses, and neuroactive ligand-receptor interaction. However, these studies are mainly hypothesis-based candidate gene studies. To identify genes beyond known mechanisms, it is highly recommended to perform hypothesis-free analyses such as genome-wide genetic association studies (genome-wide association study). Currently, there is only one genome-wide association study on chronic postsurgical pain after abdominal surgery. This study included patients undergoing hysterectomy and identified two suggestively significant single-nucleotide polymorphisms (rs117119665, rs1145324) in the meta-analysis of the discovery and replication cohort (N = 429 in total)[18]. A recent published genome-wide association study meta-analysis (including the abovementioned genome-wide association study, N = 1350 in total), identified three loci (rs138190025, rs114837251, rs3026120). Considering the relatively small sample size, conducting a genome-wide association study in a larger sample is needed to improve the statistical power.

This study aims to identify genetic variants associated with chronic postsurgical pain using genome-wide association analysis in the UK Biobank. As the prevalence of developing chronic postsurgical pain varies for specific surgeries, and it is still unclear if the mechanisms of chronic postsurgical pain are shared for different surgeries, we focus on only abdominal surgeries. A genome-wide association study was performed to investigate which single-nucleotide polymorphisms are associated with chronic postsurgical pain in abdominal surgeries. The possible underlying biological mechanisms of identified variants were investigated by functional annotation. The results of the genome-wide association study can then be used to build robust risk prediction models. The primary clinical purpose of identifying these genetic risk factors is to flag patients at high risk of developing chronic postsurgical pain before surgery. This proactive approach aims to mitigate the risk of chronic postsurgical pain and improve patient outcomes by enabling personalised and preventive care strategies.

Method

We conducted a genome-wide association study on the development of chronic postsurgical pain following various abdominal surgeries using data from the UK Biobank. These surgeries spanned multiple specialties, including urology surgery, vascular surgery, renal surgery, and both upper and lower gastrointestinal

surgeries. and suggestively significant ($P < 1\times10^{-6}$) signals were carried forward for functional annotation. This study is pre-registered at the open science framework (https://osf.io/zyb5t/).

Study cohorts and study data

The UK Biobank is a prospective cohort recruited from a general population aged 40–69 across the United Kingdom. Details about the UK Biobank [17]. Informed consent was obtained from all participants by the UK Biobank. Although participants were not directly involved in the design or conduct of this study, their participation in the UK Biobank is invaluable for this research. The results of this study will be disseminated through academic publications and shared with the UK Biobank for wider public engagement initiatives.

The general practice (general practice) prescription and surgery records data were used to define chronic postsurgical pain phenotype. The general practice prescription data dates back to the year 1985 and up to 2016. However, a cut-off was set as 1996 to ensure a relatively good data quality, considering that 96% of general practice records were computerised in the UK after 1996 [9], and general practice records before that time were sparse [10]. Surgery data are from the inpatient hospital registry and general practice surgery records. Surgery records from 1997 to 2015 were selected to align with the phenotype definition (see below) that at least one year of prescription records was available before and after the first surgery. If patients have both HR and general practice surgery records, only the HR records were considered, as the HR data is more reliable than general practice data (as indicated by the UK Biobank team).

chronic postsurgical pain phenotype definition

Figure 1 depicts the chronic postsurgical pain phenotype definition in abdominal surgeries used for the analysis based on the available data. We analyzed only the first abdominal surgery of subjects to eliminate the effects of previous abdominal surgeries. Postoperative pain was determined based on the analgesic consumption (mainly nonsteroidal anti-inflammatory drugs and opioids, the complete list of analgesics can be found in Online Supporting Information Table S1). The one-year follow-up period of patients was divided into 12 months with 30 days as an interval. If prescription records were found in an interval of the follow-up period, then a subject would be marked as experiencing pain in that interval (month). Patients with chronic postsurgical pain are defined as subjects who had analgesic consumption records for at least three ($n > 3$) (non-consecutive) months in the one-year follow-up period after their first abdominal surgery. Control subjects

are those who had analgesic consumption records less than or equal to three (n <= 3) (non-consecutive) months after their first abdominal surgery. Although chronic postsurgical pain is defined by three consecutive months of pain after surgery according to the International Association for the Study of Pain criteria, we did not apply this rule when using analgesic prescription records as a proxy to define chronic postsurgical pain as consecutive months might not be ideal for reflecting continuous presence of pain by prescription records, which depends on the total prescriptions patients receive each time and how frequently medication is delivered.

As it can take up to six months to fully recover from a major surgery or surgery with complicated post-operative course [19], an 'ordinal phenotype' was used to distinguish patients who had chronic postsurgical pain by using a more strict threshold, i.e., who had analgesic consumption records for at least six months instead of the commonly used three months cut-off point. An ordinal score ranging from 1 to 3 was assigned: score 1 for subjects using analgesics less than or equal to three months ($n \leq 3$), score 2 for subjects using analgesics from three to six months ($3 < n \leq 6$), and score 3 for subjects using analgesics beyond six months ($n > 6$) after the surgery.

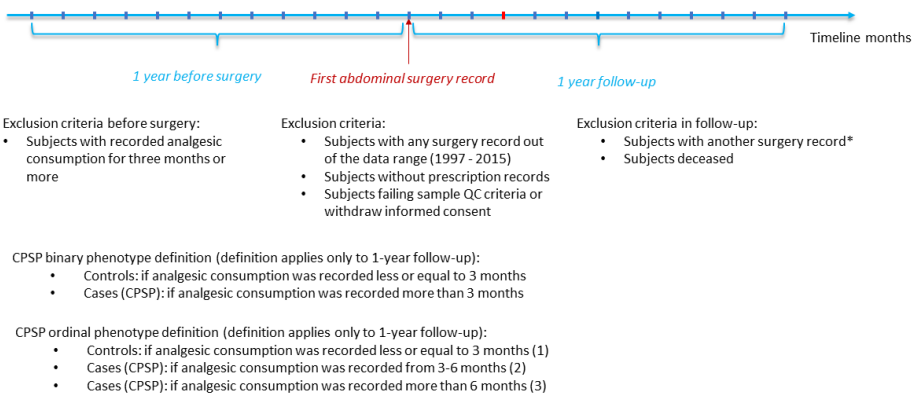

Figure 1. An illustration of chronic postsurgical pain definition. The line reflects the timeframe of surgery and prescription records of a given subject. The red arrow indicates the time of the first abdominal surgery. Pre-surgery and post-surgery follow-up periods are indicated for a period of 12 months, monthly intervals are indicated. If any analgesics were found in one of the 12 months' intervals, it indicates the subject experienced pain in that month. *: subjects with complications and two-stage surgeries were removed because it is difficult to distinguish between complications and second surgeries in the data. Red bar indicates: prescription month cut-off for cases and control. It is just for illustration as the cut-off is not based on consecutive months.

Inclusion and exclusion criteria

Subjects were included if their first abdominal surgery records were from 1997 to 2015 (See Online Supporting Information Table S2, Online Supporting Information Table S3, and Online Supporting Information Table S4 for the included abdominal surgery list in the OPCS4 code, READ v2 code, and READ v3 code, respectively).

Subjects were excluded if they met the following criteria: 1) subjects with pre-existing chronic pain (who used analgesics more than three months in the year) before the surgery; 2) subjects with any abdominal surgery records out of the data range (1997 - 2015); 3) subjects with no prescription records in the general practice data; 4) subjects with another abdominal surgery within one-year follow-up after the first surgery; 5) subjects that deceased during follow-up; 6) subjects that failed genome-wide association study sample quality control criteria (see online Supporting Information Appendix S1 for routine sample quality control criteria); 7) subjects that withdrew informed consent.

Validation of chronic postsurgical pain definition

To validate the chronic postsurgical pain definition, the following characteristics were compared between cases and controls: 1) risk factors, including demographic characteristics (age at time of surgery, gender, and body mass index), self-report chronic pain (Data Category 100048), and self-report chronic postsurgical pain (Data-Field 120005). As self-reported chronic postsurgical pain (Data-Field 120005) is not recommended for a genome-wide association study by the UK Biobank team (see supplementary method for details), we only used this data to validate our phenotype definition; 2) prescription record numbers: analgesic consumption record numbers were compared before and after surgery between cases and controls; 3) surgery types: we checked whether surgeries included in the analysis have the same percentage of cases and controls 4) finally surgery record numbers were checked: subjects with more complicated surgeries have more surgery codes linked (e.g., gastrectomy and colectomy at the same time) as patients undergoing more complicated surgeries might have higher odds of developing chronic pain [20].

Genome-wide association analysis

Routine sample and genotype quality control can be found in the online Supporting Information Appendix S1. The binary phenotype was analyzed by the linear function in GCTA (Genome-wide Complex Trait Analysis), adjusted for the following covariates: age at time of surgery, gender, assessment center, genotyping array type, first ten genetic principal components, and abdominal surgery types.

Covariates were compared by t-test for continuous variables and chi-square test for categorical variables in different groups. The significance threshold for genome-wide association study was set at the commonly accepted genome-wide threshold $P < 5 \times 10^{-8}$ [21] and a suggestively significant threshold $1 \times 10^{-6} < P < 5 \times 10^{-8}$ [22], respectively. We will refer this genome-wide association study as primary genome-wide association study and it was used for all post-genome-wide association study analysis.

The ordinal phenotype was analyzed by ordinal regression (proportional odds logistic model) in the Ordinalgenome-wide association study [23], using the same covariates as the binary analysis.

Functional annotation of single-nucleotide polymorphisms

Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) was used to identify lead single-nucleotide polymorphisms and significant independent single-nucleotide polymorphisms (single-nucleotide polymorphisms in linkage disequilibrium with the lead single-nucleotide polymorphism at $r^2 > 0.1$ and remaining statistically significant after conditioning on the lead single-nucleotide polymorphisms). The function of single-nucleotide polymorphisms in linkage disequilibrium (r2 > 0.6) with significant independent single-nucleotide polymorphisms was annotated by VEP, ANNOVAR, RegulomeDB (all in FUMA), and Haploreg. We selected some tissues/cells for Haploreg annotation (Online Supporting Information Table S5) as the regulatory effect of single-nucleotide polymorphisms varies in different tissues and cells. The annotation results include the CADD and the RegulomeDB (RDB) score. CADD is a score for deleteriousness of single nucleotide variants, and higher CADD score correlates with pathogenicity. The RDB score ranges from 1 to 7, with lower scores indicating a higher likelihood of a variant having a regulatory impact.

Pleiotropic effects of lead single-nucleotide polymorphisms are queried in the genome-wide association study Catalog and genome-wide association study Atlas. Associated traits with P-value in the original genome-wide association study passing Bonferroni correction threshold (0.05/11) will be reported.

Gene mapping and gene-based analysis

single-nucleotide polymorphisms in linkage disequilibrium ($r^2 > 0.6$) with lead single-nucleotide polymorphisms were mapped to genes by positional mapping, cis-eQTL mapping, and open chromatin mapping in FUMA. For the positional mapping, it was checked whether a single-nucleotide polymorphism was located

in a gene region (cut off 10 Kb). For cis-eQTL mapping, databases are searched to determine whether a single-nucleotide polymorphism can be linked to the expression of specific genes. Chromatin interactions are also identified based on previous data that assessed whether two genomic regions interact spatially, even if they are far away in physical distance. Significant thresholds for cis-eQTL mapping and open chromatin mapping are set as FDR < 0.05 and FDR < 1×10^{-6}, respectively.

A gene-based analysis was conducted in MAGMA (Multi-marker Analysis of GenoMic Annotation), which consists of two steps: first, single-nucleotide polymorphisms were selected to map onto genes with window size 50 kb; second, gene-based P-values were calculated based on the P-value of single-nucleotide polymorphisms mapped to a specific gene. The significant threshold for gene analysis is set as 0.05/ total gene numbers.

Validation of significant loci

Robustness analysis for suggestively significant loci was conducted by randomly splitting the dataset into two equally sized subsets five times and comparing single variant results in these sample subsets.

To check if our identified loci were also reported in other genome-wide association study studies on chronic postsurgical pain, candidate single-nucleotide polymorphisms (single-nucleotide polymorphisms that are in linkage disequilibrium ($r2 > 0.6$) with significant independent (P-value < 1×10^{-6}) single-nucleotide polymorphisms) in our study were selected to check in a genome-wide association study meta-analysis on chronic postsurgical pain. Details of this meta-analysis study can be found online [24].

RNA expression analysis

Based on the gene mapping results, we selected two genes for RNA expression analysis in adhesions of patients with chronic postsurgical pain and adhesions from patients without pain. Analysis was conducted on adhesion tissue, given their frequent occurrence as a pathological feature in patients with abdominal chronic postsurgical pain. Although the mechanisms of pain in adhesions remain incompletely elucidated, substantial evidence supports a causative role [25]. Due to the predominance of connective tissue and a low cell count in adhesions, collecting adequate RNA for quantitative polymerase chain reaction (qPCR) analysis of all identified genes is challenging. Consequently, only the two most promising genes (*SRPK2* and *PDE4D*) were selected from the identified genes for validation. The chosen genes were determined by their relevance and functions reported from

previous literature [26-28] (Online Supporting Information Table S6) and mRNA expression in normal human tissues from GTEx (obtained from Genecards). All other genes within the same locus as the chosen gene can be found in the Online Supporting Information Table S7.

The biopsies for RNA extraction were taken as part of the PainPad study (Histological and Molecular Mechanisms of Pain in Patients With Chronic Pain From Adhesions, ClinicalTrials identifier: NCT03938168). Cases were the patients who underwent surgery with adhesiolysis with an anti-adhesive agent for chronic abdominal pain, and controls were those who underwent surgery for any other indication without pain complaints. Biopsies of adhesions of all participants were stored on RNA later and fresh frozen at -80 °C.

The mRNA expression levels of the identified loci were analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Housekeeping genes were RPS11, 18S, and HPRT. The step-by-step process from RNA isolation to qPCR is described in the online Supporting Information Appendix S1. Primer sequences of the studied genes and primer validation properties can be found in Online Supporting Information Table S8 and Online Supporting Information Table S9, respectively.

The detection rates of the genes were assessed per group (cases and controls). Detection status were considered non-detectable, too low RNA yield, or detectable. The non-detectable group had a detectable housekeeping gene but non-detectable target genes. The low RNA samples had non-detectable housekeeping genes and non-detectable target genes, and were considered as a sample with too low RNA yield and/or acellular. The detection rate comparisons between the two groups were analyzed using a fisher exact test.

Expression levels of the target genes were quantified and compared between the cases and controls by independent two-tailed T-test. If patients had more than one sample to analyze, the mean expression level of the different samples was used for that patient. Samples were log2-transformed in Qbase+ and were normally distributed after transformation. A p-value <0.05 was considered statistically significant.

Results

Demographics and phenotype validation

After quality control, we identified 27,603 subjects who had undergone abdominal surgeries in the UK Biobank dataset, among which 1044 developed chronic postsurgical pain (cases). Table 1 summarises the demographics. All tested covariates (age, body mass index, gender, self-reported chronic pain, and self-reported chronic postsurgical pain) were significantly different ($P < 0.0001$) between cases and controls. Consequently, they were all adjusted in the genome-wide association study.

Table 1. Demographics of subjects with (cases) and without (controls) chronic postsurgical pain in the UK Biobank. Categorical covariates are represented as frequency (percentage) and compared by the $\chi 2$ test. Continuous covariates are presented as mean (standard deviation) and compared by an independent t-test. For types of surgery, if the OPCS4 operation code starts with 'G', 'H', or 'J', it is categorised as 'Digestive'. Codes starting with 'L' are 'Arteries veins', 'M' are 'Urinary', 'P', 'Q', or 'R' are 'Female genital tract', 'T' is 'Soft tissue', and 'Y' is 'Laparoscopy'. * Denotes that subjects with surgeries in more than one category were assigned to multiple sites.

	Controls	Cases	P value
N	26559	1044	
Females	19249 (72.5%)	684 (65.5%)	P < 0.0001
Age (years)	53.5 (10.2)	58.5 (9.0)	P < 0.0001
BMI (kg × m-2)	27.97 (5.12)	29.18 (5.47)	P < 0.0001
Self-reported back pain			P = 0.0017
Yes	5035 (70.8%)	325 (77.9%)	
No	2077	92	
Self-reported abdominal pain			P < 0.0001
Yes	2119 (60.2%)	138 (78.9%)	
No	1399	37	
Self-reported chronic postsurgical pain			P < 0.0001
Yes	584 (6.8%)	39 (17.0%)	
No	7974	191	
Types of surgery			P < 0.0001
Arteries veins	119 (88.15%)	16 (11.85%)	
Digestive	9237 (97.83%)	205 (2.17%)	
Female genital tract	10896 (98.78%)	135 (1.22%)	
Soft tissue	4045 (98.80%)	49 (1.20%)	
Urinary	2046 (97.62%)	50 (2.39%)	
Laparoscopy	362 (98.91%)	4 (1.09%)	
Multiple sites *	426 (97.04%)	13 (2.96%)	

As surgery complexity (indicated by surgery record numbers) and pre-existing pain before surgery (indicated by prescription record numbers) might bias the association, we compared these two characteristics between cases and controls. In line with our case definition, the median of surgery record numbers (P = 0.266, Online Supporting Information Figure S1A) and prescription record numbers before surgery (P < 0.0001, Online Supporting Information Figure S1B) were very similar for cases and controls, whereas cases had significantly more prescription record numbers after surgery than controls (P < 0.0001, Online Supporting Information Figure S1C). In addition, the percentage of cases is similar for different surgery types except for vascular surgery. Considering this is only a small group, we included these subjects in the genome-wide association study.

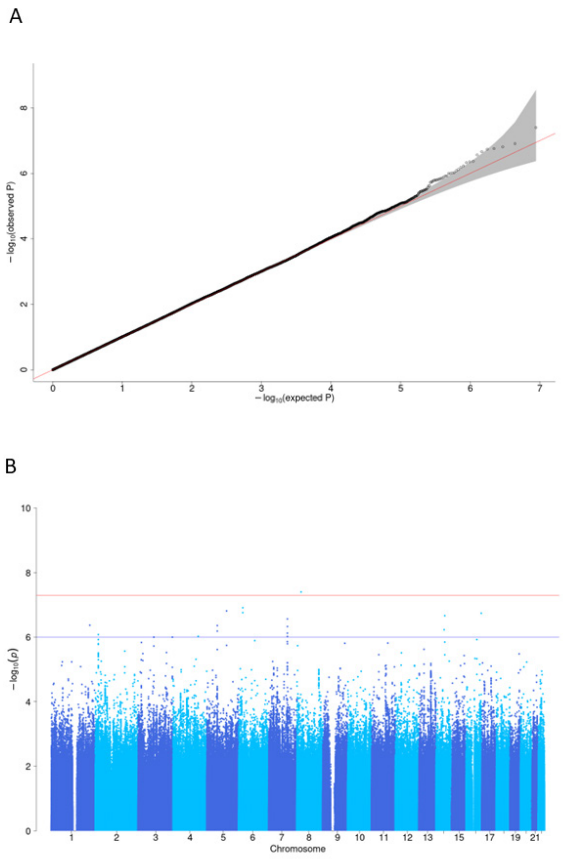

Figure 2. Q-Q plot and Manhattan plot of chronic postsurgical pain. (A) Q-Q plot of the genome-wide association study (genome-wide association study) results. The red line indicates the distribution under the null hypothesis, and the shaded area indicates the 95% confidence band. (B) Manhattan plot of the genome-wide association study results. The red line corresponds to the genome-wide significance threshold of 5×10^{-8}, whereas the blue indicates the suggestive threshold of 1×10^{-6}.

Genome-wide association analysis

A case-control genome-wide association study analysis was performed, including 8,830,911 single-nucleotide polymorphisms that passed quality control. No inflation was observed with the genomic control value of 1.00 (Figure 2A). Eleven loci surpassed the suggestively significant threshold ($P < 1 \times 10^{-6}$). Table 2 summarises the lead single-nucleotide polymorphisms in each locus, and no additional independent single-nucleotide polymorphisms (single-nucleotide polymorphisms remaining significant after conditioning on the lead single-nucleotide polymorphisms in the locus) were identified. One locus located at chromosome 8 reached genome-wide significance, in which the most significant single-nucleotide polymorphism was rs185545327 ($P = 3.99 \times 10^{-8}$) (Figure 2B, Table 2).

Table 2. Overview of the lead single-nucleotide polymorphisms passing suggestive significance in the genome-wide association study for chronic postsurgical pain. Bold font indicates the single-nucleotide polymorphism that passed the genome-wide significant threshold (5 × 10-8) in the ordinal genome-wide association study (see methods). CHR:POS chromosome number and physical position of the single-nucleotide polymorphism, EAF effect allele frequency, BETA (SE) effect size of single-nucleotide polymorphism, and standard error (SE).

single-nucleotide polymorphism	CHR:POS	Effect allele	EAF	BETA (SE)	P	Location	Nearest Gene
rs17047504	1:218376496	G	0.011	0.039 (0.008)	4.27E-07	intergenic	*RNU1-141P*
rs6531281	2:17275239	C	0.672	-0.009 (0.002)	8.32E-07	intergenic	*RN7SKP168*
rs13127505	4:141880770	T	0.107	0.013 (0.003)	9.46E-07	intronic	*RNF150*
rs56052023	5:59404369	A	0.006	0.051 (0.010)	4.39E-07	intronic	*PDE4D*
rs182762077	5:111832825	T	0.015	0.035 (0.007)	1.54E-07	intergenic	*HMGB3P16*
rs116169715	6:23158446	A	0.005	0.060 (0.011)	1.23E-07	intergenic	*RNU6-1060P*
rs146141654	7:105008889	C	0.010	0.041 (0.008)	2.74E-07	intronic	*SRPK2*
rs185545327	8:23592447	T	0.007	0.053 (0.010)	3.99E-08	ncRNA_intronic	*RP11-175E9.1: RP11-213G6.2*
rs78134813	14:65758752	A	0.007	0.050 (0.010)	5.89E-07	ncRNA_intronic	*CTD-2509G16.5*
rs117920312	14:69062922	A	0.014	0.035 (0.007)	2.17E-07	intronic	*RAD51B*
rs4843341	16:86108167	C	0.021	0.029 (0.006)	1.82E-07	intergenic	*RP11-805I24.1*

The demographic and genome-wide association study results using the ordinal phenotype were in line with the genome-wide association study using binary outcomes (Online Supporting Information Table S10, Online Supporting Information Figure S2). No additional genome-wide significant loci were identified in the ordinal genome-wide association study, but three more suggestively significant loci (rs12447350, rs144605695, and rs151022526) were identified (see online Supporting Information Table S11).

Functional annotation of single-nucleotide polymorphisms

All variants in linkage disequilibrium with lead single-nucleotide polymorphisms from the primary genome-wide association study were in the non-coding regions except for one exonic single-nucleotide polymorphism in the locus on chromosome 7 (Online Supporting Information Table S7). This single-nucleotide polymorphism was located in the exonic region of the *KMT2E* gene with a CADD score of 16.18. In the same locus, one intronic single-nucleotide polymorphism (rs138735129) near *SRPK2* showed potential regulatory functions with a RegulomeDB score of 2b. In addition, five single-nucleotide polymorphisms (rs6712392, rs138735129, rs147876663, rs76772737, and rs117920312) overlapped with all epigenomic markers of active enhancers or promoters that were checked in Haploreg (Online Supporting Information Table S7).

Pleiotropic effects of lead single-nucleotide polymorphisms were evaluated in the genome-wide association study catalog and the genome-wide association study atlas. No previously reported traits were found for lead single-nucleotide polymorphisms in the genome-wide association study catalog. In the genome-wide association study atlas, hyperthyroidism is the most significantly ($P = 1.59 \times 10^{-5}$) associated trait with lead single-nucleotide polymorphism (rs146141654). Other pleiotropic effects of lead single-nucleotide polymorphisms include regional brain volumes, lipid profiles, and depression. The complete list of associated traits passing multiple testing thresholds can be found in Online Supporting Information Table S12.

Gene mapping and gene-based analysis

After mapping genome-wide association study candidate single-nucleotide polymorphisms (single-nucleotide polymorphisms that are in linkage disequilibrium ($r2 > 0.6$) with lead single-nucleotide polymorphisms) to genes, a total of 15 genes were identified (Table 3). Eight genes were mapped by genomic location, two genes were identified by cis-eQTL mapping, seven genes were annotated as single-nucleotide polymorphisms in regions where 3D chromatin interactions occurred, and two genes were identified by at least two mapping strategies. Four genes were implicated in neurodevelopment disorders or related neurological function reported in the Genecards, and one gene was previously reported to be associated with pain (Table 3, Online Supporting Information Table S6).

Gene-based association analysis by MAGMA included 19307 genes (see Online Supporting Information Table S13 for the complete gene list). No genes remained significant after Bonferroni correction ($P = 2.59 \times 10^{-6}$).

Table 3. Genes mapped by candidate single-nucleotide polymorphisms using FUMA. single-nucleotide polymorphisms in linkage disequilibrium (r2 > 0.6) with lead single-nucleotide polymorphisms were mapped to genes. posMapsingle-nucleotide polymorphisms, the number of single-nucleotide polymorphisms mapped to this gene based on positional mapping; eqtlMapsingle-nucleotide polymorphisms, the number of single-nucleotide polymorphisms mapped to this gene based on eQTL mapping; eqtlMapminP, the minimum eQTL P-value of mapped single-nucleotide polymorphisms; eqtlMapminQ, the minimum eQTL FDR of mapped single-nucleotide polymorphisms; ciMap, "Yes" if the gene is mapped by chromatin interaction mapping; minGwasP, the minimum P-value of mapped single-nucleotide polymorphisms. * Denotes that this gene has been reported to be associated with neurodevelopmental disorders or related neurological functions in GeneCards. # Denotes an association with pain as reported in PubMed.

			Methods used for gene mapping					
Lead single-nucleotide polymorphisms	**Gene Symbol**	**chr**	**posMapsingle-nucleotide polymorphisms**	**eqtlMapsingle-nucleotide polymorphisms**	**eqtlMapminP**	**eqtlMapminQ**	**ciMap**	**minGwasP**
rs6531281	*SMC6*	2	0	6	9.50E-18	0	No	1.61E-06
	GEN1	2	0	20	3.35E-15	0	No	8.32E-07
rs13127505	*RNF150*	4	1	0	NA	NA	No	9.46E-07
rs56052023	*PDE4D* #	5	5	0	NA	NA	No	4.39E-07
rs116169715	*SOX4* *	6	0	0	NA	NA	Yes	2.23E-03
rs146141654	*LHFPL3*	7	1	0	NA	NA	No	1.07E-05
	KMT2E *	7	3	0	NA	NA	Yes	7.51E-07
	SRPK2 *	7	8	0	NA	NA	No	2.74E-07
	PUS7 *	7	2	0	NA	NA	No	4.70E-07
	RINT1	7	0	0	NA	NA	Yes	7.51E-07
rs78134813	*FUT8*	14	2	0	NA	NA	Yes	5.89E-07
rs117920312	*RAD51B*	14	2	0	NA	NA	No	2.17E-07
	ZFP36L1	14	0	0	NA	NA	Yes	2.17E-07
rs4843341	*FOXF1*	16	0	0	NA	NA	Yes	1.82E-07
	MTHFSD	16	0	0	NA	NA	Yes	1.82E-07

Validation analysis

The eleven variants that showed suggestive association with chronic postsurgical pain in the case-control genome-wide association study were included in the robustness analysis. Eight single-nucleotide polymorphisms, including the genome-wide significant loci, passed the split-half validation analysis. These loci remained at least nominally significant ($P < 0.05$) in all five iterations (Online Supporting Information Table S14). Three single-nucleotide polymorphisms (rs117920312, rs56052023, and rs78134813) failed to pass the split-half validation analysis in one iteration.

Finally, we investigated whether suggestively significant single-nucleotide polymorphisms (Table 2) identified in the case-control analysis were also associated with chronic postsurgical pain in a published genome-wide association study meta-analysis including seven independent datasets (total N = 1350). In the case single-nucleotide polymorphisms identified in our study were not present, single-nucleotide polymorphisms that are in linkage disequilibrium ($r2 > 0.6$) with the suggestively significant single-nucleotide polymorphisms identified in our study were checked (Online Supporting Information Table S15). In the end, we could include 33 single-nucleotide polymorphisms from six loci reported in both our analysis and the meta-analysis. None of these single-nucleotide polymorphisms were reported to be nominally significantly associated in the published meta-analysis [24], with the most significant single-nucleotide polymorphism rs13127505 ($P = 0.0545$) (Online Supporting Information Table S16). However, single-nucleotide polymorphisms in five loci identified from our study were not genotyped in the meta-analysis.

RNA expression analysis

Gene expression was analyzed for two identified genes (*SRPK2* and *PDE4D*) in adhesions from a cohort of patients with abdominal chronic postsurgical pain (N=31) and controls (N=29). While the results did not reach statistical significance, the detection rate was comparable for *SRPK2* ($P = 0.067$) and slightly higher for *PDE4D* ($P = 0.076$) in patients experiencing pain than in controls (Figure 3A, Online Supporting Information Table S17). The expression levels for *PDE4D* were marginally elevated in patients with abdominal chronic postsurgical pain compared to controls ($P = 0.115$), whereas the expression levels of *SRPK2* were comparable between the two groups ($P = 0.975$). However, none of these findings achieved statistical significance (Figure 3B).

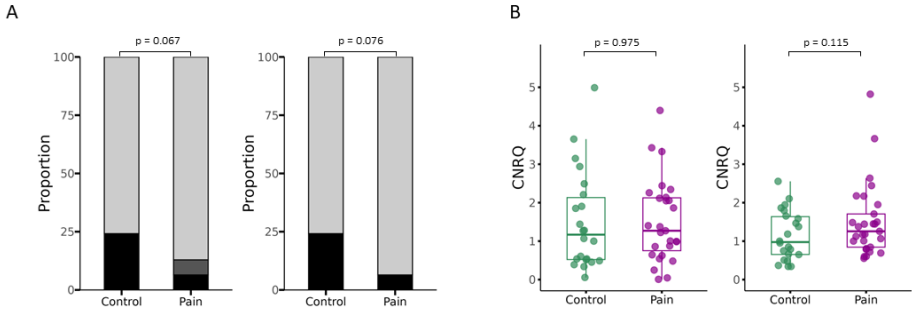

Figure 3. RNA expression analysis in adhesions comparing patients with and without chronic abdominal postsurgical pain. A) Detection rates of target genes SRPK2 (left) and PDE4D (right). Light grey, both housekeeping gene and target gene detected; dark grey, housekeeping gene detected, but target gene not detected; black, neither housekeeping gene nor target detected. B) Expression levels of identified loci in patients with chronic abdominal postsurgical pain compared and controls (left SRPK2, right PDE4D). The sample size of controls and subjects with chronic postsurgical pain are 29 and 31, respectively, for both detection rate and expression levels for both SRPK2 and PDE4D, except that the sample size of subjects with pain is 29 for SRPK2 expression level as two samples were non-detected.

Discussion

We have performed a genome-wide association study on chronic postsurgical pain using the UK Biobank and identified eleven suggestively associated loci in the case-control analysis, including one genome-wide significance ($P < 5\times10^{-8}$) locus. The genome-wide significance locus (rs185545327) was not mapped to any gene, but the suggestively significant loci on chromosome 7 (rs146141654) and chromosome 5 (rs56052023) were mapped to functionally related genes.

The mapped gene (*PDE4D*) by rs56052023 has previously been linked to pain. The PDE4 protein family is responsible for cAMP hydrolysis in nerve and immune cells [29], and PDE4 inhibition can produce potent antinociceptive activity [29] and reduce neuroinflammation [27] in animal models. Consistent with prior literature findings, this study showed a trend of elevated PDE4 expression adhesions from chronic postsurgical pain patients although the results were not significant. Considering the antinociceptive effect of PDE4 inhibitors in chronic neuropathic pain in mice and the role of neuroinflammation implicated in the development of chronic pain after acute injury [30], further investigation into PDE4 inhibitors as a potential treatment for postsurgical pain is warranted [29]. However, the lead

single-nucleotide polymorphism in this loci failed to pass robustness analysis by randomly splitting the dataset into two equally sized subsets.

Besides *PDE4D*, mapped genes involved in other neurological functions might also be potential candidates for pain development. SRPK2 is involved in neuronal apoptosis in neurons in vitro and in vivo [28]. Accumulating evidence suggests the role of neuronal apoptosis in the pathogenesis of chronic pain, but the involvement of specific molecular signaling and inhibition of neuronal apoptosis in alleviating pain is still unclear [31]. Therefore, this locus might be worth validating and curating further hypotheses. In addition, three mapped genes are linked to neurodevelopment disorders. As pain is a common comorbid symptom associated with several central nervous system disorders such as Alzheimer's disease [32], depression [33], and Huntington's disease [34], this comorbidity might indicate shared underlying genetic mechanisms. *KMT2E* might be an interesting candidate as it involves several neurodevelopmental syndromes. A mutation in this gene is known to cause a rare neurodevelopmental disorder with abdominal pain symptoms [35]. The other two genes, *PUS7* and *SOX4,* are involved in complex neurodevelopmental disorders [36, 37].

The pleiotropic effects analysis of the lead single-nucleotide polymorphisms showed that four variants were associated with regional brain volumes (e.g., cerebellum, pallidum) in previous studies. These pleiotropic effects might link to chronic postsurgical pain as previous studies showed various anatomic sites of altered brain morphology are involved in pain perception and modulation [38, 39]. However, it is unclear whether regional brain volumes mediate the associations between single-nucleotide polymorphisms and chronic postsurgical pain, which might be interesting to investigate in future research. In addition, depression and lipid profiles were identified as pleiotropic effects for lead single-nucleotide polymorphisms. Considering the phenotypic correlation between pain and these traits [40-42], single-nucleotide polymorphisms with these pleiotropic effects might be a good candidate worth further investigation.

Unfortunately, the single-nucleotide polymorphism heritability was too low (with a negative value) to carry forward for any genetic correlation analysis. According to our cases/control sample size and assuming a prevalence of 20% chronic postsurgical pain development, this study had 80% power to detect associations of single-nucleotide polymorphisms allele frequency greater than 0.15 with a relative risk greater than 1.29. Considering the moderate heritability in other chronic pain phenotypes (median~ 45%) [43], the low heritability in this study reflects the "missing heritability" problem in genome-wide association study. The heritability

calculated in genome-wide association study (single-nucleotide polymorphism-heritability) is based on a subset of common genetic variants, which tends to be lower than in twin studies. This is because the genotyped single-nucleotide polymorphisms in genome-wide association study are perhaps not in incomplete linkage disequilibrium with the causal variants [44], and the effects of non-additive effects, rare variants, and structural variants are undetected. This result also indicates that chronic postsurgical pain development is a multifactorial trait with combined clinical, environmental, and genetic effects [4].

In the replication phase, although eight lead single-nucleotide polymorphisms were replicated in the self-split analysis, only one single-nucleotide polymorphism (rs13127505) was marginally close to the nominal significance threshold in the genome-wide association study meta-analysis that was published previously [24]. The last validation analysis was limited as single-nucleotide polymorphisms from our results were unavailable in part of the cohorts from the meta-analysis (see Online Supporting Information Table S16), leading to a even smaller sample size (N < 1350) for validation. Most likely, the missing single-nucleotide polymorphisms is related to the allele frequency of the single-nucleotide polymorphisms, as UK Biobank includes rare markers (<1% MAF) in the genotyping array and low-frequency variants (1-5%) imputated in European populations. A common variant might pass the minor allele frequency threshold for inclusion in a large cohort (such as UK Biobank) but not in small cohorts like the chronic postsurgical pain meta-analysis (N= 1350). In addition, the genome-wide association study meta-analysis differed from our study population as it consists of surgery cohorts in different anatomical regions rather than only abdominal regions, and it is still unclear whether the genetic susceptibility of chronic postsurgical pain development is similar for various surgeries. However, there are no overlapping loci between our study and the genome-wide association study on chronic postsurgical pain focusing on abdominal surgery only (the study mentioned in the introduction earlier) [33] as all identified suggestively loci in that studies were on chromosome 15 but there are no suggestively loci on chromosome 15 in our study. This is probably due to the small cohort and thus underpowered results in that study.

One strength of this study is that we included many surgeries in the abdominal region to reach a large sample size. Furthermore, we demonstrated modest increased detection rates and expression levels of one genome-wide association study-identified gene (*PDE4D*) in adhesions from patients with chronic postsurgical pain compared to adhesions from patients without pain, which strengthens the findings of our genome-wide association study analysis.

The limitation of this study was that rather than using chronic postsurgical pain measurement directly, we used a proxy to define chronic pain by using analgesic prescription records after operations, representing a group of patients with more severe pain as not all patients having chronic pain will use analgesics. In other words, patients who developed chronic postsurgical pain without using analgesics were ruled out from the cases, reflected by the low prevalence of chronic postsurgical pain in this study. The low prevalence is in line with the incidence of severe chronic postsurgical pain in previous studies [4]. Therefore, the total burden of chronic postsurgical pain following abdominal surgery is probably underestimated in this study. Besides, our cases definition can also include subjects using analgesics for conditions other than chronic postsurgical pain. The discrepancy can also be found in the demographic characteristics between our study and epidemiological studies. For instance, the percentage of self-reported chronic pain in cases and controls is consistent with previous literature but not age and gender. Younger age and female gender are associated with chronic postsurgical pain development [4] but in our study these two characteristics are associated in the opposite direction. Possibly, this discrepancy can be attributed to differences in the chronic postsurgical pain definition. As mentioned earlier, we defined chronic postsurgical pain by medication use, while in other studies it is often defined by questionnaires. Additionally, discrepancies in healthcare-seeking behaviour between sex and age groups might contribute to the differences [45], e.g., some patients seeking analgesia while others return to outpatient clinics for diagnosis. Another limitation was that the time period for this study (between 1997 and 2015), surgical techniques and also peri-operative pain management has changed significantly and introduces unmeasured confounders. For instance, changes in prescribed analgesic plans over time may impact our pain phenotype definition.

In conclusion, we identified eleven loci associated with chronic postsurgical pain development after abdominal surgeries in a large-scale population from the UK Biobank. Two of these single-nucleotide polymorphisms were closely related to genes related to pain (*SRPK2*, *PDE4D*), and *PDE4D* showed a modest increase in adhesions from a cohort of patients with chronic postsurgical pain and patients without any chronic pain. However, analysing these associations in sufficiently large cohorts with direct chronic postsurgical pain measurement is highly recommended to validate this association. Future studies will be essential to fully elucidate the genetic mechanisms and underlying biology of chronic postsurgical pain, which could facilitate the prevention and intervention of chronic postsurgical pain in the long term.

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Data availability

Summary statistics of the primary analysis are available at DANS archive (https://doi.org/10.17026/LS/X9DUS1).

Gene mapping results are available at FUMA (https://fuma.ctglab.nl/browse/689).

Programming and statistical code used in the phenotype definition and data analysis can be found at GitHub (https://github.com/lisongmiller/UKB_GWAS_CPSP_abdominal).

Author contributions

Song Li analyzed the data and prepared the manuscript. Masja K. Toneman, Regina L.M. van Boekel, Richard P. G. ten Broek, and Kris C. P. Vissers contributed to the phenotype definition and revised the manuscript. Judith P.M. Mangnus contributed to the RNA expression analysis. Stefano Strocchi helped with data preparation and analysis. Marieke J.H. Coenen conceptualised the study, supervised the overall project, and revised the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest disclosures

The authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary method

Self-reported CPSP in UKB

Prescription records were used to determine whether subjects from the UK Biobank (UKB) developed chronic postsurgical pain (CPSP). This surrogate phenotype was used as the UKB team does not recommend using self-reported CPSP (Data-Field 120005) to run a GWAS because there were significant issues with case definition due to the difficulties with assessing chronicity and determining sub-types of neuropathic pain. Essentially, there is a lack of granular detail in the questionnaires regarding the timing of onset of pain. They also pointed out that there is a balance between the level of detail in the responses and the time taken to complete the questionnaire, which influenced how much information was captured. Therefore, we use analgesic consumption records to define CPSP.

Routine sample and genotype QC

Subjects meeting the following criteria will be included for analysis: Subjects with consistent self-reported and genetically determined sex, genetically determined white British ancestry, without putative sex-chromosome aneuploidy, not considered outliers due to missing heterozygosity, individual call rate > 90%, all relevant covariates are available.

Markers on autosomes that meet the following criteria will be included: SNPs with an imputation quality score (INFO scores) of greater than 0.8, Minor Allele Frequency (MAF) > 0.005, Hardy-Weinberg equilibrium (HWE) test P > 10^{-6}, Genotyping call rate > 95%.

Heritability and genetic correlation

Heritability was calculated in LDSC. The genetic correlation of CPSP with other traits is also investigated in LDSC. Summary statistics of selected traits are downloaded from GWAS atlas, including diagnoses - main ICD10: R10 abdominal and pelvic pain (atlas ID: 3682), Pain type(s) experienced in last month: stomach or abdominal pain (atlas ID: 3573), back pain (PMID 30747904), neck/shoulder pain for 3+ months (PMID: 31427789), knee pain for 3+ months (PMID: 31427789), headaches for 3+ months (PMID: 31427789), depression (PMID 30718901), body mass index (PMID: 30239722). The significant threshold for genetic correlation is set as 0.05/total number of tested correlations.

Study cohort for RNA expression analysis

The biopsies for RNA extraction were taken as part of the PainPad study (Histological and Molecular Mechanisms of Pain in Patients With Chronic Pain From Adhesions, ClinicalTrials identifier: NCT03938168).

The inclusion criteria for cases are: 1) patients suffered from chronic pain for more than 12 months after their last surgery; 2) insufficient improvement of pain after conservative treatments for at least 6 months; 3) pre-operative work-up with cineMRI (a noninvasive mapping technique for adhesions) showing expected beneficial outcomes of adhesiolysis, in accordance to our current standard practice for adhesion-related pain.

The inclusion criteria for controls are: 1) patients between 18 and 75 years old scheduled for elective abdominal reoperation; 2) without chronic abdominopelvic pain or other diseases or syndromes that cause chronic pain (e.g., rheumatic arthritis). In these patients, the presence of adhesions was expected based on their surgical history. If no adhesions were found during the operation, control patients were replaced.

A potential subject with chronic pain will be excluded from participation in this study in the following cases: 1) contra-indications for general anaesthesia and re-operation; 2) inability to acquire informed consent.

All patients were included over a period of 18 months at the surgical departments of the Radboudumc, MUMC+ and the Pantein Hospital in Boxmeer. The cohorts were compatible in regard to age, gender and the number of reoperations. In this analysis, we analysized samples from 31 patients with chronic pain and 29 controls without chronic pain.

The study protocol was reviewed and approved by the medical ethical committee. All patients gave written informed consent for their participation in the PAIN-PAD study.

RNA isolation procedure and qPCR analysis

RNA isolation of the frozen biopsies was performed with the Qiagen micro kit (Cat. No. 74004, Qiagen). Diluted in RLT buffer, and treated with proteinase K (cat. No. 19131, Qiagen. To remove the DNA the DNase mixture, containing DNase and RDD buffer (Qiagen DNase kit. Cat. No. 79256, Qiagen was used. Quantification of the purified RNA was carried by the nanodrop (ND-1000 UV-Vis Spectrophotometer,

Thermo fisher scientific). Previously isolated RNA was used to create cDNA with the use of the iScript cDNA synthesis kit (cat. No. 1708891, BioRAD) and the cDNA synthesis protocol was run in a biorad thermocycler T100 (ID 68567).

The cDNA was used to perform a Reverse Transcriptase quantitative Polymerase Chain Reaction (RT qPCR). A Biorad 96-well plate (cat. No. HSP9601/ HSP9631) was used to measure the fluorescence of the samples. Samples were measured in triplicate to control tissue heterogeneity. Each well contained 2.5 µl cDNA and 10 µl mastermix. The mastermix consists of 6.25 µl SYBRgreen (ref. nr. A25742), containing all essentials for a qPCR, 0.5 µl of both the forward and reversed primer and 2.75 µl milliQ. RT qPCR was run on a Biorad CFX connect (ID 63181). The qPCR was ran consisting of 7 minutes denaturation period at 95°C, followed by 40 amplification cycles consisting of 15 seconds at 95°C and a 1 minute annealing period at 60°C.

Online Supporting Information Supplementary figures

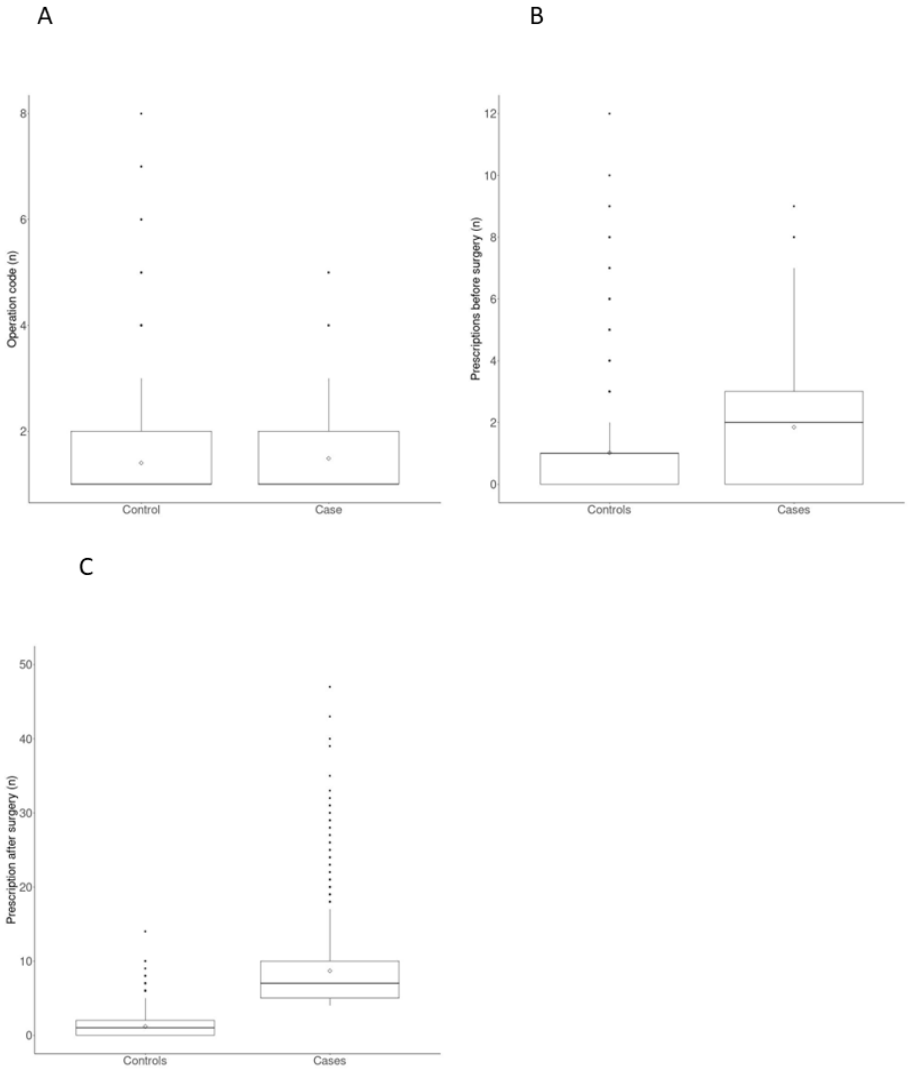

Figure S1. Prescription and operation record numbers for controls and cases. A. operation record numbers, B. prescription record numbers before the operation, C. prescription record numbers after the operation.

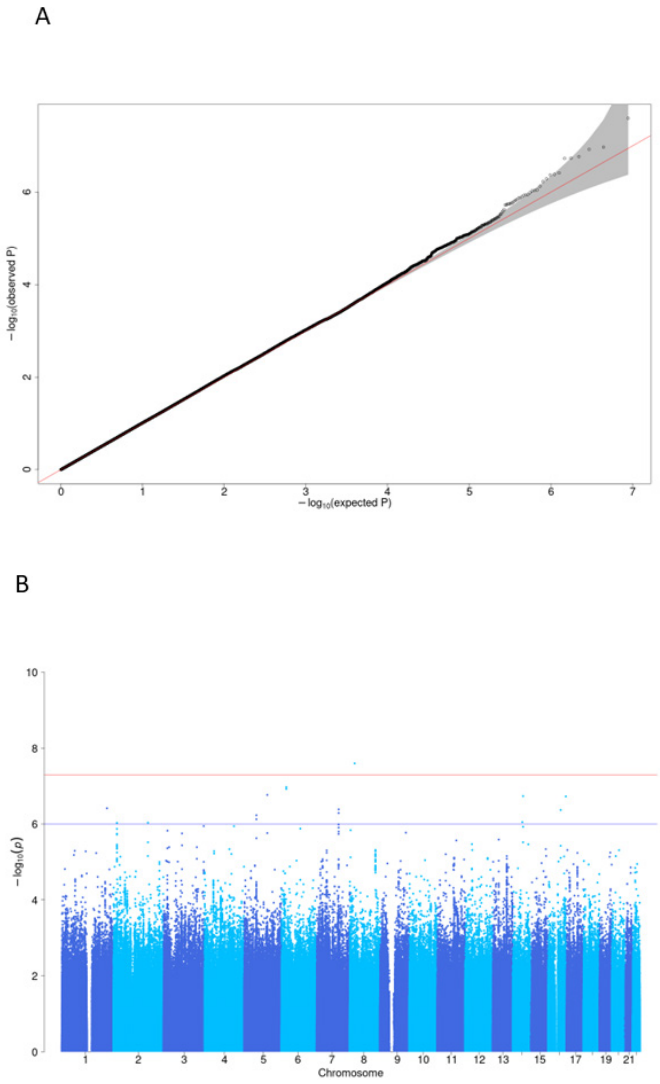

Figure S2. Q-Q plot and Manhattan plot of the GWAS analysis on post-surgical pain using the ordinal phenotype.

Online Supporting Information Supplementary tables

This section lists most of the supplementary tables. Tables S2-S4 are not included here due to their length but can be accessed online at the following link: https://github.com/lisongmiller/UKB_GWAS_CPSP_abdominal/tree/main/supp_data

Table S1. Chemical name of selected drugs.

BNF_chemical_name	Category
ALOXIPRIN	NSAID
LYSINE ASPIRIN	NSAID
ASPIRIN	NSAID
ASPIRIN & CAFFEINE	NSAID
ASPIRIN & PAPAVERETUM	NSAID
ASPIRIN & PARACETAMOL	NSAID
ASPIRIN COMBINED PREPARATIONS	NSAID
ACECLOFENAC	NSAID
ACEMETACIN	NSAID
ALFENTANIL HYDROCHLORIDE	OPIOID
AMITRIPTYLINE EMBONATE	TCA
AMITRIPTYLINE HYDROCHLORIDE	TCA
ASPIRIN,PARACETAMOL & CODEINE	OPIOID
ASPIRIN,PHENACETIN & CODEINE(CODEINE CO)	OPIOID
AZAPROPAZONE	NSAID
BUPRENORPHINE	OPIOID
BUPRENORPHINE HYDROCHLORIDE	OPIOID
BUTRIPTYLINE	TCA
CARBAMAZEPINE	ANTICONVULSANT
CELECOXIB	NSAID
CLONIDINE HYDROCHLORIDE	α2-agonist
CO-CODAMOL (CODEINE PHOS/PARACETAMOL)	OPIOID
CO-CODAPRIN (CODEINE PHOS/ASPIRIN)	OPIOID
CODEINE PHOSPHATE	OPIOID
CO-DYDRAMOL (DIHYDROCODEINE/PARACET)	OPIOID
CO-PROXAMOL (DEXTROPROP HCL/PARACET)	OPIOID
DEXIBUPROFEN	NSAID
DEXKETOPROFEN	NSAID
DEXTROMORAMIDE TARTRATE	OPIOID

Table S1. Continued

BNF_chemical_name	Category
DEXTROPROPOXYPHENE	OPIOID
DIAMORPHINE HYDROCHLORIDE (SYSTEMIC)	OPIOID
DIAMORPHINE HYDROCHLORIDE (TOP)	OPIOID
DICLOFENAC POTASSIUM	NSAID
DICLOFENAC SODIUM	NSAID
DIFLUNISAL	NSAID
DIHYDROCODEINE TARTRATE	OPIOID
DIPIPANONE HYDROCHLORIDE	OPIOID
DIPYRONE SODIUM	Metamizole
ESKETAMINE HYDROCHLORIDE	NMDA receptor antagonist
ETODOLAC	NSAID
ETORICOXIB	NSAID
FENBUFEN	NSAID
FENOPROFEN	NSAID
FENTANYL	OPIOID
FENTANYL CITRATE	OPIOID
FLURBIPROFEN	NSAID
GABAPENTIN	ANTICONVULSANT
GABAPENTIN (NEUROPATHIC PAIN)	ANTICONVULSANT
HYDROMORPHONE HYDROCHLORIDE	OPIOID
IBUPROFEN	NSAID
IBUPROFEN LYSINE	NSAID
IBUPROFEN SODIUM DIHYDRATE	NSAID
INDOMETACIN	NSAID
KETAMINE	NMDA receptor antagonist
KETOPROFEN	NSAID
KETOROLAC TROMETAMOL	NSAID
LORNOXICAM	NSAID
LUMIRACOXIB	NSAID
MEFENAMIC ACID	NSAID
MELOXICAM	NSAID
MEPTAZINOL HYDROCHLORIDE	OPIOID
METHADONE HYDROCHLORIDE	OPIOID
MORPHINE	OPIOID
MORPHINE ANHYDROUS	OPIOID
MORPHINE HYDROCHLORIDE	OPIOID

Table S1. Continued

BNF_chemical_name	Category
MORPHINE SULFATE	OPIOID
MORPHINE TARTRATE & CYCLIZINE TARTRATE	OPIOID
NABUMETONE	NSAID
NALBUPHINE HYDROCHLORIDE	OPIOID
NAPROXEN	NSAID
NAPROXEN SODIUM	NSAID
NEFOPAM HYDROCHLORIDE	NSAID
NIMESULIDE	NSAID
OXYCODONE	OPIOID
OXYCODONE HCL/NALOXONE HCL	OPIOID
OXYCODONE HYDROCHLORIDE	OPIOID
PAPAVERETUM	OPIOID
PARACETAMOL	NSAID
PARACETAMOL & CAFFEINE	NSAID
PARACETAMOL & CODEINE PHOSPHATE	OPIOID
PARACETAMOL & IBUPROFEN	NSAID
PARACETAMOL COMBINED PREPARATIONS	NSAID
PARECOXIB SODIUM	NSAID
PENTAZOCINE HYDROCHLORIDE	OPIOID
PENTAZOCINE LACTATE	OPIOID
PETHIDINE HYDROCHLORIDE	OPIOID
PHENAZOCINE HYDROBROMIDE	OPIOID
PHENYLBUTAZONE	NSAID
PIROXICAM	NSAID
POWDERED OPIUM	OPIOID
PREGABALIN	ANTICONVULSANT
REMIFENTANIL HYDROCHLORIDE	OPIOID
ROFECOXIB	NSAID
SODIUM SALICYLATE	NSAID
SULINDAC	NSAID
TAPENTADOL HYDROCHLORIDE	OPIOID
TENOXICAM	NSAID
TIAPROFENIC ACID	NSAID
TOLFENAMIC ACID	NSAID
TOLMETIN	NSAID
TRAMADOL HYDROCHLORIDE	OPIOID

Table S1. Continued

BNF_chemical_name	Category
VALDECOXIB	NSAID
KETAMINE HYDROCHLORIDE	ANTIDEPRESSANT
BENORILATE	NSAID
BUPIVACAINE HYDROCHLORIDE	ANAESTHETIC
BUPIVACAINE HYDROCHLORIDE & FENTANYL CIT	OPIOID
BUPRENORPH HCL/NALOXONE HCL	OPIOID
DESIPRAMINE HYDROCHLORIDE	TCA
DULOXETINE HYDROCHLORIDE	SSRI
FLUFENAMIC ACID	NSAID
LEVACETYLMETHADOL HYDROCHLORIDE	OPIOID
LEVOBUPIVACAINE HYDROCHLORIDE	Local Anesthetic
LEVORPHANOL TARTRATE	OPIOID
NORTRIPTYLINE	TCA
OXCARBAZEPINE	ANTICONVULSANT
OXYPHENBUTAZONE	NSAID
ROPIVACAINE HYDROCHLORIDE	ANAESTHETIC
VENLAFAXINE	SNRI

Table S2. Selected OPCS4 codes of abdominal operation.
Table S3. Selected READ v2 codes of abdominal operation.
Table S4. Selected READ v3 codes of abdominal operation.

(Table S2-S4 can be found online.)

Table S5. Selected cells and tissues for functional annotation of the SNPs in Haploreg.

Epigenome ID (EID)	Group	Mnemonic	Description
E007	ES-deriv	ESDR.H1.NEUR.PROG	H1 Derived Neuronal Progenitor Cultured Cells
E009	ES-deriv	ESDR.H9.NEUR.PROG	H9 Derived Neuronal Progenitor Cultured Cells
E010	ES-deriv	ESDR.H9.NEUR	H9 Derived Neuron Cultured Cells
E052	Myosat	MUS.SAT	Muscle Satellite Cultured Cells
E053	Neurosph	BRN.CRTX.DR.NRSPHR	Cortex derived primary cultured neurospheres
E054	Neurosph	BRN.GANGEM.DR.NRSPHR	Ganglion Eminence derived primary cultured neurospheres
E055	Epithelial	SKIN.PEN.FRSK.FIB.01	Foreskin Fibroblast Primary Cells skin01
E056	Epithelial	SKIN.PEN.FRSK.FIB.02	Foreskin Fibroblast Primary Cells skin02
E057	Epithelial	SKIN.PEN.FRSK.KER.02	Foreskin Keratinocyte Primary Cells skin02
E058	Epithelial	SKIN.PEN.FRSK.KER.03	Foreskin Keratinocyte Primary Cells skin03
E059	Epithelial	SKIN.PEN.FRSK.MEL.01	Foreskin Melanocyte Primary Cells skin01
E061	Epithelial	SKIN.PEN.FRSK.MEL.03	Foreskin Melanocyte Primary Cells skin03
E067	Brain	BRN.ANG.GYR	Brain Angular Gyrus
E068	Brain	BRN.ANT.CAUD	Brain Anterior Caudate
E069	Brain	BRN.CING.GYR	Brain Cingulate Gyrus
E070	Brain	BRN.GRM.MTRX	Brain Germinal Matrix
E071	Brain	BRN.HIPP.MID	Brain Hippocampus Middle
E072	Brain	BRN.INF.TMP	Brain Inferior Temporal Lobe
E073	Brain	BRN.DL.PRFRNTL.CRTX	Brain_Dorsolateral_Prefrontal_Cortex
E074	Brain	BRN.SUB.NIG	Brain Substantia Nigra
E081	Brain	BRN.FET.M	Fetal Brain Male
E082	Brain	BRN.FET.F	Fetal Brain Female
E089	Muscle	MUS.TRNK.FET	Fetal Muscle Trunk
E090	Muscle	MUS.LEG.FET	Fetal Muscle Leg
E100	Muscle	MUS.PSOAS	Psoas Muscle
E107	Muscle	MUS.SKLT.M	Skeletal Muscle Male
E108	Muscle	MUS.SKLT.F	Skeletal Muscle Female
E120	ENCODE2012	MUS.HSMM	HSMM Skeletal Muscle Myoblasts Cells
E121	ENCODE2012	MUS.HSMMT	HSMM cell derived Skeletal Muscle Myotubes Cells
E125	ENCODE2012	BRN.NHA	NH-A Astrocytes Primary Cells
E126	ENCODE2012	SKIN.NHDFAD	NHDF-Ad Adult Dermal Fibroblast Primary Cells
E127	ENCODE2012	SKIN.NHEK	NHEK-Epidermal Keratinocyte Primary Cells

Table S6. Literature supporting the association of identified genes with pain or neurological functions.

Gene	Title	Pubmed ID
PDE4D	COX inhibitors downregulate PDE4D expression in a clinical model of inflammatory pain	18288087
PDE4D	PDE4B as a microglia target to reduce neuroinflammation	27038323
PDE4D	Intrathecal injection of phosphodiesterase 4B-specific siRNA attenuates neuropathic pain in rats with L5 spinal nerve ligation	26706904
PDE4D	Inhibition of phosphodiesterase-4 in the spinal dorsal horn ameliorates neuropathic pain via cAMP-cytokine-Cx43 signaling in mice	35156776
SOX4	De Novo SOX4 Variants Cause a Neurodevelopmental Disease Associated with Mild Dysmorphism	30661772
SRPK2	Interaction of Akt-phosphorylated SRPK2 with 14-3-3 mediates cell cycle and cell death in neurons	19592491
PUS7	PUS7 deficiency in human patients causes profound neurodevelopmental phenotype by dysregulating protein translation	35144859
PUS7	PUS7 mutations impair pseudouridylation in humans and cause intellectual disability and microcephaly	30778726
KMT2E	O'Donnel-Luria-Rodan Syndrome: New gene variant identified in Romania (A case report)	35481221

Table S7. Functional annotation of candidate SNPs associated with chronic postsurgical pain.

IndSigSNP	rsID	effect allele	MAF	gwasP	beta	se	r2	nearestGene
rs17047504	rs17047504	G	0.0129	4.27E-07	0.0394	0.0078	1.0000	*RNU1-141P*
rs6531281	rs2342546	T	0.2555	4.69E-05	-0.0077	0.0019	0.6471	*RN7SKP168*
	rs2090310	C	0.2535	7.27E-05	-0.0076	0.0019	0.6575	*RN7SKP168*
	rs925788	A	0.2535	9.81E-05	-0.0074	0.0019	0.6575	*RN7SKP168*
	rs7564158	T	0.4672	1.13E-03	-0.0053	0.0016	0.6060	*RN7SKP168*
	rs6712392	T	0.3489	7.64E-06	-0.0077	0.0017	0.9957	*RN7SKP168*
	rs11422020	A	0.3489	NA	NA	NA	0.9957	*RN7SKP168*
	rs6755572	T	0.3469	7.89E-06	-0.0077	0.0017	0.9871	*RN7SKP168*
	rs10164520	T	0.3469	4.01E-06	-0.0079	0.0017	0.9872	*RN7SKP168*
	rs10200324	A	0.3489	4.01E-06	-0.0079	0.0017	0.9957	*RN7SKP168*
	rs1515980	T	0.3489	7.85E-06	-0.0077	0.0017	0.9957	*RN7SKP168*
	rs1515982	G	0.3489	3.49E-06	-0.0080	0.0017	0.9957	*RN7SKP168*
	rs1515983	A	0.3489	3.59E-06	-0.0080	0.0017	0.9957	*RN7SKP168*
	rs906492	G	0.3489	3.11E-06	-0.0080	0.0017	0.9957	*RN7SKP168*
	rs4324323	C	0.3489	3.34E-06	-0.0080	0.0017	0.9957	*RN7SKP168*
	rs13013588	C	0.3489	5.01E-06	-0.0079	0.0017	0.9957	*RN7SKP168*
	rs10177601	C	0.3499	1.63E-06	-0.0082	0.0017	1.0000	*RN7SKP168*
	rs1807869	G	0.3499	1.19E-06	-0.0084	0.0017	1.0000	*RN7SKP168*
	rs1515988	T	0.3499	1.68E-06	-0.0083	0.0017	1.0000	*RN7SKP168*
	rs1039742	G	0.3499	1.61E-06	-0.0083	0.0017	1.0000	*RN7SKP168*
	rs6531281	C	0.3499	8.32E-07	-0.0085	0.0017	1.0000	*RN7SKP168*
	rs6718117	T	0.3539	8.79E-06	-0.0077	0.0017	0.9657	*RN7SKP168*
	rs6531282	A	0.3529	9.02E-06	-0.0077	0.0017	0.9614	*RN7SKP168*
rs13127505	rs13127505	T	0.1113	9.46E-07	0.0132	0.0027	1.0000	*RNF150*
rs56052023	rs78419998	T	0.0070	NA	NA	NA	0.8732	*PDE4D*
	rs55907841	A	0.0060	4.41E-04	0.0402	0.0114	0.7470	*PDE4D*
	rs144788536	T	0.0080	6.53E-07	0.0520	0.0105	1.0000	*PDE4D*
	rs56052023	A	0.0080	4.39E-07	0.0509	0.0101	1.0000	*PDE4D*
	rs138414269	A	0.0070	2.42E-06	0.0505	0.0107	0.8732	*PDE4D*
rs182762077	rs77324010	A	0.0159	1.80E-06	0.0310	0.0065	1.0000	*HMGB3P16*
	rs182762077	T	0.0159	1.54E-07	0.0351	0.0067	1.0000	*HMGB3P16*

	func	CADD	RDB	H3K4me1	H3K4me3	H3K27ac	H3K9ac	DNase
	intergenic	0.298	7	No	No	No	No	No
	intergenic	0.264	7	Yes	No	No	No	No
	intergenic	3.549	7	Yes	No	Yes	No	No
	intergenic	3.102	6	No	No	No	No	No
	intergenic	0.856	7	No	No	No	No	No
	intergenic	2.121	5	Yes	Yes	Yes	Yes	No
	intergenic	14.490	NA	Yes	Yes	No	No	No
	intergenic	1.394	5	Yes	No	No	No	No
	intergenic	14.780	5	Yes	No	No	No	No
	intergenic	0.097	5	Yes	No	No	No	No
	intergenic	0.109	6	Yes	No	No	No	No
	intergenic	0.104	6	Yes	No	No	No	No
	intergenic	8.511	7	Yes	No	No	No	No
	intergenic	0.420	7	Yes	No	No	No	No
	intergenic	3.532	7	No	No	No	No	No
	intergenic	0.863	6	Yes	Yes	No	No	No
	intergenic	3.010	5	Yes	No	No	No	No
	intergenic	1.448	7	Yes	No	Yes	No	No
	intergenic	3.329	7	Yes	No	Yes	No	No
	intergenic	2.773	7	Yes	No	Yes	Yes	Yes
	intergenic	5.511	5	Yes	No	Yes	No	Yes
	intergenic	0.185	7	Yes	No	No	No	No
	intergenic	1.999	6	Yes	No	No	No	No
	intronic	1.827	7	Yes	No	No	No	No
	intronic	0.764	6	No	No	No	No	No
	intronic	0.531	7	No	No	Yes	Yes	No
	intronic	0.189	6	No	No	No	No	No
	intronic	0.434	7	No	No	No	No	No
	intronic	6.137	6	No	No	No	No	No
	intergenic	0.136	7	No	No	No	No	No
	intergenic	1.206	6	No	No	No	No	No

Table S7. Continued

IndSigSNP	rsID	effect allele	MAF	gwasP	beta	se	r2	nearestGene
rs116169715	rs78068757	G	0.0099	9.66E-03	0.0278	0.0107	0.9072	*RP1-209A6.1*
	rs79944167	T	0.0099	6.06E-04	0.0368	0.0107	0.9072	*RP1-209A6.1*
	rs548495600	C	0.0099	3.06E-03	0.0311	0.0105	0.9072	*RP1-209A6.1*
	rs79599998	T	0.0099	3.56E-04	0.0377	0.0106	0.9072	*RP1-209A6.1*
	rs140951849	C	0.0099	3.06E-03	0.0333	0.0113	0.9072	*RP1-209A6.1*
	rs76693184	C	0.0099	2.35E-03	0.0345	0.0114	0.9072	*RP1-209A6.1*
	rs115555457	C	0.0129	2.23E-03	0.0273	0.0089	0.6928	*RP1-209A6.1*
	rs116759763	A	0.0099	1.74E-07	0.0590	0.0113	0.9072	*RP11-108N13.1*
	rs191594571	T	0.0149	3.40E-03	0.0258	0.0088	0.7274	*RP11-108N13.1*
	rs188193055	A	0.0109	NA	NA	NA	1.0000	*RNU6-1060P*
	rs116169715	A	0.0109	1.23E-07	0.0599	0.0113	1.0000	*RNU6-1060P*
rs146141654	rs140813881	C	0.0080	1.07E-05	0.0322	0.0073	0.7470	*LHFPL3*
	rs150298466	T	0.0050	7.88E-06	0.0337	0.0075	0.8317	*KMT2E*
	rs189360229	T	0.0060	1.38E-06	0.0347	0.0072	1.0000	*KMT2E*
	rs149720943	T	0.0060	7.51E-07	0.0354	0.0072	1.0000	*KMT2E:SRPK2*
	rs138735129	C	0.0060	6.30E-06	0.0327	0.0072	1.0000	*SRPK2*
	rs7809613	G	0.0060	9.56E-07	-0.0422	0.0086	1.0000	*SRPK2*
	rs2385555	T	0.0050	9.65E-06	-0.0329	0.0074	0.8317	*SRPK2*
	rs1204071	C	0.0040	3.66E-05	-0.0298	0.0072	0.6640	*SRPK2*
	rs147876663	G	0.0060	6.20E-06	0.0326	0.0072	1.0000	*SRPK2*
	rs146141654	C	0.0060	2.74E-07	0.0410	0.0080	1.0000	*SRPK2*
	rs184869978	A	0.0050	1.61E-06	0.0484	0.0101	0.8317	*PUS7*
	rs151022526	T	0.0050	4.70E-07	0.0507	0.0101	0.8317	*PUS7*
rs185545327	rs185545327	T	0.0050	3.99E-08	0.0527	0.0096	1.0000	*RP11-175E9.1:RP11-213G6.2*
rs78134813	rs143962940	C	0.0089	NA	NA	NA	0.8871	*CTD-2509G16.5*
	rs78134813	A	0.0080	5.89E-07	0.0498	0.0100	1.0000	*CTD-2509G16.5*
	rs141876033	A	0.0070	NA	NA	NA	0.8732	*FUT8*
	rs139034722	G	0.0129	1.04E-04	0.0359	0.0093	0.6092	*FUT8*
rs117920312	rs76772737	C	0.0239	3.53E-06	0.0306	0.0066	0.8696	*RAD51B*
	rs117920312	A	0.0209	2.17E-07	0.0354	0.0068	1.0000	*RAD51B*
rs4843341	rs4843341	C	0.0408	1.82E-07	0.0290	0.0056	1.0000	*RP11-805I24.1*
	rs79347073	A	0.0258	1.41E-04	0.0245	0.0064	0.6032	*RP11-805I24.1*

	func	CADD	RDB	H3K4me1	H3K4me3	H3K27ac	H3K9ac	DNase
	ncRNA_intronic	1.105	6	No	No	No	No	No
	ncRNA_intronic	0.713	5	Yes	No	No	No	No
	ncRNA_intronic	1.542	NA	No	No	No	No	No
	ncRNA_intronic	0.899	6	Yes	No	No	No	No
	ncRNA_intronic	4.282	7	No	No	No	No	No
	ncRNA_intronic	7.979	6	No	No	No	No	No
	ncRNA_intronic	1.102	7	Yes	No	Yes	No	No
	intergenic	7.686	6	No	No	No	No	No
	intergenic	0.728	7	No	No	No	No	No
	intergenic	0.729	7	Yes	No	No	No	No
	intergenic	2.248	7	No	No	No	No	No
	intronic	3.361	6	Yes	No	Yes	Yes	No
	intronic	9.952	7	Yes	No	Yes	Yes	No
	intronic	6.876	6	Yes	No	Yes	No	No
	exonic	16.180	4	Yes	No	Yes	Yes	No
	intronic	3.626	2b	Yes	Yes	Yes	Yes	Yes
	intronic	1.187	5	Yes	No	Yes	Yes	No
	intronic	0.638	7	Yes	No	Yes	Yes	No
	intronic	0.439	6	Yes	No	No	Yes	No
	intronic	1.300	4	Yes	Yes	Yes	Yes	Yes
	intronic	0.774	4	Yes	No	Yes	Yes	Yes
	intronic	11.250	6	No	No	Yes	No	No
	intronic	0.956	7	No	No	No	No	No
	ncRNA_intronic	0.560	6	No	No	No	No	No
	ncRNA_intronic	0.421	7	Yes	No	Yes	No	No
	ncRNA_intronic	0.633	5	Yes	No	Yes	Yes	Yes
	intronic	1.401	6	Yes	No	No	No	No
	intronic	1.021	5	Yes	No	No	No	No
	intronic	2.169	5	Yes	Yes	Yes	Yes	Yes
	intronic	9.329	4	Yes	Yes	Yes	Yes	Yes
	intergenic	3.915	2b	Yes	No	Yes	Yes	No
	ncRNA_exonic	2.049	4	Yes	Yes	No	Yes	No

Table S8. Primer sequences of studied genes in qPCR analysis. Forward and reverse sequence for each primer and amplicon length in base pairs. Primers were designed using Primer-Blast, but SRPK2 was based on literature.

Gene	Forward primer	Reverse primer	Amplicon length (bp)
RPS11	5′ AGAGGACCATTGTCATCCGC	5′ AGACATGTTCTTGTGGCGCT	83
HPRT	5′ GGATTTGAAATTCCAGACAAGTTT	5′ GCGATGTCAATAGGACTCCAG	171
18S	5′ AAACGGCTACCACATCCAAG	5′ CGCTCCCAAGATCCAACTAC	250
SRPK2	5′CCTCGTTGTTCTCTGGATCCTTAGAACCTG	5′TCTATGGAGCGGTACTGACGCGTCTG	298
PDE4D	5′ AGACCCTACAGACCAGGCAC	5′ GACACTTGATTTCCAGACCGAC	115

Table S9. Primer validation properties of target genes and housekeeping genes. To create a validation curve for each primer, a series of dilutions were made starting at 1:2.5 or 1:5 dilution and was diluted 2x every two wells. Samples were measured in duplicate, and a no template control was taken along, also in duplicate. Primers with an efficacy between 90%-110% and correlation coefficient (R2 value) close to 1 were considered to be validated.

Primer	E-value	R^2 value	Linear dynamic range (cq)	Specific (yes/no)	Primer valid (yes/no)
RPS11	96.60%	0.994	15.27 – 20.72	Yes	Yes
HPRT	91.80%	0.993	25.38 – 29.42	Yes	Yes
18S	98.50%	0.99	24.48 – 28.55	Yes	Yes
SRPK2-1	96.60%	0.998	28.34 – 32.99	Yes	Yes
PDE4D-3	100.60%	0.999	23.16 – 28.20	Yes	Yes

Table S10. Demographic characteristics of participants based on the ordinal post-surgical pain phenotype.

	Slight pain	Moderate pain	Chronic post-surgical pain	P value
N	26559	752	292	
Females	19249	506	178	
Age (years)	53.5 (10.2)	58.3 (9.00)	59.1 (9.08)	P < 0.0001
BMI (kg × m-2)	28.0 (5.12)	29.0 (5.22)	29.6 (6.08)	P < 0.0001
Self-reported back pain				7.40E-03
Yes	5035	242	83	
No	2077	69	23	
Self-reported abdominal pain				P < 0.0001
Yes	2119	100	38	
No	1399	27	10	
Self-reported CPSP				P < 0.0001
Yes	584	30	9	
No	7974	137	54	
Types of surgery				P < 0.0001
Arteries Veins	110 (81.481)	15 (11.111)	10 (7.407)	
Digestive	8999 (95.308)	320 (3.389)	123 (1.303)	
Female Genital Tract	10718 (97.163)	237 (2.148)	76 (0.689)	
Soft_tissue	358 (97.814)	5 (1.366)	3 (0.82)	
Urinary	411 (93.622)	18 (4.1)	10 (2.278)	
Laparoscopy	3970 (96.971)	90 (2.198)	34 (0.83)	
Multiple Sites	1993 (95.086)	67 (3.197)	36 (1.718)	

Table S11. Lead SNPs passing the suggestive significance level in the GWAS using ordinal chronic postsurgical pain phenotype.

SNP	CHR:POS	Effect allele frequency	P
rs17047504	1:218376496	0.01	3.85E-07
rs6531281	2:17275239	0.33	9.39E-07
rs144605695	2:167065038	0.01	9.23E-07
rs56052023	5:59404369	0.01	5.89E-07
rs182762077	5:111832825	0.01	1.71E-07
rs116169715	6:23158446	0.01	1.06E-07
rs151022526	7:105108669	0.01	4.13E-07
rs185545327	8:23592447	0.01	2.53E-08
rs78134813	14:65758752	0.01	8.94E-07
rs117920312	14:69062922	0.01	1.84E-07
rs12447350	16:61148234	0.01	4.27E-07
rs4843341	16:86108167	0.02	1.87E-07

Table S12. Pleiotropic effects of lead SNPs. PMID: pubmed ID, N: sample size in the reported GWAS. EA: effect allele. NEA, non-effect allele. Note that no pleiotropic effects were found for rs78134813.

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs146141654	4375	30367059	2018	Endocrine	Hyperthyroidism	1.59E-05	51823	C	G
rs182762077	4724	BioRxiv: https://doi.org/10.1101/288632	2019	Neurological	Inferior fronto-occipital fasciculus mode of anisotropy	4.22E-05	17706	C	T
rs6531281	4537	31676860	2019	Neurological	Right pallidum	6.59E-05	19629	G	C
rs56052023	4049	28358823	2017	Body Structures	Achilles tendon injury	8.43E-05	102979	NA	NA
rs146141654	964	27005778	2016	Metabolic	Triglycerides in very large VLDL	9.08E-05	21548	C	G
rs146141654	904	27005778	2016	Metabolic	Triglycerides in large VLDL	1.24E-04	21239	C	G
rs146141654	972	27005778	2016	Metabolic	Triglycerides in chylomicrons and extremely large VLDL	1.33E-04	21540	C	G
rs17047504	4238	31015401	2019	Environmental	Antiglaucoma preparations and miotics	1.60E-04	100868	G	T
rs146141654	961	27005778	2016	Metabolic	Total lipids in very large VLDL	1.67E-04	19273	C	G
rs146141654	953	27005778	2016	Metabolic	VLDL diameter	1.71E-04	19273	C	G
rs117920312	4532	31676860	2019	Neurological	Right cerebellum exterior	2.18E-04	19629	G	A
rs146141654	3464	31427789	2019	Metabolic	Impedance measures - Arm fat percentage (left)	2.30E-04	379699	C	G
rs117920312	4633	31676860	2019	Neurological	Right cerebellum exterior	2.96E-04	21821	G	A
rs146141654	908	27005778	2016	Metabolic	LDL diameter	3.23E-04	19273	G	C
rs146141654	901	27005778	2016	Metabolic	Total lipids in large VLDL	3.38E-04	18960	C	G
rs116169715	3392	31427789	2019	Psychiatric	Longest period of depression	3.98E-04	54608	A	G
rs6531281	4638	31676860	2019	Neurological	Right pallidum	4.36E-04	21821	G	C
rs146141654	3460	31427789	2019	Metabolic	Impedance measures - Arm fat percentage (right)	4.47E-04	379752	C	G

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs117920312	4740	BioRxiv: https://doi.org/10.1101/288648	2019	Neurological	Cingulum (hippocampus) radial diusivities	5.24E-04	17706	A	G
rs146141654	900	27005778	2016	Metabolic	Free cholesterol in large VLDL	5.25E-04	21238	C	G
rs117920312	4534	31676860	2019	Neurological	Right thalamus proper	5.31E-04	19629	G	A
rs146141654	963	27005778	2016	Metabolic	Phospholipids in very large VLDL	5.64E-04	21237	C	G
rs117920312	4521	31676860	2019	Neurological	Left thalamus proper	5.71E-04	19629	G	A
rs146141654	902	27005778	2016	Metabolic	Concentration of large VLDL particles	6.49E-04	18960	C	G
rs6531281	3358	31427789	2019	Social Interactions	Father's age	7.07E-04	86941	C	G
rs13127505	2053	24084763	2013	Neoplasms	Advanced adenoma	7.17E-04	1406	A	G
rs146141654	898	27005778	2016	Metabolic	Total cholesterol in large VLDL	8.53E-04	21235	C	G
rs13127505	2465	28240269	2017	Cell	AURKB - Aurora kinase B	8.90E-04	1000	NA	NA
rs117920312	4519	31676860	2019	Neurological	Left cerebellum exterior	9.10E-04	19629	G	A
rs116169715	3188	31427789	2019	Cardiovascular	Pulse rate (automated reading)	9.51E-04	361411	G	A
rs146141654	899	27005778	2016	Metabolic	Cholesterol esters in large VLDL	9.71E-04	18960	C	G
rs117920312	4696	BioRxiv: https://doi.org/10.1101/288604	2019	Neurological	Cingulum (hippocampus) mean diusivities	9.74E-04	17706	A	G
rs56052023	969	27005778	2016	Metabolic	Total lipids in chylomicrons and extremely large VLDL	1.02E-03	18960	A	G
rs117920312	4620	31676860	2019	Neurological	Left cerebellum exterior	1.05E-03	21821	G	A
rs13127505	4119	29403010	2018	Metabolic	Aspartate aminotransferase	1.06E-03	134154	C	T
rs117920312	4635	31676860	2019	Neurological	Right thalamus proper	1.08E-03	21821	G	A

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs17047504	3540	31427789	2019	Activities	Reason for glasses/contact lenses: For long-sightedness, i.e. for distance and near, but particularly for near tasks like reading (called 'hypermetropia')	1.12E-03	78647	T	G
rs117920312	4622	31676860	2019	Neurological	Left thalamus proper	1.13E-03	21821	G	A
rs6531281	858	27005778	2016	Metabolic	3Lhydroxybutyrate	1.15E-03	24154	C	G
rs146141654	3555	31427789	2019	Activities	Medication for cholesterol, blood pressure, diabetes, or take exogenous hormones: Blood pressure medication	1.16E-03	207533	C	G
rs146141654	3200	31427789	2019	Environment	Job involves mainly walking or standing	1.18E-03	220728	C	G
rs6531281	3684	31427789	2019	Infection	Diagnoses - main ICD10: R31 Hematuria	1.19E-03	300791	G	C
rs182762077	979	27989323	2017	Immunological	Hepatocyte growth factor	1.25E-03	8292	T	C
rs17047504	3570	31427789	2019	Neurological	Pain type(s) experienced in last month: Headache	1.30E-03	385698	G	T
rs4843341	3409	31427789	2019	Environment	Education - Qualifications	1.32E-03	318526	C	T
rs6531281	4233	31015401	2019	Environmental	Antimigraine preparations	1.40E-03	119844	G	C
rs116169715	4002	29500382	2018	Psychiatric	Guilty feelings (GUILT)	1.45E-03	265139	G	A
rs182762077	3239	31427789	2019	Psychiatric	Exposure to tobacco smoke outside home	1.49E-03	327794	T	C
rs146141654	4302	30664634	2019	Metabolic	Arms-arm fat ratio (female)	1.49E-03	195068	C	G
rs6531281	3314	31427789	2019	Ear, Nose, Throat	Hearing difficulty/problems with background noise	1.51E-03	378722	G	C
rs146141654	3270	31427789	2019	Metabolic	Comparative body size at age 10	1.51E-03	379749	C	G

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs146141654	3465	31427789	2019	Metabolic	Impedance measures - Arm fat mass (left)	1.52E-03	379663	C	G
rs146141654	1226	27694991	2016	Neurological	Intracranial Volume	1.56E-03	26577	NA	NA
rs116169715	3452	31427789	2019	Metabolic	Impedance measures - Leg fat percentage (right)	1.61E-03	379806	G	A
rs146141654	3461	31427789	2019	Metabolic	Impedance measures - Arm fat mass (right)	1.68E-03	379725	C	G
rs146141654	3445	31427789	2019	Metabolic	Impedance measures - Body Mass Index (BMI)	1.72E-03	379831	C	G
rs56052023	963	27005778	2016	Metabolic	Phospholipids in very large VLDL	1.78E-03	21237	A	G
rs56052023	3835	27863252	2016	Immunological	Basophil percentage of granulocytes (two-way meta)	1.79E-03	131536	A	G
rs146141654	970	27005778	2016	Metabolic	Concentration of chylomicrons and extremely large VLDL particles	1.79E-03	18960	C	G
rs13127505	3204	31427789	2019	Activities	Number of days/week walked 10+ minutes	1.80E-03	379927	C	T
rs17047504	4284	30804560	2019	Respiratory	FEV1/FVC ratio	1.82E-03	321047	T	G
rs4843341	3282	31427789	2019	Social Interactions	Number of full brothers	1.85E-03	380062	T	C
rs4843341	3366	31427789	2019	Reproduction	Age at menopause (last menstrual period) (female)	1.92E-03	119160	C	T
rs146141654	3435	31427789	2019	Metabolic	Body Mass Index	1.93E-03	385336	C	G
rs6531281	254	25772697	2015	Immunological	CD3 on DN T	2.04E-03	669	C	G
rs6531281	240	25772697	2015	Immunological	CD27 on IgG+B	2.08E-03	669	C	G

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs17047504	4596	31676860	2019	Neurological	Right postcentral	2.12E-03	21821	T	G
rs116169715	3185	31427789	2019	Metabolic	Waist circumference	2.17E-03	385932	G	A
rs116169715	3456	31427789	2019	Metabolic	Impedance measures - Leg fat percentage (left)	2.18E-03	379786	G	A
rs116169715	3295	31427789	2019	Psychiatric	Guilty feelings	2.18E-03	376361	G	A
rs146141654	3441	31427789	2019	Metabolic	Impedance measures - Body fat percentage	2.25E-03	379615	C	G
rs116169715	3508	31427789	2019	Nutritional	Non-butter spread type details: Other low or reduced fat spread	2.27E-03	201144	G	A
rs17047504	3537	31427789	2019	Environment	Attendance/disability/mobility allowance: Disability living allowance	2.34E-03	383632	G	T
rs182762077	3250	31427789	2019	Nutritional	Pork intake	2.42E-03	384328	T	C
rs146141654	863	27005778	2016	Metabolic	Double bonds in fatty acids	2.49E-03	15728	G	C
rs6531281	3556	31427789	2019	Activities	Medication for cholesterol, blood pressure, diabetes, or take exogenous hormones: Hormone replacement therapy	2.49E-03	207533	G	C
rs17047504	4495	31676860	2019	Neurological	Right postcentral	2.49E-03	19629	T	G
rs13127505	2778	28240269	2017	Cell	SYNCRIP - Heterogeneous nuclear ribonucleoprotein Q	2.51E-03	1000	NA	NA
rs146141654	3458	31427789	2019	Metabolic	Impedance measures - Leg fat-free mass (left)	2.54E-03	379766	C	G
rs6531281	527	24816252	2014	Metabolic	Lipid::Long chain fatty acid::pentadecanoate (15:0)	2.56E-03	7502	G	C

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs17047504	4282	30804560	2019	Respiratory	FEV1	2.57E-03	321047	T	G
rs146141654	3459	31427789	2019	Metabolic	Impedance measures - Leg predicted mass (left)	2.60E-03	379761	C	G
rs146141654	860	27005778	2016	Metabolic	CH2 groups to double bonds ratio	2.61E-03	13532	C	G
rs13127505	4373	30367059	2018	Endocrine	Free thyroxine (FT4, male)	2.63E-03	22455	T	C
rs146141654	4332	30583798	2018	Psychiatric	Erectile dysfunction	2.67E-03	223805	C	G
rs146141654	3468	31427789	2019	Metabolic	Impedance measures - Trunk fat percentage	2.69E-03	379600	C	G
rs56052023	961	27005778	2016	Metabolic	Total lipids in very large VLDL	2.75E-03	19273	A	G
rs146141654	3738	31427789	2019	Psychiatric	Depression - Lifetime number of depressed periods	2.77E-03	57986	C	G
rs146141654	969	27005778	2016	Metabolic	Total lipids in chylomicrons and extremely large VLDL	2.80E-03	18960	C	G
rs116169715	3341	31427789	2019	Reproduction	Number of live births (female)	2.86E-03	208434	G	A
rs4843341	3460	31427789	2019	Metabolic	Impedance measures - Arm fat percentage (right)	2.87E-03	379752	T	C
rs146141654	3865	27863252	2016	Immunological	Red cell distribution width (two-way meta)	2.90E-03	131520	G	C
rs56052023	898	27005778	2016	Metabolic	Total cholesterol in large VLDL	2.99E-03	21235	A	G
rs6531281	4524	31676860	2019	Neurological	Left pallidum	3.00E-03	19629	G	C
rs116169715	3288	31427789	2019	Psychiatric	Fed-up feelings	3.03E-03	378357	G	A
rs182762077	4720	BioRxiv: https://doi.org/10.1101/288628	2019	Neurological	External capsule mode of anisotropy	3.05E-03	17706	C	T

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs4843341	4302	30664634	2019	Metabolic	Arms-arm fat ratio (female)	3.05E-03	195068	T	C
rs17047504	4676	BioRxiv: https://doi.org/10.1101/288584	2019	Neurological	External capsule axial diusivities	3.12E-03	17706	G	T
rs6531281	300	25772697	2015	Immunological	CD4mem:%Th1* (3)	3.12E-03	669	C	G
rs146141654	4256	30952852	2019	Psychiatric	Sleep midpoint	3.20E-03	84810	C	G
rs6531281	295	25772697	2015	Immunological	CD4mem:%Th17	3.22E-03	669	C	G
rs17047504	4334	30552067	2018	Respiratory	Asthma	3.25E-03	30810	G	T
rs4843341	3554	31427789	2019	Activities	Medication for cholesterol, blood pressure, diabetes, or take exogenous hormones: Cholesterol lowering medication	3.26E-03	207533	T	C
rs4843341	3386	31427789	2019	Activities	Work/job satisfaction	3.29E-03	128621	T	C
rs6531281	3211	31427789	2019	Activities	Frequency of stair climbing in last 4 weeks	3.29E-03	382731	G	C
rs13127505	2498	28240269	2017	Cell	STAB2 - Stabilin-2	3.30E-03	1000	NA	NA
rs6531281	281	25772697	2015	Immunological	CD8:%Act(25+38+RO+)	3.31E-03	669	G	C
rs17047504	4670	BioRxiv: https://doi.org/10.1101/288578	2019	Neurological	Anterior limb of internal capsule axial diusivities	3.35E-03	17706	G	T
rs146141654	971	27005778	2016	Metabolic	Phospholipids in chylomicrons and extremely large VLDL	3.41E-03	21542	C	G
rs17047504	4233	31015401	2019	Environmental	Antimigraine preparations	3.70E-03	119844	G	T
rs146141654	927	27005778	2016	Metabolic	Triglycerides in medium VLDL	3.76E-03	21241	C	G
rs6531281	301	25772697	2015	Immunological	CD4mem:%PD1-R6+	3.77E-03	669	C	G

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs56052023	900	27005778	2016	Metabolic	Free cholesterol in large VLDL	3.86E-03	21238	A	G
rs17047504	4452	31676860	2019	Neurological	Left isthmus cingulate	3.88E-03	19629	T	G
rs117920312	4533	31676860	2019	Neurological	Right cerebellum white matter	3.90E-03	19629	G	A
rs185545327	856	27005778	2016	Metabolic	Ratio of bisLallylic bonds to double bonds in lipids	3.95E-03	13524	C	T
rs116169715	3441	31427789	2019	Metabolic	Impedance measures - Body fat percentage	3.96E-03	379615	G	A
rs117920312	4509	31676860	2019	Neurological	Cerebellar vermal lobules VIII X	3.96E-03	19629	G	A
rs116169715	39	27046643	2016	Cognitive	Memory	4.04E-03	112067	G	A
rs182762077	4671	BioRxiv: https://doi.org/10.1101/288579	2019	Neurological	Average across all tracts axial diusivities	4.04E-03	17706	C	T
rs182762077	4676	BioRxiv: https://doi.org/10.1101/288584	2019	Neurological	External capsule axial diusivities	4.09E-03	17706	C	T
rs146141654	3469	31427789	2019	Metabolic	Impedance measures - Trunk fat mass	4.11E-03	379578	C	G
rs116169715	3460	31427789	2019	Metabolic	Impedance measures - Arm fat percentage (right)	4.11E-03	379752	G	A
rs56052023	3834	27863252	2016	Immunological	Basophil percentage of white cells (two-way meta)	4.15E-03	131863	A	G
rs17047504	3351	31427789	2019	Mortality	Had other major operations	4.17E-03	206807	G	T
rs146141654	3442	31427789	2019	Metabolic	Impedance measures - Whole body fat mass	4.27E-03	379203	C	G
rs17047504	4316	30643251	2019	Psychiatric	Smoking cessation	4.36E-03	312821	G	T
rs116169715	3435	31427789	2019	Metabolic	Body Mass Index	4.37E-03	385336	G	A

Table S12. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs116169715	3558	31427789	2019	Activities	Medication for pain relief, constipation, heartburn: Ibuprofen (e.g. Nurofen)	4.37E-03	382089	G	A
rs13127505	2242	28240269	2017	Cell	AIF1 - Allograft inflammatory factor 1	4.42E-03	1000	NA	NA
rs17047504	3333	31427789	2019	Activities	Frequency of heavy DIY in last 4 weeks	4.45E-03	153377	T	G
rs116169715	3995	29500382	2018	Psychiatric	Fed-up feelings (FED_UP)	4.46E-03	266208	G	A
rs56052023	964	27005778	2016	Metabolic	Triglycerides in very large VLDL	4.46E-03	21548	A	G

Table S13. Genes identified by gene-based analysis in MAGMA. N: effective sample size. NPARAM: the number of relevant parameters used in the model. ZSTAT: z statistics. (Here lists top 20 candidates. The complete list can be found online.)

GENE	CHR	START	STOP	NSNPS	NPARAM	N	ZSTAT	P	SYMBOL
ENSG00000135824	1	182565239	182703711	384	49	27453	3.5625	1.84E-04	*RGS8*
ENSG00000107882	10	104213744	104443292	608	25	27520	3.535	2.04E-04	*SUFU*
ENSG00000138111	10	104171149	104286802	305	29	27506	3.529	2.09E-04	*TMEM180*
ENSG00000138107	10	104188986	104312482	349	22	27507	3.521	2.15E-04	*ACTR1A*
ENSG00000138764	4	78028304	78404542	1976	41	27484	3.5158	2.19E-04	*CCNG2*
ENSG00000164111	4	122539110	122668268	604	54	27478	3.462	2.68E-04	*ANXA5*
ENSG00000141200	17	51850239	51952573	387	34	27481	3.4051	3.31E-04	*KIF2B*
ENSG00000107874	10	104133002	104242418	235	43	27482	3.3577	3.93E-04	*CUEDC2*
ENSG00000198836	3	193260933	193465612	668	41	27460	3.3326	4.30E-04	*OPA1*
ENSG00000120055	10	104159594	104261300	254	35	27496	3.206	6.73E-04	*C10orf95*
ENSG00000131459	5	179677690	179830387	686	58	27331	3.1292	8.76E-04	*GFPT2*
ENSG00000116213	1	3497331	3619325	475	66	27338	3.1182	9.10E-04	*WRAP73*
ENSG00000158109	1	3491566	3596691	412	54	27355	3.1028	9.58E-04	*TPRG1L*
ENSG00000229729	3	88058424	88238123	457	19	27512	3.0322	1.21E-03	*RP11-159G9.5*
ENSG00000115598	2	102753433	102906462	531	38	27446	3.0255	1.24E-03	*IL1RL2*
ENSG00000163320	3	88051094	88249035	502	20	27512	3.0167	1.28E-03	*CGGBP1*
ENSG00000124406	4	42360390	42709122	1357	61	27379	2.9879	1.40E-03	*ATP8A1*
ENSG00000163624	4	85454132	85622491	384	27	27449	2.9781	1.45E-03	*CDS1*
ENSG00000107872	10	104128946	104232893	207	44	27472	2.9779	1.45E-03	*FBXL15*
ENSG00000179021	3	88148893	88267879	258	8	27539	2.9772	1.45E-03	*C3orf38*

Table S14. Robustness analysis of lead SNPs.

SNP	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
rs116169715	9.30E-08	3.98E-02	4.05E-04	9.34E-05	3.18E-04	6.88E-05	2.56E-02	4.57E-08	1.47E-05	1.15E-03
rs117920312	2.59E-05	2.50E-03	2.92E-04	2.35E-04	2.98E-02	3.09E-07	2.84E-01	7.48E-10	1.09E-07	3.97E-02
rs13127505	8.02E-05	3.12E-03	3.10E-06	1.97E-02	2.66E-03	7.60E-05	2.24E-02	2.97E-06	2.30E-06	3.12E-02
rs146141654	1.19E-04	5.78E-04	2.41E-04	2.57E-04	3.06E-04	3.36E-04	2.68E-04	2.35E-04	9.71E-03	2.73E-06
rs17047504	2.09E-05	3.95E-03	5.73E-05	1.49E-03	1.56E-02	1.61E-06	7.55E-03	7.10E-06	2.46E-03	2.99E-05
rs182762077	4.25E-06	4.49E-03	4.30E-05	7.01E-04	2.96E-02	5.05E-08	3.36E-03	8.04E-06	5.59E-05	7.38E-04
rs185545327	5.06E-03	5.95E-07	1.08E-05	5.61E-04	1.20E-05	8.09E-04	7.86E-06	1.02E-03	3.85E-05	2.26E-04
rs4843341	3.93E-05	1.34E-03	1.23E-02	1.16E-06	2.31E-05	2.37E-03	8.01E-05	7.27E-04	9.38E-05	6.54E-04
rs56052023	2.12E-01	2.32E-08	1.76E-01	8.08E-08	1.72E-05	4.42E-03	2.40E-03	3.26E-05	7.07E-03	7.94E-06
rs6531281	1.54E-02	8.18E-06	7.59E-06	1.09E-02	7.58E-06	1.66E-02	3.30E-05	5.38E-03	9.20E-05	2.14E-03
rs78134813	7.22E-04	2.21E-04	1.04E-08	2.90E-01	2.89E-04	4.79E-04	1.67E-02	3.69E-06	8.13E-04	1.89E-04

Table S15. SNPs in LD (r2 > 0.6) with lead SNPs queried in the meta-analysis.

Lead SNP	Proxy SNP	R2	MAF
rs116169715	rs116169715	1.000	0.011
rs116169715	rs188193055	1.000	0.011
rs116169715	rs181467670	1.000	0.011
rs116169715	rs116759763	1.000	0.011
rs116169715	rs76693184	1.000	0.011
rs116169715	rs140951849	1.000	0.011
rs116169715	rs79599998	1.000	0.011
rs116169715	rs548495600	1.000	0.011
rs116169715	rs79944167	1.000	0.011
rs116169715	rs78068757	1.000	0.011
rs116169715	rs191594571	0.663	0.017
rs116169715	rs115555457	0.663	0.017
rs117920312	rs117920312	1.000	0.017
rs117920312	rs76772737	0.746	0.022
rs117920312	rs146688500	0.746	0.022
rs13127505	rs13127505	1.000	0.110
rs146141654	rs146141654	1.000	0.006
rs146141654	rs147876663	1.000	0.006
rs146141654	rs1204071	1.000	0.006
rs146141654	rs2385555	1.000	0.006
rs146141654	rs7809613	1.000	0.006
rs146141654	rs184869978	1.000	0.006
rs146141654	rs151022526	1.000	0.006
rs146141654	rs188349781	1.000	0.006
rs146141654	rs559936485	1.000	0.006
rs146141654	rs558166804	1.000	0.006
rs146141654	rs138735129	1.000	0.006
rs146141654	rs143342390	1.000	0.006
rs146141654	rs149720943	1.000	0.006
rs146141654	rs189360229	1.000	0.006
rs146141654	rs1271116617	1.000	0.006
rs146141654	rs537090648	1.000	0.006
rs146141654	rs140813881	1.000	0.006
rs17047504	rs17047504	1.000	0.006
rs17047504	rs561565222	1.000	0.006
rs17047504	rs573304760	1.000	0.006

Table S15. Continued

Lead SNP	Proxy SNP	R2	MAF
rs17047504	rs555172230	1.000	0.006
rs17047504	rs375410493	1.000	0.006
rs17047504	rs541573906	1.000	0.006
rs17047504	rs185306761	1.000	0.006
rs17047504	rs187247586	1.000	0.006
rs17047504	rs560921551	1.000	0.006
rs17047504	rs567548441	1.000	0.006
rs17047504	rs184696675	1.000	0.006
rs182762077	rs182762077	1.000	0.011
rs182762077	rs77324010	1.000	0.011
rs182762077	rs114837332	1.000	0.011
rs182762077	rs1758157657	1.000	0.011
rs182762077	rs1758154874	1.000	0.011
rs182762077	rs79798796	1.000	0.011
rs182762077	rs116197008	1.000	0.011
rs182762077	rs183632075	1.000	0.011
rs182762077	rs114779418	1.000	0.011
rs4843341	rs4843341	1.000	0.022
rs4843341	rs79347073	1.000	0.022
rs4843341	rs79596063	0.796	0.028
rs4843341	rs146106361	0.746	0.017
rs4843341	rs144196587	0.746	0.017
rs4843341	rs78380532	0.746	0.017
rs4843341	rs13380539	0.659	0.033
rs56052023	rs56052023	1.000	0.006
rs56052023	rs851285	1.000	0.006
rs56052023	rs144788536	1.000	0.006
rs56052023	rs148582675	1.000	0.006
rs56052023	rs78419998	1.000	0.006
rs56052023	rs144391307	1.000	0.006
rs56052023	rs561337849	1.000	0.006
rs56052023	rs181980221	1.000	0.006
rs56052023	rs183902862	1.000	0.006
rs56052023	rs559679869	1.000	0.006
rs56052023	rs182736701	1.000	0.006
rs6531281	rs6531281	1.000	0.313

Table S15. Continued

Lead SNP	Proxy SNP	R2	MAF
rs6531281	rs1039742	1.000	0.313
rs6531281	rs1515988	1.000	0.313
rs6531281	rs1807869	1.000	0.313
rs6531281	rs10177601	1.000	0.313
rs6531281	rs13013588	1.000	0.313
rs6531281	rs4324323	1.000	0.313
rs6531281	rs906492	1.000	0.313
rs6531281	rs1515983	1.000	0.313
rs6531281	rs1515982	1.000	0.313
rs6531281	rs1515980	1.000	0.313
rs6531281	rs10200324	1.000	0.313
rs6531281	rs6755572	1.000	0.313
rs6531281	rs11422020	1.000	0.313
rs6531281	rs6712392	1.000	0.313
rs6531281	rs6718117	0.975	0.319
rs6531281	rs6531282	0.975	0.319
rs6531281	rs10164520	0.975	0.308
rs6531281	rs925788	0.786	0.264
rs6531281	rs2090310	0.786	0.264
rs6531281	rs2342546	0.761	0.269
rs6531281	rs10553506	0.622	0.423
rs6531281	rs7564158	0.622	0.423
rs78134813	rs78134813	1.000	0.006
rs78134813	rs143962940	1.000	0.006
rs78134813	rs565111600	1.000	0.006
rs78134813	rs191456211	1.000	0.006
rs78134813	rs540592563	1.000	0.006
rs78134813	rs141876033	1.000	0.006
rs78134813	rs571244819	1.000	0.006
rs78134813	rs202247281	1.000	0.006
rs78134813	rs544262238	1.000	0.006
rs78134813	rs538542446	1.000	0.006
rs185545327	rs185545327	1.000	0.007

Table S16. P-values of candidate SNPs reported in the CPSP meta-analysis. CPSP: chronic postsurgical pain.

lead SNP	Reported Marker	Allele1	Freq1	Effect	StdErr	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
rs4843341	rs4843341	t	0.9719	-0.6091	0.4044	0.132	-??-???	0	0.333	1	0.5638
rs4843341	rs79596063	a	0.0298	0.3884	0.3476	0.2638	+?-+???	0	0.856	2	0.6519
rs4843341	rs146106361	a	0.0231	0.73	0.5483	0.183	+??????	0	0	0	1
rs4843341	rs78380532	c	0.9769	-0.6995	0.5497	0.2032	-??????	0	0	0	1
rs4843341	rs13380539	a	0.9693	-0.1904	0.3822	0.6183	-?+????	48.3	1.936	1	0.1642
rs4843341	rs144196587	t	0.0215	0.8179	0.5665	0.1488	+??????	0	0	0	1
rs4843341	rs79347073	a	0.0231	0.8116	0.5454	0.1367	+??????	0	0	0	1
rs6531281	rs10177601	t	0.2992	-0.0896	0.0955	0.3481	-++---+	0	2.757	6	0.8386
rs6531281	rs6718117	t	0.6915	0.0598	0.0945	0.5268	+-++++-	0	3.149	6	0.7899
rs6531281	rs1515988	t	0.7007	0.0894	0.0955	0.3492	+--+++-	0	2.424	6	0.8769
rs6531281	rs1515983	a	0.7019	0.0801	0.0955	0.4019	+--+++-	0	2.021	6	0.9177
rs6531281	rs906492	a	0.2978	-0.0793	0.0955	0.4063	-++---+	0	2.027	6	0.9172
rs6531281	rs10200324	a	0.7028	0.0772	0.0954	0.4185	+--+++-	0	2.037	6	0.9163
rs6531281	rs1515982	c	0.2972	-0.0772	0.0954	0.4185	-++---+	0	2.037	6	0.9163
rs6531281	rs925788	a	0.7828	0.0783	0.1043	0.4528	+--+++-	20.5	7.548	6	0.2731
rs6531281	rs13013588	t	0.298	-0.0827	0.0955	0.3865	-++---+	0	2.436	6	0.8756
rs6531281	rs6531282	a	0.6913	0.0558	0.0944	0.5548	+-++++-	0	3.147	6	0.7902
rs6531281	rs2090310	t	0.2185	-0.0795	0.1043	0.446	-++---+	20.1	7.507	6	0.2765
rs6531281	rs4324323	t	0.298	-0.0827	0.0955	0.3865	-++---+	0	2.436	6	0.8756
rs6531281	rs10164520	a	0.2971	-0.078	0.0955	0.4138	-++---+	0	2.023	6	0.9176
rs6531281	rs7564158	t	0.5845	0.09	0.0869	0.3003	+-++++-	0	4.052	6	0.6696

Table S16. Continued

lead SNP	Reported Marker	Allele1	Freq1	Effect	StdErr	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
rs6531281	rs1515980	t	0.7127	-0.0104	0.2279	0.9635	??-????	0	0	0	1
rs6531281	rs1039742	a	0.299	-0.0935	0.0956	0.3277	-++---+	0	3.146	6	0.7903
rs6531281	rs6712392	t	0.7039	0.071	0.0956	0.4578	+--+++-	0	2.18	6	0.9024
rs6531281	rs2342546	t	0.7804	0.0843	0.1042	0.4183	+--+++-	23.4	7.831	6	0.2507
rs6531281	rs6531281	c	0.701	0.0935	0.0956	0.3277	+--+++-	0	3.146	6	0.7903
rs6531281	rs1807869	a	0.2997	-0.0889	0.0955	0.3519	-++---+	0	2.438	6	0.8754
rs6531281	rs6755572	t	0.7051	0.0754	0.0955	0.4301	+--+++-	0	2.301	6	0.8901
rs13127505	rs13127505	t	0.1036	0.3056	0.159	0.05454	++?+-+-	32	7.358	5	0.1954
rs117920312	rs76772737	t	0.9855	-0.5943	0.8742	0.4966	??-????	0	0	0	1
s146141654	rs143342390	a	0.0169	0.4871	0.6357	0.4435	+??????	0	0	0	1
rs182762077	rs77324010	a	0.0189	0.4883	0.7667	0.5242	??+????	0	0	0	1
rs182762077	rs182762077	t	0.0178	0.6458	0.7907	0.4141	??+????	0	0	0	1

Table S17. RNA Expression analysis in adhesions of patients with and without chronic abdominal postsurgical pain

Samples	18S CNRQ	HPRT CNRQ	RPS11 CNRQ	PDE4D CNRQ	SRPK2 CNRQ	Trait
M04	1.13E+00	6.73E-01	1.32E+00	7.79E-01	4.72E-01	Control
M10	1.01E+00	9.82E-01	1.01E+00	8.47E-01	4.89E-01	Control
M11	1.20E+00	1.29E+00	6.48E-01	2.03E+00	2.60E+00	Control
M13	8.87E-01	1.07E+00	1.05E+00	7.90E-01	6.08E-01	Control
M14	9.63E-01	8.08E-01	1.29E+00	1.90E+00	1.09E+00	Control
M15	1.02E+00	1.83E+00	5.37E-01	3.85E-01	5.82E-01	Control
M16	1.15E+00	1.11E+00	7.88E-01	3.73E-01	5.45E-01	Control
M17	9.32E-01	6.72E-01	1.60E+00	1.01E+00	5.76E-01	Control
M18	1.14E+00	5.62E-01	1.56E+00	5.51E-01	4.23E-01	Control
M19	9.21E-01	1.24E+00	8.77E-01	2.13E+00	1.87E+00	Control
M20	1.17E+00	9.04E-01	9.47E-01	2.57E+00	2.96E+00	Control
M22	4.81E-01	9.80E-01	2.12E+00	4.08E-01	6.64E-02	Control
M24	1.16E+00	1.07E+00	8.03E-01	1.20E+00	3.70E+00	Control
M25	NaN	NaN	NaN	NaN	NaN	Control
M26	9.23E-01	1.14E+00	9.50E-01	4.80E-01	3.39E-01	Control
M27	1.26E+00	8.00E-01	9.96E-01	1.83E+00	2.25E+00	Control
M28	NaN	NaN	NaN	NaN	NaN	Control
M29	1.28E+00	1.04E+00	7.46E-01	6.67E-01	1.30E+00	Control
M31	NaN	NaN	NaN	NaN	NaN	Control
M32	9.02E-01	9.40E-01	1.18E+00	1.67E+00	1.45E+00	Control
M33	NaN	NaN	NaN	NaN	NaN	Control
M34	1.19E+00	1.19E+00	7.05E-01	1.63E+00	5.13E+00	Control
M35	9.45E-01	9.47E-01	1.12E+00	1.47E+00	1.91E+00	Control
M36	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	Control
M6	NaN	NaN	NaN	NaN	NaN	Control
M8	NaN	NaN	NaN	NaN	NaN	Control
M9	1.11E+00	1.72E+00	5.24E-01	7.42E-01	3.52E+00	Control
N02	8.73E-01	9.44E-01	1.21E+00	1.39E+00	1.29E+00	Control
M02	NaN	NaN	NaN	NaN	NaN	Control
B04	9.04E-01	9.92E-01	1.21E+00	2.25E+00	1.93E+00	Pain
B05	9.20E-01	8.55E-01	1.28E+00	2.22E+00	1.25E+00	Pain
B06	7.45E-01	1.07E+00	1.26E+00	7.07E-01	8.96E-01	Pain
B07	1.11E+00	7.49E-01	1.20E+00	1.03E+00	6.57E-01	Pain
B08	1.03E+00	7.77E-01	1.31E+00	1.30E+00	1.02E+00	Pain
B09	7.26E-01	9.45E-01	1.46E+00	6.24E-01	5.65E-01	Pain

Table S17. Continued

Samples	18S CNRQ	HPRT CNRQ	RPS11 CNRQ	PDE4D CNRQ	SRPK2 CNRQ	Trait
B10	1.30E+00	9.62E-01	8.02E-01	1.47E+00	4.49E+00	Pain
B11	6.48E-01	6.23E-01	2.48E+00	9.86E-01	NaN	Pain
B12	1.21E+00	1.06E+00	7.86E-01	1.47E+00	3.39E+00	Pain
B14	1.37E+00	9.21E-01	7.96E-01	1.15E+00	2.41E+00	Pain
B16	8.69E-01	9.98E-01	1.15E+00	2.46E+00	3.45E+00	Pain
B18	2.44E-01	1.71E+00	2.39E+00	8.75E-01	NaN	Pain
B19	8.24E-01	8.55E-01	1.42E+00	1.21E+00	4.68E-02	Pain
B20	9.86E-01	1.09E+00	9.30E-01	4.83E+00	2.45E+00	Pain
B21	1.78E+00	1.23E-01	4.58E+00	1.82E+00	1.52E-02	Pain
B24	6.43E-01	9.38E-01	2.31E+00	1.30E+00	2.77E+00	Pain
B25	9.03E-01	1.21E+00	9.16E-01	3.70E+00	2.07E+00	Pain
B26	8.35E-01	1.37E+00	8.93E-01	1.53E+00	2.33E+00	Pain
B27	9.49E-01	8.43E-01	1.25E+00	1.52E+00	1.02E+00	Pain
B30	1.07E+00	6.88E-01	1.36E+00	2.74E+00	1.46E+00	Pain
B31	1.13E+00	6.70E-01	1.33E+00	1.11E+00	5.01E-01	Pain
B32	1.04E+00	6.93E-01	1.40E+00	1.44E+00	1.33E+00	Pain
B36	8.64E-01	8.11E-01	1.43E+00	2.02E+00	1.02E+00	Pain
B37	9.03E-01	7.29E-01	1.52E+00	8.47E-01	2.60E-01	Pain
B41	9.18E-01	7.90E-01	1.38E+00	1.49E+00	6.44E-01	Pain
B43	1.02E+00	9.81E-01	9.99E-01	1.18E+00	2.12E+00	Pain
B44	8.84E-01	1.14E+00	1.03E+00	1.74E+00	2.09E+00	Pain
M1	1.45E+00	1.04E+00	6.66E-01	5.80E-01	1.44E+00	Pain
N01	1.02E+00	8.30E-01	1.30E+00	7.55E-01	9.01E-01	Pain
B28	NaN	NaN	NaN	NaN	NaN	Pain
B42	NaN	NaN	NaN	NaN	NaN	Pain

Chapter 5

Genome-wide association study on chronic postsurgical pain in the UK Biobank

Song Li [1], Masja K. Toneman [2], Luda Diatchenko [3], Marc Parisien [3], Kris C. P. Vissers [4], Richard P.G. ten Broek [2], Regina L.M. van Boekel [4, 5] Marieke J.H. Coenen [6,*]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[2] Department of Surgery, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands.

[3] Faculty of Dental Medicine and Oral Health Sciences, Department of Anesthesia, Faculty of Medicine, Alan Edwards Centre for Research on Pain, McGill University, Montreal, Quebec, Canada.

[4] Department of Anesthesiology, Pain and Palliative Medicine, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[5] Research Department Emergency and Critical Care, HAN University of Applied Sciences, School of Health Studies, Nijmegen, the Netherlands.

[6] Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands.

*Corresponding author

Marieke J.H. Coenen, Ph.D., Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands. M.Coenen@erasmusmc.nl

Abstract

Background. Chronic postsurgical pain (CPSP) persists beyond the expected healing period following surgery, imposing a substantial burden on patients' overall well-being. Unfortunately, CPSP often remains under-diagnosed and undertreated. To better understand the mechanism of CPSP development, this study explores to identify genetic variants associated with CPSP.

Methods. A genome-wide association study (GWAS) was conducted in a cohort of 95,931 individuals from the UK biobank who had undergone different surgical procedures. Three analyses were performed: (1) case control analysis (2923 cases with CPSP and 93008 controls), (2) ordinal analysis in 3 groups based on time of analgesics use (n=95931) and (3) a meta-analysis combining our dataset with a recent publication (n=97281).

Results. In the case control analysis one genetic locus within *GLRA3* displayed a genome-wide significant ($P < 2.5 \times 10^{-8}$) association with CPSP, and nine loci displayed suggestively significant associations ($P < 1 \times 10^{-6}$). The ordinal analysis aligned with the case control analysis with an additional locus (rs140330443) reaching genome-wide significance. In the meta-analysis with the recently published dataset the SNP rs17298280 in the *GLRA3* gene remained significant ($P = 2.19 \times 10^{-9}$).

Conclusion. This study contributes new insights into the genetic factors associated with CPSP. The top hit *GLRA3* is known for involvement in prostaglandin E2(PGE2)-induced pain processing pathways. Our study provides a foundation for future investigations into the function of these risk variants and the mechanisms underlying CPSP by offering summary statistics. However, further validation in other cohorts is required to confirm these findings.

Keywords
Chronic postsurgical pain, Genome-wide association study, Genetics, Risk Prediction, UK Biobank

Introduction

Approximately, 23 million individuals experience chronic postsurgical pain (CPSP) annually [1]. CPSP has been linked to a reduced overall quality of life, placing a substantial emotional and physical burden on patients [2].

The occurrence of CPSP (5% to 85%) depends on the surgical site, type of surgery, likelihood of nerve damage, and perioperative factors [3]. CPSP is still underdiagnosed and undertreated [4]. The management of CPSP might be improved by using individualized risk prediction for clinical decision-making [5]. Although several identified CPSP risk factors have been included in risk prediction models[6, 7], adequate prediction in clinical practice has not been achieved [8].

Accuracy of CPSP prediction could be enhanced by incorporating genetic factors into prediction models. However, identifying genetic factors remains challenging [9]. Two recent systematic reviews on genetic association studies of (chronic) postsurgical pain showed that only three variants (*OPRM1* rs1799971, *COMT* rs4680, and *KCNS1* rs734784) remained significantly associated with CPSP after meta-analysis [10, 11]. In addition, the focus of genetic association studies has been on acute pain (such as analgesic requirements and pain score after surgeries [12-14]), and most previous studies are candidate gene studies, which might overlook the beyond-known mechanisms. Only three genome-wide association studies (GWASes) on CPSP have been published in relatively small cohorts (few hundred to 1,700 subjects), showing inconsistent results [12, 14, 15]. However, hypothesis-free methods (such as GWAS) in large cohorts are needed to discover the genetic background of CPSP further. Furthermore, polygenic risk scores (PRS) based on GWASes has the potential to explain more phenotypic variance compared to single variant tests. Incorporating PRS in prediction models for chronic (postsurgical) pain might improve predictive accuracy [4].

This paper aims to identify genetic variants associated with CPSP in selected surgeries using data from the UK Biobank (UKB) through a GWAS. Exploratory goals were to investigate shared genetic factors and genetic correlations among CPSP development in different surgeries and other phenotypically related traits reported in previous research.

Method

We performed a meta-analysis GWAS for CPSP development (using a binary outcome) after major and minor surgeries, referred to as the main analysis in the following text. The results were carried forward for post-GWAS analyses. Figure 1 depicts the study workflow. This study was pre-registered (Open Science Framework (https://osf.io/h6cr9/).

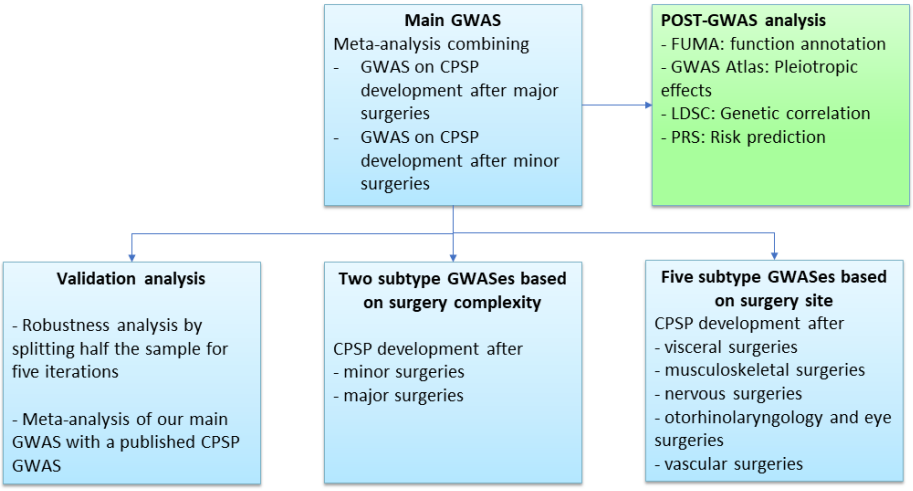

Figure 1. Analysis overview. UKB, UK biobank; GWAS, genome-wide association analysis; CPSP, chronic postsurgical pain; FUMA, an integrative web-based platform for functional mapping and annotation of genome-wide association studies; LDSC, linkage disequilibrium score regression; PRS, polygenic risk score.

Study cohorts

UK Biobank (UKB) data were used for the analyses. A description of the cohort can be found in the supplementary methods.

Inclusion and exclusion criteria

Subjects were considered eligible for inclusion if their initial surgery records were dated between 1997 and 2015. Only initial surgeries were selected for analysis as prior surgical procedures may affect CPSP development. Table S1 lists included surgeries, surgery complexity, and corresponding OPCS4 code. After quality control procedures (Figure 2), subjects were divided into two groups (i.e., major and minor surgeries) based on the complexity of the surgeries.

Subjects were excluded if they met the following criteria: 1) failing routine GWAS sample quality control (QC) (supplementary methods); 2) withdrawing informed

consent; 3) surgery records falling outside the established date range; 4) undergoing another surgery within the one-year follow-up period after their initial surgery; 5) deceased during follow-up; 6) without GP registration data (an release of GP data was used which covered approximately 45% of all UKB participants resulting in the exclusion of 122049 participants); 7) without any prescription records in the GP data; 8) pre-existing chronic pain as suggested by more than three months of analgesic records within the year before surgery.

CPSP phenotype definition

Postoperative pain was determined based on moment of the surgery and prescription records. After initial surgery, one-year analgesic prescription records were extracted following the surgery event, primarily encompassing nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (see Table S2 for included drugs). This follow-up period of 12 months was divided into intervals of 30 days, and the presence of analgesic prescription records within a specific interval denoted pain treatment during that particular month.

Based on the duration of analgesic prescription, patients were dichotomized into two groups: cases, defined as those with a minimum of four (consecutive or non-consecutive) months of analgesic consumption, and controls, comprising individuals with three or fewer months of analgesic use. Sample sizes and the number of cases and controls for all GWASes can be found in Table S3.

Given that a complete recovery from major surgery can extend over six months [16], an ordinal score ranging from 1 to 3 was used. Score 1: analgesics use for three months or less; score 2 analgesics use between three to six months; score 3 analgesic use exceeding six months following their surgical procedure (see supplementary materials).

Genome-wide association analyses

We conducted two GWASes for CPSP development after major or minor surgery in the UK biobank. The main analysis was performed with the binary phenotype (cases with CPSP and controls without CPSP) combining results from major and minor surgeries in a meta-analysis. Major and minor surgeries were not directly combined in one analysis as the genetic background between these two might be different. Next we performed an analysis based on the ordinal phenotype (see above), as an exploratory analysis. For validation purposes a meta-analysis combining our GWAS meta-analysis with a published paper [14] on CPSP GWAS meta-analysis was performed. In the published study, patients undergoing hysterectomy, mastectomy, abdominal surgery, hernia repair, or knee surgery were recruited and genotyped,

and post-surgical pain was assessed 3–6 months in a Numeric Rating Scale (NRS, 0-10 scale) after surgery.

Statistical power was calculated for the main GWAS with the following parameters: 2,923 cases and 93,008 controls, significance level as 2.5×10^{-8} (corrected for multiple testing, the same test for binary phenotype, ordinal phenotype), CPSP prevalence of 0.03 (based on the observed prevalence in the participants included in the analysis), the minimum effect allele frequency of 0.1, and the minimum effect allele relative risk of 1.3.

Seven subtype GWASes were performed, including two based on surgery complexity (CPSP development after either major surgeries or minor surgeries) and five GWASes based on different surgery site. Details of the GWAS meta-analysis, subtype GWASes, and the post-GWAS analyses can be found in the supplementary methods. The number of selected genetic principal components was based on a scree plot (Figure S1).

Validation of significant loci

For GWAS-identified loci, the lead SNPs are the most statistically significant SNPs within each locus. Independent SNPs are those in linkage disequilibrium (LD, with $r^2 > 0.6$) with the lead SNP and that remain statistically significant after conditioning on the lead SNPs. A robustness analysis for suggestively significant loci ($P < 1 \times 10^{-6}$) was performed by splitting the main GWAS datasets into two equally sized subsets five times and then comparing the single variant results within these subsets for validation.

To determine whether the identified loci in this study were also reported in a published GWAS on CPSP, candidate SNPs (in LD $r^2 > 0.6$) with suggestively significant independent SNPs in our study were examined in that study [14]. Additionally, to compare our results with the published study on CPSP, we use PRS to investigate whether our GWAS meta-analysis results can predict CPSP development in their study.

To validate previous published results on CPSP we checked p-values for three variants that showed statistically significant association with CPSP in two systematic reviews [10, 11] in our dataset. In addition, significant results from two earlier published GWAS studies [12, 14] were checked. In the introduction, we describe three previously published GWASes. We did not include the analysis of van Reij *et al*. [15] as this dataset was also included in the paper of Parisien *et al*. [14].

Risk prediction by polygenic risk score

To predict an individual's genetic predisposition to CPSP development across major and minor surgeries, we constructed regression models using GWAS results after major surgeries to predict CPSP development after minor surgeries. Typically, a PRS is built from the GWAS results, where an individual's genetic risk is the sum of all their risk alleles weighted by the significance of the corresponding allele [17]. Two models were built: the null model, which included only GWAS covariates as predictive factors, and the PRS model, which incorporated PRS and GWAS covariates. The variance explained by the PRS model subtracted from the variance explained by the null model is the variance explained by the PRS. The PRS were generated utilizing the LDpred2 algorithm with the 'auto' option [18]. This option facilitates direct estimation of model parameters from available data without external training data.

The same approach was applied to predict the risk of self-reported CPSP (Data-Field ID: 120005) within the UKB. To avoid sample overlap, individuals included in the GWAS were excluded from subjects with self-reported CPSP.

Results

Demographics and phenotype validation

After QC procedures, we identified 26,670 subjects with major surgeries and 69,261 subjects with minor surgeries within the UKB dataset (Figure 2). Demographics of the combined dataset (major and minor surgeries) is available in Table 1. Pre-existing pain before surgery was similar between cases and controls indicated by prescription record numbers before surgery. In line with our selection criteria, cases consumed significantly more analgesics than controls after surgery ($P < 0.0001$) (Figure S2).

All tested covariates were significantly different between cases and controls and are incorporated as covariates in the GWAS. In addition, the median surgery record numbers (Figure S2 A) were similar between cases and controls, indicating no potential influence of surgery complexity on our phenotype definition. Besides our main GWAS analysis combining major and minor surgeries in a meta-analysis, an ordinal analysis and an analysis combining the data with a recent publication, we also performed several sub-type analyses.

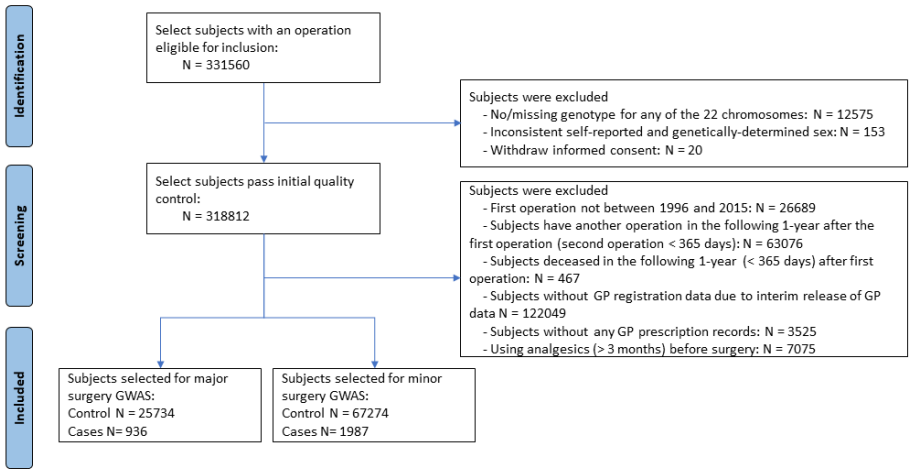

Figure 2. Flowchart of patient selection for chronic postsurgical pain.

Table 1. Demographic of participants without CPSP (control) and with CPSP (cases) after selected surgeries in the UK biobank.

	Controls	Cases	P value
N	93008	2923	
Females (%)	52228 (56.15%)	1495 (51.15%)	P < 0.0001
Age (years)	52.6 (9.45)	57.6 (8.32)	P < 0.0001
BMI (kg × m-2)	27.48 (4.733)	28.71 (5.054)	P < 0.0001
Types of surgery			P < 0.0001
Visceral	61267 (97.57)	1529 (2.43)	
Musculoskeletal	14244 (96.00)	593 (4.00)	
Otorhinolaryngology and eye	8120 (98.02)	164 (1.98)	
Nerves	3116 (93.86)	204 (6.14)	
Multiple sites *	3238 (92.49)	263 (7.51)	
Vascular	3023 (94.68)	170 (5.32)	
Opioid user	44325 (47.66%)	2418 (82.72%)	
Median opioid prescription number (SD) #	3 (28.32)	12 (63.29)	
Median analgesic prescription numbers (SD)	0 (0.71)	7 (4.76)	
Median analgesic prescription months (SD)	0 (0.60)	5 (1.84)	

* Subjects with more than one surgery subtype are classified as multiple sites. # The median of opioid prescription number is calculated based on participants that used opioids (opioid users). Age and BMI (body mass index) are presented as mean ± standard deviation. Types of surgery is presented as the count and percentage of controls or cases in each surgery types. Opioid user is presented as the count and percentage of opioid user in cases and controls, respectively. The opioid/analgesic prescription number is present as median (SD) as the distribution is highly skewed. SD, standard deviation.

Genome-wide association analysis

In the meta-analysis combining the GWAS after major and minor surgeries (main GWAS), no inflation was observed in the results (Figure S3 C). A single locus on chromosome 4 reached genome-wide significance (Figure S4 C). Within this locus, the most significant SNP was rs17298280 in the intronic region of the *GLRA3* gene (P-value = 2.17×10^{-9}). The minor allele (G) frequency in case and controls are 0.163 and 0.193, respectively. Within the same locus, an additional independent SNP remained statistically significant after conditioning on the lead SNP. Table 2 summarizes the lead SNPs within each locus that surpassed the suggestively significant threshold ($P < 1 \times 10^{-6}$). Ordinal GWAS results were consistent with the GWAS meta-analysis (Table S4, Figure S5), with an additional locus (rs140330443 within *CRAMP1L*) reaching genome-wide significance.

In the meta-analysis encompassing GWAS after major surgeries, GWAS after minor surgeries, and the published CPSP study [14], among all suggestively significant SNPs, only two SNPs (rs17298280, rs12143186) were genotyped in our subjects and all cohorts from the published CPSP study. The SNP rs17298280 remained the most significant locus ($P = 2.19 \times 10^{-09}$, Figure S6). The C allele of this SNP positively associated with CPSP in the GWAS after major surgeries, GWAS after minor surgeries, and two cohorts from the published CPSP study, but showed negative association in the remaining cohorts of the published study. Lead SNPs reaching suggestive significance in this meta-analysis are presented in Table S5.

Power and heritability analysis

The power for the meta-analysis of GWAS after major and minor surgeries was 0.693. The liability scale heritability for the GWAS after major surgeries was 0.1202 ± 0.1170, and for GWAS after minor surgeries is 0.0091 ± 0.0617. However, the heritability for the main GWAS (combining the GWAS after major and minor surgeries) was unmeasurable (below zero).

Functional annotation of SNPs

All variants in LD with the lead SNPs were non-coding variants (Table S6). In the genome-wide significant locus (*GLRA3*), rs11133053 had the highest CADD score of 11.41. Within the same locus, two intronic SNPs (rs144351495, rs17298280) were associated with active enhancers or promoters in multiple tissues, indicating potential regulatory functions. Rs17298280 also had a promising Regulome DB score (2a) for a potential regulatory SNP.

Table 2. Overview of the lead SNPs passing the suggestive significance level in the GWAS meta-analysis on chronic postsurgical pain.

SNP	CHR:POS	Effect allele	MAF	BETA (SE)	Location	Nearest_ Gene	Direction	P	HetPVal	Major GWAS P	Minor GWAS P
rs17298280	**4:175634898**	**C**	**0.207**	**0.0059 (0.0010)**	**intronic**	***GLRA3***	**++**	**2.17E-09**	**2.63E-01**	**8.10E-05**	**2.86E-06**
rs184832856	2:139555831	A	0.006	-0.0256 (0.0049)	intergenic	*NXPH2*	--	1.64E-07	5.55E-01	2.93E-02	1.35E-06
rs140330443	16:1672582	A	0.005	0.0240 (0.0046)	intronic	*CRAMP1L*	++	1.75E-07	5.01E-03	3.49E-07	2.06E-03
rs186819635	1:95071179	A	0.003	0.0252 (0.0049)	intergenic	*RP11-86H7.6*	++	2.70E-07	2.74E-01	1.09E-01	4.63E-07
rs12143186	1:68737833	T	0.111	-0.0062 (0.0012)	intergenic	*COX6B1P7*	--	3.20E-07	3.88E-02	1.43E-05	5.84E-04
rs10032594	4:175645417	C	0.335	-0.0042 (0.0008)	intronic	*GLRA3*	--	4.08E-07	8.47E-01	1.90E-02	6.01E-06
rs145636748	1:95059856	A	0.008	0.0197 (0.0039)	intergenic	*RP11-86H7.6*	++	5.46E-07	8.25E-01	8.57E-03	1.69E-05
rs117130005	17:19604614	T	0.017	0.0173 (0.0035)	intronic	*SLC47A2*	++	6.26E-07	5.16E-01	2.63E-03	4.94E-05
rs2160419	13:72314346	A	0.014	-0.0182 (0.0037)	intronic	*DACH1*	--	7.70E-07	9.05E-01	1.66E-02	1.32E-05
rs138470454	10:14101741	C	0.007	0.0247 (0.0050)	intronic	*FRMD4A*	++	9.75E-07	6.02E-01	4.59E-02	5.82E-06

Bold font indicates the SNP that passed the genome-wide significant threshold (5×10^{-8}). CHR:POS coordination of the SNP. MAF, Minor allele frequency, which is obtained in FUMA based on the 1000 Genomes Project Phase 3 data. BETA (SE) effect size and standard error. HetPVal, heterogeneity P- value.

The pleiotropic effects of lead SNPs in the meta-analysis were evaluated in the GWAS Atlas and GWAS Catalog. A list of associated traits that passed multiple testing thresholds is provided in Table S7 and Table S8. In the GWAS Atlas, 79 traits surpassed the significance threshold, with a focus on psychiatric and neurological traits. Notably, depression emerged as the most significantly associated trait with the lead SNP rs140330443 (P-value 4.26 x 10^{-8}). Other phenotypically correlated traits were identified, such as posttraumatic stress disorder, alcoholic drinks, BMI, and sitting height. Furthermore, in the GWAS Catalog, red blood cell count was identified as an associated trait for lead SNP rs12143186.

Gene mapping and gene-based analysis

After mapping GWAS candidate SNPs to genes, we identified 42 genes (Table 3). Thirteen genes were mapped based on genomic location, six were identified through cis-expression quantitative trait locus (cis-eQTL) mapping, and 29 were annotated by SNPs located within 3D chromatin interaction regions. Eight genes were identified through at least two of these mapping strategies. Fourteen genes were related to pain or relevant neurological functions, based on PubMed or Genecards.

Gene-based association analysis in MAGMA was performed for 19296 genes (Table S9). However, no statistically significant associations were identified (Bonferroni-corrected P-value 2.59 x 10^{-6}).

Validation analysis

In our robustness analysis, eight of the ten variants in the meta-analysis successfully passed the validation with nominal significance ($P < 0.05$) in all five iterations (Table S10).

Candidate SNPs from our meta-analysis including major and minor surgeries were checked in the published CPSP study [14], none of these SNPs passed the Bonferroni-corrected p-value threshold (0.05/31). One SNP (rs62066262) passed the nominal significance threshold but was only genotyped in one cohort (Table S11).

In the validation analysis using meta GWAS results to predict CPSP in the published CPSP study based on a polygenic risk score, the variance explained by the null model (with all GWAS covariates) was 8.23%. After introducing the PRS into the model, the explained variance only increased by 0.000144%. Utilizing only the GWAS after major surgeries results for the PRS validation analysis, produced comparable findings (data not shown).

Table 3. Genes mapped by SNPs in LD (r2 > 0.6) with lead SNPs in FUMA.

				Methods used for gene mapping				
Lead SNPs	**Gene Symbol**	**chr**	**posMapSNPs**	**eqtlMapSNPs**	**eqtlMapminP**	**eqtlMapminQ**	**ciMap**	**minGwasP**
rs17298280	*ADAM29*	4	0	NA	NA	NA	Yes	2.55E-09
	GLRA3 #	4	9	PsychENCODE_eQTLs	2.63E-04	1.94E-02	Yes	2.17E-09
rs117130005	*ALDH3A2* *	17	0	NA	NA	NA	Yes	6.26E-07
	SLC47A2	17	2	NA	NA	NA	No	6.26E-07
	ULK2 *	17	1	GTEx/v8/Cells_Cultured_fibroblasts:GTEx/v8/Skin_Sun_Exposed_Lower_leg	2.01E-05	3.44E-10	No	2.60E-06
	AKAP10 *	17	0	eQTLGen_cis_eQTLs	4.13E-12	0	No	2.60E-06
	LGALS9B	17	0	eQTLGen_cis_eQTLs	8.55E-07	2.70E-03	No	4.76E-05
rs12143186	*WLS* #	1	0	eQTLGen_cis_eQTLs	6.21E-14	0	No	1.71E-05
rs138470454	*FRMD4A* *	10	1	NA	NA	NA	No	9.75E-07
	HSPA14	10	0	NA	NA	NA	Yes	9.75E-07
rs140330443	*CRAMP1L*	16	1	NA	NA	NA	No	1.75E-07
	LA16c-431H6.6	16	1	NA	NA	NA	No	1.75E-07
	TSR3	16	0	NA	NA	NA	Yes	NA
	GNPTG	16	0	NA	NA	NA	Yes	NA
	HN1L	16	0	NA	NA	NA	Yes	NA
	MAPK8IP3 *	16	0	NA	NA	NA	Yes	NA
	HAGH	16	1	NA	NA	NA	No	NA
	FAHD1	16	2	NA	NA	NA	No	NA
	MEIOB	16	2	NA	NA	NA	Yes	NA
	HS3ST6 #	16	0	NA	NA	NA	Yes	NA

Table 3. Continued

			Methods used for gene mapping					
Lead SNPs	**Gene Symbol**	**chr**	**posMapSNPs**	**eqtlMapSNPs**	**eqtlMapminP**	**eqtlMapminQ**	**ciMap**	**minGwasP**
	MSRB1 *	16	0	NA	NA	NA	Yes	NA
	RPL3L	16	0	NA	NA	NA	Yes	NA
	NDUFB10	16	0	NA	NA	NA	Yes	NA
	RPS2	16	0	NA	NA	NA	Yes	NA
	RNF151	16	0	NA	NA	NA	Yes	NA
	TBL3	16	0	NA	NA	NA	Yes	NA
	NOXO1	16	0	NA	NA	NA	Yes	NA
	AC005606.1	16	0	NA	NA	NA	Yes	NA
	GFER	16	0	NA	NA	NA	Yes	NA
	SYNGR3	16	0	NA	NA	NA	Yes	NA
	NTN3 #	16	0	NA	NA	NA	Yes	NA
	*TBC1D24 **	16	0	NA	NA	NA	Yes	NA
	PRSS33	16	0	NA	NA	NA	Yes	NA
rs145636748; rs145636748: rs186819635	*F3 **	1	1	NA	NA	NA	Yes	2.70E-07
rs184832856	*TMED5*	1	0	NA	NA	NA	Yes	1.70E-03
	CCDC18	1	0	NA	NA	NA	Yes	1.70E-03
	ARHGAP29	1	1	NA	NA	NA	No	1.70E-03
	TMEM56	1	0	NA	NA	NA	Yes	1.70E-03
	RWDD3 #	1	0	NA	NA	NA	Yes	1.70E-03

Table 3. Continued

				Methods used for gene mapping				
Lead SNPs	**Gene Symbol**	**chr**	**posMapSNPs**	**eqtlMapSNPs**	**eqtlMapminP**	**eqtlMapminQ**	**ciMap**	**minGwasP**
rs186819635; rs145636748	*ABCD3*	1	1	eQTLGen_cis_eQTLs	2.38E-08	1.15E-04	No	1.51E-04
rs184832856	*HNMT #*	2	0	NA	NA	NA	Yes	1.64E-07
rs2160419	*DACH1*	13	3	NA	NA	NA	No	7.70E-07

posMapSNPs, the number of positional mapping SNPs to this gene; eqtlMapSNPs, the number of eQTL mapping SNPs to this gene; eqtlMapminP, the minimum eQTL P-value of mapped SNPs; eqtlMapminQ, the minimum eQTL FDR of mapped SNPs; ciMap, "Yes" indicates chromatin interaction mapping; minGwasP, the minimum P-value of mapped SNPs. NA, not applicable.

this gene has been reported to be associated with pain as reported in PubMed.

* this gene has been reported to be associated with neurodevelopmental disorders or related neurological functions in GeneCards

Three SNPs reported to be associated with chronic pain from two published systematic reviews were checked in our dataset, none of the three SNPs showed nominal statistical significance (Table S12). Genome-wide significant variants identified by previously performed GWASes were checked as well. Only one of the four variants (rs114837251, near *MAP9/GUCY1A1/GUCY1B1*) showed nominal significance in our study (Table S13).

Risk prediction

In the PRS model using the GWAS after major surgeries results to predict CPSP development after minor surgeries, the null model (including only GWAS covariates) explained 9.85% of the variance. Upon introducing the PRS score, there was a negligible increase of 0.0024% in explained variance.

In the PRS model using the meta-analysis GWAS results to predict self-reported CPSP in the UK Biobank, the null model accounted for 0.3235% of the variance, and the PRS score contributed to a slight increase of 0.01865% in explained variance. We conducted the same analysis using the GWAS after major surgeries results, yielding comparable results (data not shown).

Subtype GWASes and Genetic correlations

Subtype GWASes were performed based on surgery complexity and surgery site, and results can be found in Figure S4, Figure S7, Table S14, and Table S15.

CPSP development after different subtype surgeries showed non-significant moderate correlation coefficients. The same trend was observed for other phenotypically correlated traits (Table S16, Table S17, Figure S8). More information these two analyses can be found in the supplementary materials.

Discussion

We identified one genome-wide significant locus (*GLRA3*) associated with chronic postsurgical pain using the UKB in the meta-analysis of major and minor surgeries. This SNP remains genome-wide significant in the meta-analysis, which integrates data from our genome-wide association results (both major surgeries and minor surgeries) and a published CPSP study [14].

The genome-wide significant locus is mapped to the *GLRA3* gene, encoding a protein (GlyRα3) of the glycine receptor subfamily, which are widely distributed

throughout the central nervous system. GlyRα3 plays an important role in down-regulation of neuronal excitability and contributes to generation of inhibitory postsynaptic current. The role of GlyRα3 varies by the nature of pain. In an inflammatory pain mouse model, elevated COX2 led to the spinal release of PGE2, which inactivated GlyRα3 via phosphorylation. This GlyRα3-mediated inactivation of inhibitory neurons contributes to the central mechanisms of chronic inflammatory pain [19, 20]. By contrast, GlyRα3 appears to have little or no role in several neuropathic pain models [21, 22]. For instance, in partial sciatic nerve ligation model, Glra3$^{-/-}$ mice show normal pain behavior, mechanical allodynia, and thermal sensitivities [20]. This all suggests that inflammatory pain might be an important underlying mechanism of CPSP.

Genes identified through suggestively significant loci may involve in pain-related processes, as evidenced by gene expression studies. Notable examples include WLS, exhibiting high expression in chronic neuropathic pain patients [23]; RWDD3 downregulated following sciatic nerve ligation [24]; reduced expression of Netrin-3 (NTN3) is strongly associated with the severity of diabetic neuropathic pain in a diabetic mouse model [25]; HNMT has been linked to the morphine dosage requirements in cancer pain patients [26]. However, transcriptome-wide association analysis to find gene expression associated with CPSP by utilizing S-PrediXcan in specific tissues showed no significant association (after Bonferroni correction) for the genes identified (data not shown). Furthermore, although some other genes are not directly implicated in pain, they are associated with other neurological functions that might contribute to pain development. For instance, HS3ST6 is involved in hereditary angioedema, characterized by acute episodic cutaneous or submucosal angioedema, often accompanied by abdominal pain [27].

In the validation analysis of our identified SNPs, only one SNP (rs62066262) was replicated in a previously published study on CPSP [14] with nominal significance. In the validation of previously identified SNPs in our study, only one SNP (rs114837251) approached the nominal significance threshold (P = 0.05059) in our meta-analysis. It is important to note that our study is not a perfect replication of the previous study and vice versa, given several key distinctions. First, many SNPs in our study were not genotyped in their study, such as rs2160419, which was only genotyped in one out of six cohorts. Moreover, the phenotype definition was different between the two studies. In the published study chronic postsurgical pain was assessed directly via a numeric rating scale or focusing acute pain within 48 hours after surgery, our study utilized a surrogate pain phenotype based on analgesic consumption. However, the previously published study emphasized the

potential role of the adaptive immune system in CPSP development, aligning with our findings that implicate inflammatory pain as a significant component of CPSP.

The pleiotropic effects analysis of the lead SNPs reveals traits known to be linked with CPSP development, such as psychiatric traits (depression, posttraumatic stress disorder, and anxiety) and BMI [7, 28]. Additionally, the GWAS catalog indicates SNPs with pleiotropic effects of red blood cell count. This connection between red blood cell counts and pain is intriguing as red cell distribution width has been linked to chronicity in nonspecific low back pain [29].

The low estimated SNP heritability in our study reflects the well-known "missing heritability" problem in GWASes. This phenomenon can be attributed to SNP-based heritability calculations based on a subset of common genetic variants. As a result, the heritability estimation tends to be lower than those observed in twin studies. Several factors contribute to this disparity, including that the genotyped SNPs in GWASes are not in complete linkage disequilibrium with the causal variants; non-additive effects, rare variants, and structural variants often are undetected in SNP-based heritability assessments.

Although PRS analysis in our study lacks power due to insignificant GWAS results, we should not overlook the potential of using PRS as a potential predictor of CPSP. [30-32]. There is a growing movement to incorporate PRS in risk prediction for clinical care [33, 34]. In the context of CPSP, risk prediction is in its initial stages. Two studies explored building a PRS model for CPSP [4, 35]. The models integrated PRS into risk assessment exhibit a higher predictive accuracy for CPSP than non-genetic models [4]. To replicate and validate these findings, further studies need to include large-scale cohorts to construct robust PRS models, which underscore the need for large-scale GWASes efforts. An example of such an initiative is the PPG cohort at our research center [36].

One of the strengths of our study is the inclusion of different types of surgeries, leading to a substantial sample size. This large sample size allowed us to investigate the common genetic underpinnings of CPSP across various surgical procedures. However, it is crucial to acknowledge the limitations of our study. Instead of directly measuring CPSP, we employed a proxy method relying on analgesic prescription records to define chronic pain. The advantage of this phenotype definition is that it captures more severe pain symptoms necessitating medication as indicated by the relatively low prevalence of CPSP in our study. We cross checked our phenotype definition with the self-reported CPSP in the UKB (after removing subjects

without prescriptions) as shown in Table S18. The agreement between these two classifications was compared by unweighted Cohen's Kappa (κ = 0.02), which only shows slight agreement.. Subjects included as controls in our analysis indicated to suffer from CPSP in the self-reported data, this can be explained by two reasons. First, our definition did not include all surgeries and only considered the first surgery for analysis. Thus, subjects may develop CPSP from subsequent surgeries. Second, evidence indicates that there are patients experiencing significant pain but opting not to pursue treatment [37]. It might happen that subjects with CPSP do not always seek prescriptions. Subjects without CPSP in the self-reported data but marked as cases in our phenotype definition can be explained by several factors: subject having high pain tolerance, effective pain control by prescribed analgesics, and analgesics prescribed for conditions other than chronic postsurgical pain. Additionally, as the UKB team mentioned, the chronicity and location of CPSP are not well-documented in their questionnaire and should not be seen as the standard for examining CPSP and advices not to use this phenotype for GWASes. Therefore, we did not use this phenotype in our analysis but used analgesic consumption one year after an operation as phenotype. Additionally, CPSP development could be a combination of genuine CPSP and a suboptimal response to analgesics. Despite these limitations, the substantial sample size included in our study potentially mitigates this issue. Another limitation is that our study lacks statistical power, as it did not meet the commonly accepted threshold of 0.8. Although our sample size is relatively large (N = 95,931), complex traits require even larger sample sizes to fully elucidate the genetic architecture of such traits, as they are influenced by numerous common variants with very small effect sizes. For example, the estimated heritability of height in studies before 2010 was only 5%, much lower than the pedigree-based heritability estimate of 80%, despite those studies being considered large at the time (n = 1,000 to 10,000) [38]. A closer estimation to the pedigree-based heritability was achieved in a more recent GWAS meta-analysis with sample size exceeding 250,000 for height [39]. Thus, while our sample size is substantial, it is still relatively small for precise heritability estimation.

Our study provides a foundation by offering summary statistics for future investigations into the function of these risk variants and the mechanisms underlying CPSP. In subsequent research, we advocate for conducting GWASes with a substantial sample size and consistent phenotype definition, as this will be instrumental in advancing risk identification and tailoring personalized treatments for individuals at risk of CPSP.

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Data availability

Summary statistics of the primary analysis are available at DANS archive (https://doi.org/10.17026/LS/486TJZ). Gene mapping results are available at FUMA (https://fuma.ctglab.nl/snp2gene/266351).

Author contributions

SL analyzed the data and prepared the manuscript. LD and MP provided the data for the PRS analysis. RLMvB, MKT, RPGtB, and KCPV contributed to the phenotype definition. MJHC conceptualized the study, supervised the overall project. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest disclosures

The authors declare that they have no conflicts of interest.

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Supplementary methods

Study cohorts

UK biobank is a prospective cohort comprising individuals recruited from the general population aged 40 to 69 across the United Kingdom (UK), as described in more detail elsewhere [1]. The phenotype definition was based on the primary care (general practitioners, GP) data within UKB, a longitudinal dataset encompassing structured diagnoses and prescription information. Notably, at the time of our analysis (2023 July), the interim release of GP data covered approximately 45% of all UKB participants (all provided informed consent as part of the UKB).

Note of phenotype definition

The surgery complexity information was sourced from the Clinical Coding and Schedule Development Group [2] and the clinician's expertise. In this study, surgeries categorized as major, xmajor, and complex were collectively classified as major, while minor and intermediate were grouped as minor (Table S1).

Notably, the phenotype definition did not completely follow the IASP CPSP definition criteria requiring consecutive prescription records. As the analgesic prescription records were used as a proxy to CPSP, this approach was not ideal to perfectly capture the continuous presence of pain because it depended on the total prescriptions received by patients and the frequency of medication delivery.

Oridinal phenotype definition

Specifically, an ordinal score ranging from 1 to 3 was assigned: a score of 1 was assigned to individuals using analgesics for three months or less ($n \leq 3$), a score of 2 denoted those using analgesics for a duration between three to six months ($3 < n \leq 6$), and a score of 3 was assigned for those with analgesic use exceeding six months ($n > 6$) following their surgical procedure. The ordinal phenotype GWAS was conducted only in a single analysis that included both major and minor surgeries.

Heritability analysis

Liability-scale (SNP-based) heritability was calculated by using LDSC [3] with Europeans from the 1000 Genomes Project [4] as the LD reference panel. The major histocompatibility complex region (chr6: 26–34 Mb) was excluded. This calculation was based on several assumptions: a population prevalence of 0.10 for individuals who have undergone surgeries in general, a CPSP prevalence of 0.04 for subjects with major surgeries, and a CPSP prevalence of 0.03 for subjects with minor surgeries and in the meta-analysis.

GWAS Routine Sample and genotype QC

Subjects meeting the following criteria were included for analysis: Subjects with consistent self-reported and genetically determined sex, genetically determined white British ancestry, without putative sex-chromosome aneuploidy, not considered outliers due to missing heterozygosity, individual call rate > 90%, all relevant covariates are available.

Markers on autosomes that meet the following criteria were included: SNPs with an imputation quality score (INFO scores) of greater than 0.8, Minor Allele Frequency (MAF) > 0.005, Hardy-Weinberg equilibrium (HWE) test P > 10^{-6}, Genotyping call rate > 95%.

Subtype Genome-wide association analyses

We conducted two subtype GWASes based on surgery complexity, i.e., GWAS on CPSP development after major or minor surgery. Both analyses followed the same analytical procedures using the linear mixed model function in GCTA [5]. In GCTA, MLM-based tool (fastGWA) controls for population stratification by principal components and for relatedness by a sparse genetic relationship matrix [5]. The same set of covariates applied to all GWASes models, including age at time of surgery, gender, assessment center, genotyping array type, the first five genetic principal components, and surgery types. The number of selected genetic principal components was based on a scree plot (Figure S1). Covariates were compared using t-tests for continuous variables and chi-square tests for categorical variables across different groups. Significance thresholds for GWAS were set at commonly accepted levels: $P < 5 \times 10^{-8}$ for genome-wide significance [6] and a suggestively significant threshold of $1 \times 10^{-6} < P < 5 \times 10^{-8}$ [7].

We conducted another five subtype GWASes based on surgery type, to explore the genetic correlations between CPSP development for various surgeries. These subtypes encompassed visceral surgeries, musculoskeletal surgeries, nervous surgeries, otorhinolaryngology and eye surgeries, and vascular surgeries, which could be major and minor surgeries. Notably, individuals who undergoned two different types of surgery on the same day (such as both visceral and musculoskeletal surgeries) will be included in our GWAS meta-analysis (see below) but excluded from this subtype-specific GWAS analysis. For this subtype-specific GWAS analysis, we focused on the binary phenotypes. These analyses followed the same analytical procedures as the subtype analyses based on surgery complexity mentioned above.

GWAS Meta-analysis

To explore if loci were potentially involved in CPSP development across major and minor surgeries, we conducted a meta-analysis using the fixed-effect inverse-variance weighted model in METAL, integrating the GWAS results on CPSP development after major and minor surgeries. Please note that there was not overlap for either cases or controls between GWAS on CPSP development after major surgeries and GWAS on CPSP development after minor surgeries as subjects were partitioned by their first major / minor surgery. The results of this meta-analysis will be used for subsequent post-GWAS analyses. The ordinal phenotype for the meta-analysis of GWAS on CPSP development after major or minor surgeries was analyzed with the ordinal regression in OrdinalGWAS [8]. Significance thresholds for GWAS meta-analysis of CPSP after major or minor surgeries were set at: $P < 1.7 \times 10^{-8}$ for genome-wide significance to account for the multiple testing correction (for binary and ordinal phenotype, and the meta-analysis mentioned below).

Additionally, to further enhance the statistical power to identify novel loci associated with CPSP and validate our GWAS findings, another meta-analysis was performed encompassing our GWAS on CPSP development after major surgeries, GWAS on CPSP development after minor surgeries, and a previously published GWAS on CPSP [9]. For the previously published CPSP study, summary statistics from the GWAS on binary CPSP outcomes were provided for each subcohort. We conducted a meta-analysis of CPSP after major surgery, CPSP after minor surgery, and the six subcohorts from the previously published study.

Functional annotation of SNPs

FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies) was used to identify lead SNPs and significant independent SNPs, which are SNPs in linkage disequilibrium (LD, with $r^2 > 0.6$) with the lead SNP and remain statistically significant after conditioning on the lead SNPs. To understand the SNPs' functions and identify potential regulatory SNPs, all SNPs in LD ($r^2 > 0.6$) with significant independent SNPs were annotated by Variant Effect Predictor, ANNOVAR, RegulomeDB (all within FUMA), and Haploreg.

Additionally, we explored potential pleiotropic effects associated with lead SNPs by querying the GWAS Catalog and GWAS Atlas. Traits that passed the Bonferroni correction threshold (0.05/10) in the original GWAS reported in the database are presented in the results section.

Gene mapping and gene-based analysis

SNPs in LD ($r^2 > 0.6$) with lead SNPs were mapped to genes through three approaches in FUMA: positional mapping, cis-expression quantitative trait locus (cis-eQTL) mapping, and open chromatin mapping. In the positional mapping strategy, we assessed whether SNPs fall within a gene region (10 kilobases window). In the eQTL mapping, SNPs were searched in the database for association with gene expression levels. The significance threshold is FDR < 0.05 for cis-eQTL mapping. Open chromatin mapping can identify chromatin interactions based on prior spatial interaction data, even if these interactions occurred over considerable physical distances. The significance threshold in open chromatin mapping was set at FDR $< 1x\ 10^{-6}$. Following the gene mapping process, we searched GeneCards and PubMed to investigate whether the candidate genes were functionally related to pain or neurological functions, providing further insights into their potential role in CPSP.

A gene-based analysis was conducted in MAGMA. SNPs were selected to map onto genes with a window size of 50 kb.Gene-based P-values were calculated based on the P-value of SNPs mapped to a specific gene. The significant threshold for gene analysis is set as 0.05/total number of genes included in the analysis (N=19296).

Genetic correlation

To investigate whether there are genetic confounders between phenotypic correlation with CPSP, genetic correlation analysis was performed: 1) between CPSP development after major and minor surgery, using GWAS on CPSP development after major surgeries and GWAS on CPSP development after minor surgeries results; 2) between CPSP development after different surgery types, utilizing the subtype-specific GWAS results mentioned earlier.

In addition, phenotypically correlated traits with CPSP were also investigated. A list of included traits can be found below. Given that the meta-analysis GWAS heritability result is unmeasurable, we utilized the GWAS on CPSP development after major surgeries results for genetic correlation with phenotypically correlated traits. The significance threshold for genetic correlation was set at 0.05 divided by the total number of tested correlations (0.05/15).

The genetic correlation of CPSP with other traits includes pain types experienced in the last month (headache, neck or shoulder pain, stomach or abdominal pain, hip pain, knee pain), ICD10 diagnosis of abdominal and pelvic pain (R10) as main or secondary diagnoses, ever vs. never smokers, and the use of opioids for personal consumption, which all are sourced from the GWAS Atlas. Additional traits from

relevant papers, including back pain [10]), neck/shoulder pain for 3+ months [11], knee pain for 3+ months [11], headaches for 3+ months [11], depression [12], and body mass index [13].

Self-reported CPSP in UKB

The self-reported CPSP data was obtained from the UK Biobank's Experience of Pain questionnaire (Data-Field 120005). Participants were provided with response options including "Yes," "No," "Do not know," and "Prefer not to answer." It is important to note that the information collected through this questionnaire are insufficient to conduct Genome-Wide Association Studies (GWAS) related to pain. As per the UKB "It is essential to carefully record the duration, location, intensity and quality of pain as well as the temporal relationship to predisposing factors and co-morbidities (such as sleep, anxiety and depression). Whilst UK Biobank gathered data on pain during the baseline assessment, the level of phenotyping is not sufficient to undertake any pain-related GWAS.

Supplementary results

Genome-wide association analysis

In the GWAS after major and minor surgeries analysis, no inflation was observed in the results, as evidenced by genomic control values of 1.00 and 1.01, respectively (Figure S3 for QQ-plot). No genome-wide significant hits were identified ($P < 5 \times 10\text{-}8$) in either of these analyses (Figure S4A, Figure S4B). Lead SNPs surpassing the suggestively significant threshold are presented in Table S14 and Table S15 for surgery subtypes based on complexity or sites, respectively (see Figure S4 and S7 for Manhattan plots).

Genetic correlation

Genetic correlation analysis between the GWAS after major and minor surgeries was not feasible due to the low heritability observed in the GWAS after minor surgeries.

Genetic correlation analysis between the GWAS after major surgeries and other phenotypically correlated traits, showed strongest regression coefficient (rg) with the published CPSP study [14]. Most chronic pain phenotypes displayed a positive genetic correlation, some exceptions are self-reported abdominal pain and abdominal pain as secondary diagnosis. Similarly, other phenotypically related traits, such as BMI and depression, also correlate positively. Important to note none of these genetic correlations reached statistical significance (Figure S8, Table S16).

No statistically significant genetic correlations were identified between subtype surgeries. However, rg values exhibited substantial magnitudes. Positive correlations were observed among musculoskeletal, vascular, and nerve subtypes, while negative correlations were noted with other surgery subtypes (Figure S8, Table S17).

Supplementary figures

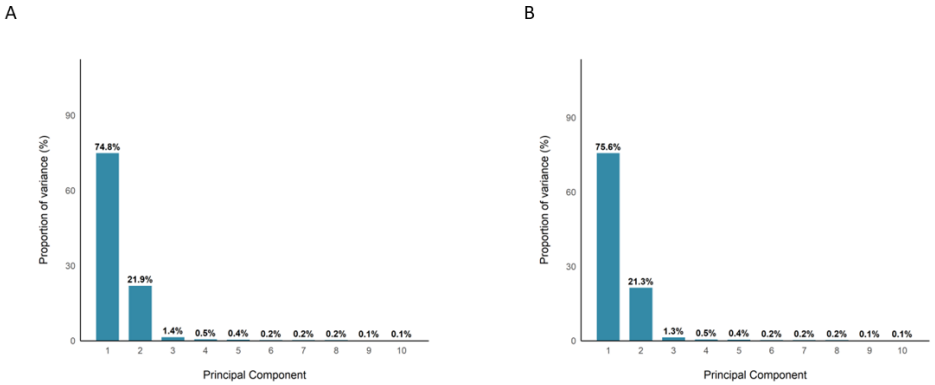

Figure S1. Percentage of variances explained by principal components in subjects with major (A) and minor (B) surgeries.

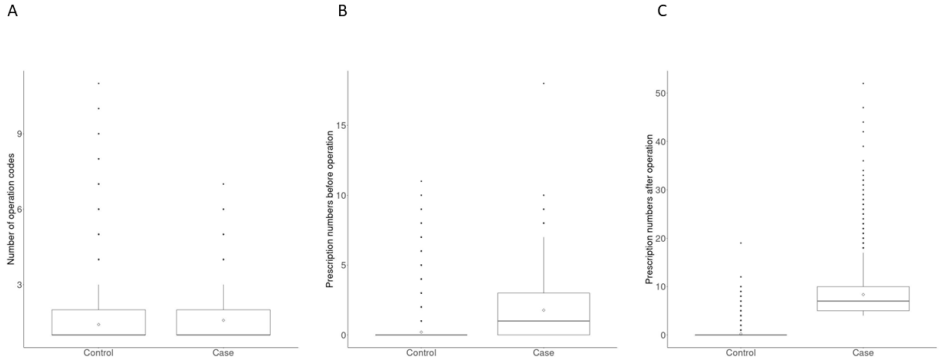

Figure S2. Number of operation codes (A), analgesic prescription numbers before (B) and after (C) surgeries in subjects included in the main GWAS on CPSP development after major and minor surgeries.

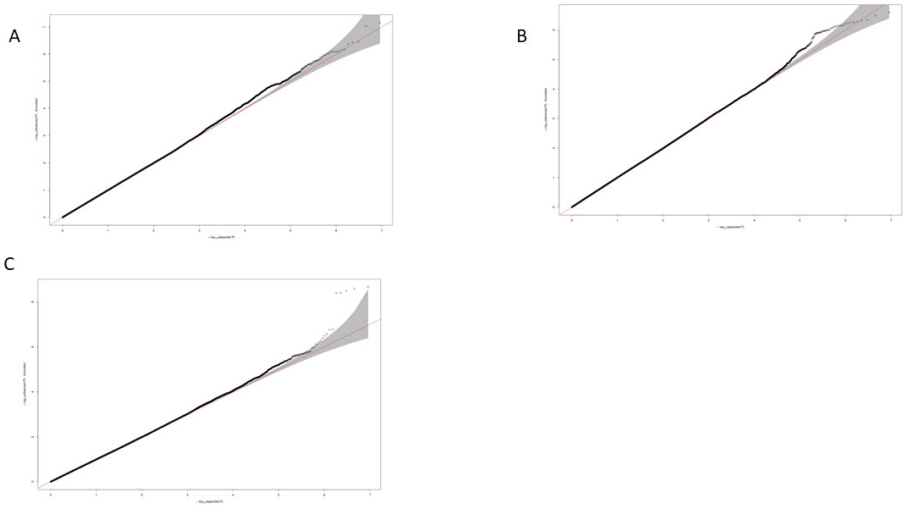

Figure S3. QQ plot of GWAS on chronic post-surgical pain in subjects undergone major (A), minor (B) and meta-analysis of major and minor surgeries (C).

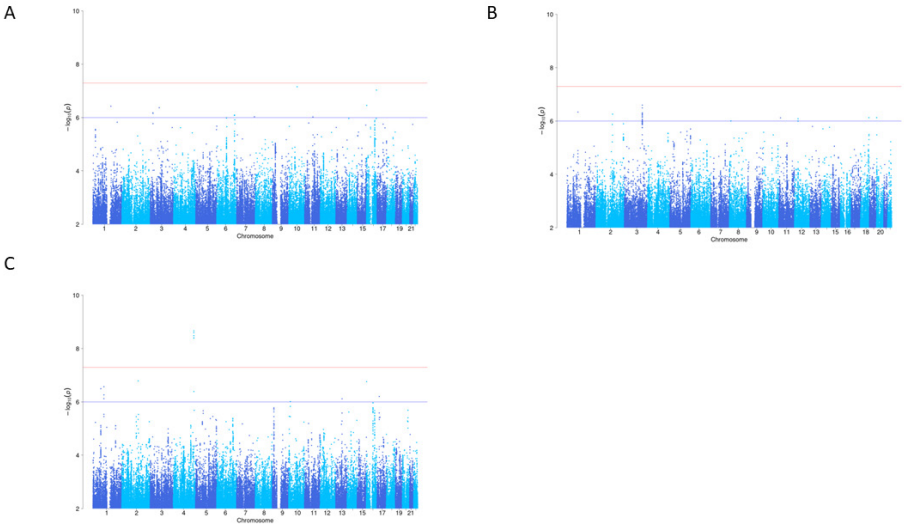

Figure S4. Manhattan plot of genome-wide association analysis on chronic postsurgical pain. (A) GWAS on CPSP after selected major surgeries. (B) GWAS on CPSP after selected minor surgeries. (C) Meta analysis of GWAS on CPSP after selected major and minor surgeries. The red line corresponds to the genome-wide significance threshold of 5×10^{-8}, whereas the blue indicates the suggestive threshold of 1×10^{-6}. GWAS, genome-wide association analysis; CPSP, chronic postsurgical pain.

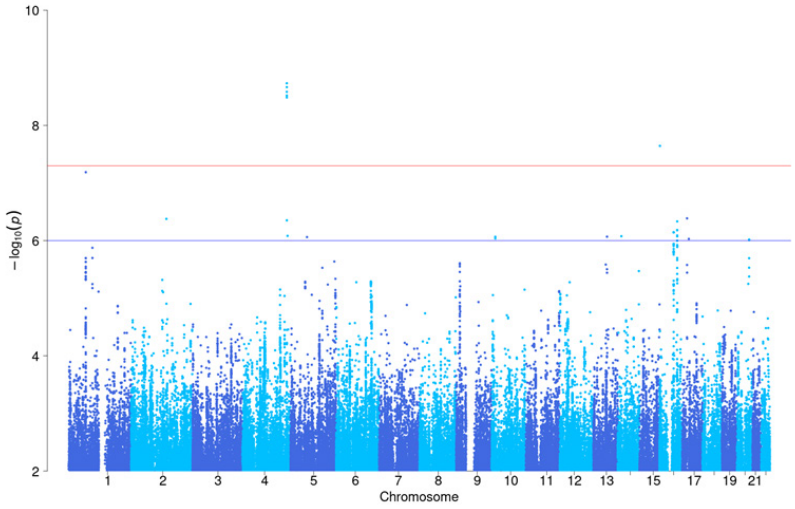

Figure S5. Manhattan plot of genome-wide association analysis on chronic postsurgical pain after selected major and minor surgeries using ordinal phenotype.

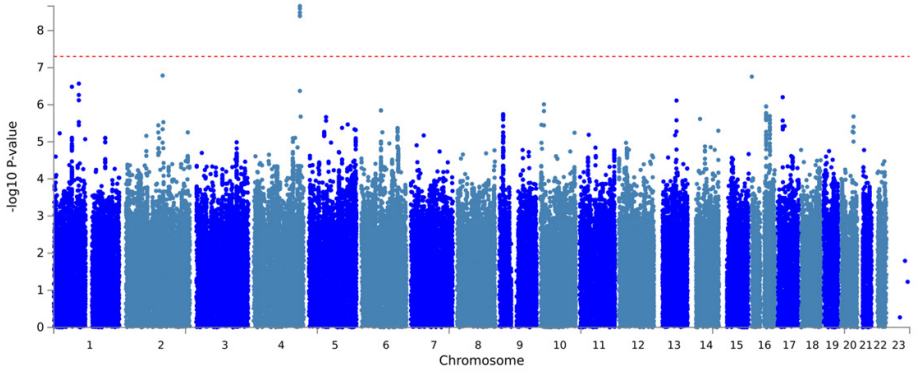

Figure S6. Meta-analysis of GWAS on CPSP development after major surgeries, GWAS on CPSP development after minor surgeries, and publish chronic postsurgical pain study.

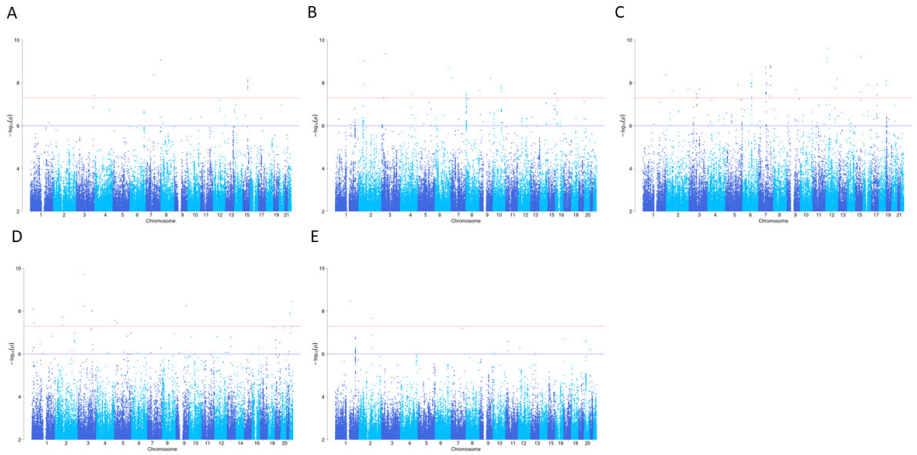

Figure S7. Subtype GWASes in subjects undergone musculoskeletal (A), nervous (B), otorhinolaryngology and eye surgeries (C), vascular (D), visceral surgery (E). GWAS, genome-wide association study.

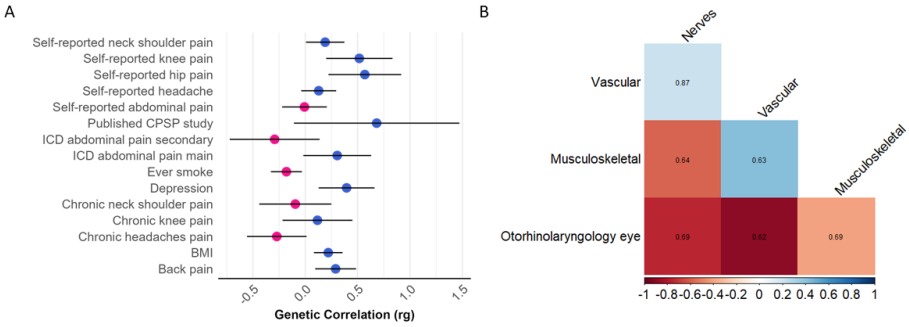

Figure S8. Genetic correlations between (A) CPSP and other traits. Pink indicates negative correlation, and blue indicates positive correlation. Bar indicates standard error. (B) Surgery subtypes within CPSP. The bottom color scale indicates the range from negative correlation to positive correlation. The number in each square indicates P-value of the correlation.

Supplementary tables

This section lists most of the supplementary tables. Table 1 is not included here due to their length but can be accessed online at the following link: https://github.com/lisongmiller/UKB_GWAS_CPSP_all_operations /supp_data

Table S1. Selected OPCS4 codes of operations. (This Table can be found online.)

Table S2. Chemical name of selected drugs.

BNF_chemical_name	Category
ALOXIPRIN	NSAID
LYSINE ASPIRIN	NSAID
ASPIRIN	NSAID
ASPIRIN & CAFFEINE	NSAID
ASPIRIN & PAPAVERETUM	NSAID
ASPIRIN & PARACETAMOL	NSAID
ASPIRIN COMBINED PREPARATIONS	NSAID
ACECLOFENAC	NSAID
ACEMETACIN	NSAID
ALFENTANIL HYDROCHLORIDE	OPIOID
AMITRIPTYLINE EMBONATE	TCA
AMITRIPTYLINE HYDROCHLORIDE	TCA
ASPIRIN,PARACETAMOL & CODEINE	OPIOID
ASPIRIN,PHENACETIN & CODEINE(CODEINE CO)	OPIOID
AZAPROPAZONE	NSAID
BUPRENORPHINE	OPIOID
BUPRENORPHINE HYDROCHLORIDE	OPIOID
BUTRIPTYLINE	TCA
CARBAMAZEPINE	ANTICONVULSANT
CELECOXIB	NSAID
CLONIDINE HYDROCHLORIDE	α2-agonist
CO-CODAMOL (CODEINE PHOS/PARACETAMOL)	OPIOID
CO-CODAPRIN (CODEINE PHOS/ASPIRIN)	OPIOID
CODEINE PHOSPHATE	OPIOID
CO-DYDRAMOL (DIHYDROCODEINE/PARACET)	OPIOID
CO-PROXAMOL (DEXTROPROP HCL/PARACET)	OPIOID
DEXIBUPROFEN	NSAID

Table S2. Continued

BNF_chemical_name	Category
DEXKETOPROFEN	NSAID
DEXTROMORAMIDE TARTRATE	OPIOID
DEXTROPROPOXYPHENE	OPIOID
DIAMORPHINE HYDROCHLORIDE (SYSTEMIC)	OPIOID
DIAMORPHINE HYDROCHLORIDE (TOP)	OPIOID
DICLOFENAC POTASSIUM	NSAID
DICLOFENAC SODIUM	NSAID
DIFLUNISAL	NSAID
DIHYDROCODEINE TARTRATE	OPIOID
DIPIPANONE HYDROCHLORIDE	OPIOID
DIPYRONE SODIUM	Metamizole
ESKETAMINE HYDROCHLORIDE	NMDA receptor antagonist
ETODOLAC	NSAID
ETORICOXIB	NSAID
FENBUFEN	NSAID
FENOPROFEN	NSAID
FENTANYL	OPIOID
FENTANYL CITRATE	OPIOID
FLURBIPROFEN	NSAID
GABAPENTIN	ANTICONVULSANT
GABAPENTIN (NEUROPATHIC PAIN)	ANTICONVULSANT
HYDROMORPHONE HYDROCHLORIDE	OPIOID
IBUPROFEN	NSAID
IBUPROFEN LYSINE	NSAID
IBUPROFEN SODIUM DIHYDRATE	NSAID
INDOMETACIN	NSAID
KETAMINE	NMDA receptor antagonist
KETOPROFEN	NSAID
KETOROLAC TROMETAMOL	NSAID
LORNOXICAM	NSAID
LUMIRACOXIB	NSAID
MEFENAMIC ACID	NSAID
MELOXICAM	NSAID
MEPTAZINOL HYDROCHLORIDE	OPIOID
METHADONE HYDROCHLORIDE	OPIOID
MORPHINE	OPIOID

Table S2. Continued

BNF_chemical_name	Category
MORPHINE ANHYDROUS	OPIOID
MORPHINE HYDROCHLORIDE	OPIOID
MORPHINE SULFATE	OPIOID
MORPHINE TARTRATE & CYCLIZINE TARTRATE	OPIOID
NABUMETONE	NSAID
NALBUPHINE HYDROCHLORIDE	OPIOID
NAPROXEN	NSAID
NAPROXEN SODIUM	NSAID
NEFOPAM HYDROCHLORIDE	NSAID
NIMESULIDE	NSAID
OXYCODONE	OPIOID
OXYCODONE HCL/NALOXONE HCL	OPIOID
OXYCODONE HYDROCHLORIDE	OPIOID
PAPAVERETUM	OPIOID
PARACETAMOL	NSAID
PARACETAMOL & CAFFEINE	NSAID
PARACETAMOL & CODEINE PHOSPHATE	OPIOID
PARACETAMOL & IBUPROFEN	NSAID
PARACETAMOL COMBINED PREPARATIONS	NSAID
PARECOXIB SODIUM	NSAID
PENTAZOCINE HYDROCHLORIDE	OPIOID
PENTAZOCINE LACTATE	OPIOID
PETHIDINE HYDROCHLORIDE	OPIOID
PHENAZOCINE HYDROBROMIDE	OPIOID
PHENYLBUTAZONE	NSAID
PIROXICAM	NSAID
POWDERED OPIUM	OPIOID
PREGABALIN	ANTICONVULSANT
REMIFENTANIL HYDROCHLORIDE	OPIOID
ROFECOXIB	NSAID
SODIUM SALICYLATE	NSAID
SULINDAC	NSAID
TAPENTADOL HYDROCHLORIDE	OPIOID
TENOXICAM	NSAID
TIAPROFENIC ACID	NSAID
TOLFENAMIC ACID	NSAID

Table S2. Continued

BNF_chemical_name	Category
TOLMETIN	NSAID
TRAMADOL HYDROCHLORIDE	OPIOID
VALDECOXIB	NSAID
KETAMINE HYDROCHLORIDE	ANTIDEPRESSANT
BENORILATE	NSAID
BUPIVACAINE HYDROCHLORIDE	ANAESTHETIC
BUPIVACAINE HYDROCHLORIDE & FENTANYL CIT	OPIOID
BUPRENORPH HCL/NALOXONE HCL	OPIOID
DESIPRAMINE HYDROCHLORIDE	TCA
DULOXETINE HYDROCHLORIDE	SSRI
FLUFENAMIC ACID	NSAID
LEVACETYLMETHADOL HYDROCHLORIDE	OPIOID
LEVOBUPIVACAINE HYDROCHLORIDE	Local Anesthetic
LEVORPHANOL TARTRATE	OPIOID
NORTRIPTYLINE	TCA
OXCARBAZEPINE	ANTICONVULSANT
OXYPHENBUTAZONE	NSAID
ROPIVACAINE HYDROCHLORIDE	ANAESTHETIC
VENLAFAXINE	SNRI

Table S3. Case and control numbers in different GWASes.

GWAS	Controls	Cases	Total
Selected major surgeries in the UK biobank	25734	936	26670
Selected minor surgeries in the UK biobank	67274	1987	69261
Meta analysis of GWAS on major with minor	93008	2923	95931
Meta analysis of GWAS on major with minor ordinal phenotype	N/A	N/A	95931 *
Meta analysis of GWAS on major with minor + published meta	N/A	N/A	97281 †
Subtype GWAS musculoskeletal surgeries	14244	593	14837
Subtype GWAS nervous surgeries	3116	204	3320
Subtype GWAS otorhinolaryngology and eye surgeries	8120	164	8284
Subtype GWAS vascular surgeries	3023	170	3193
Subtype GWAS visceral surgeries	61267	1529	62796

* Ordinal phenotype for time of analgesic use for three months or less ($n \leq 3$), between three to six months ($3 < n \leq 6$), exceeding six months ($n > 6$) are 93008, 2030, 893, respectively. † As we use the summary statistics from Marc Parisien et al., the exact case/controls numbers are not available.

Table S4. Lead SNPs passing the suggestive significance level in the GWAS on CPSP development after major or minor surgeries using ordinal chronic postsurgical pain phenotype.

SNP	CHR_POS	Effect_ allele	MAF	Location	Nearest_Gene	P
rs17298280	4:175634898	C	0.207	intronic	*GLRA3*	1.85E-09
rs140330443	16:1672582	G	0.005	intronic	*CRAMP1L*	2.27E-08
rs12143186	1:68737833	T	0.111	intergenic	*COX6B1P7*	6.48E-08
rs28733998	15:45016686	C	0.001	intergenic	*TRIM69*	1.13E-07
rs117130005	17:19604614	C	0.017	intronic	*SLC47A2*	4.10E-07
rs184832856	2:139555831	A	0.006	intergenic	*NXPH2*	4.19E-07
rs10032594	4:175645417	C	0.335	intronic	*GLRA3*	4.44E-07
rs141868733	16:70004702	A	0.033	intergenic	*PDXDC2P*	4.63E-07
rs13339005	16:55934159	G	0.163	intronic	*CES5A*	7.12E-07
rs186819635	1:95071179	C	0.003	intergenic	*RP11-86H7.6*	7.82E-07
rs149599100	4:178428709	A	0.03	ncRNA_intronic	*RP11-130F10.1*	8.26E-07
rs118008920	14:35450086	G	0.007	ncRNA_intronic	*RP11-85K15.2*	8.38E-07
rs2160419	13:72314346	A	0.014	intronic	*DACH1*	8.53E-07
rs147169428	10:14061145	C	0.006	intronic	*FRMD4A*	8.56E-07
rs192245625	5:65181671	A	0.008	intergenic	*NLN*	8.68E-07
rs138470454	10:14101741	G	0.007	intronic	*FRMD4A*	9.16E-07
rs113003452	17:26694117	C	0.007	ncRNA_intronic	*TMEM199: CTB-96E2.3: VTN: CTB-96E2.2: SARM1*	9.34E-07
rs144243735	20:48311052	T	0.019	intronic	*B4GALT5*	9.57E-07

CHR_POS: chromosome and position. MAF, minor allele frequency. BETA_SE: effect size and standard error.

Table S5. Lead SNPs passing the suggestive significance level in the meta-analysis of major, GWAS on CPSP development after minor surgeries and the published CPSP study. The symbol in the direction column: '+' represent positive association, '-' represents negative association, '?' represents not genotyped. The order of symbol in the direction column represent cohorts from: GWAS on CPSP development after major surgeries, GWAS on CPSP development after minor surgeries, hysterectomy cohort (cohort acronym HYS), abdomen and knee cohort (cohort acronym ABKNEE), mastectomy cohort (cohort acronym PMPS), hernia cohort (cohort acronym HRN), knee replacement cohort (cohort acronym TKR), knee arthroplasty cohort (cohort acronym TANK).

SNP	CHR_POS	Effect_allele	MAF	BETA_SE	Location	Nearest_Gene	Direction	P	HetPVal	P_major	P_minor
rs12143186	1:68737833	T	0.111	-0.0062 (0.0012)	intergenic	*COX6B1P7*	----+++-	3.27E-07	2.95E-01	1.43E-05	5.84E-04
rs186819635	1:95071179	A	0.003	0.0252 (0.0049)	intergenic	*RP11-86H7.6*	++??????	2.70E-07	2.74E-01	1.09E-01	4.63E-07
rs184832856	2:139555831	A	0.006	-0.0256 (0.0049)	intergenic	*NXPH2*	--??????	1.64E-07	5.55E-01	2.93E-02	1.35E-06
rs17298280	4:175634898	C	0.207	0.0059 (0.0010)	intronic	*GLRA3*	++--+--+	2.19E-09	9.77E-01	8.10E-05	2.86E-06
rs138470454	10:14101741	C	0.007	0.0247 (0.0050)	intronic	*FRMD4A*	++??????	9.75E-07	6.02E-01	4.59E-02	5.82E-06
rs2160419	13:72314346	A	0.014	-0.0182 (0.0037)	intronic	*DACH1*	--??-???	7.70E-07	9.93E-01	1.66E-02	1.32E-05
rs140330443	16:1672582	A	0.005	0.0240 (0.0046)	intronic	*CRAMP1L*	++??????	1.75E-07	5.01E-03	3.49E-07	2.06E-03
rs117130005	17:19604614	T	0.017	0.0173 (0.0035)	intronic	*SLC47A2*	++??????	6.26E-07	5.16E-01	2.63E-03	4.94E-05

CHR_POS: chromosome and position. MAF, minor allele frequency. BETA_SE: effect size and standard error. P: meta-analysis P -values. HetPVal: Heterogeneity p-value. P_major: P value from major GWAS. P_minor: P-value from minor GWAS.

Table S6. Functional annotation of candidate SNPs associated with chronic postsurgical pain.

IndSigSNP	rsID	Effect_allele	MAF	GWASP	BETA	SE	r2
rs12143186	rs12754452	A	0.09245	4.02E-04	-0.0045	0.0013	0.696919
rs12143186	rs4655580	T	0.08648	1.37E-05	0.0058	0.0013	0.679724
rs12143186	rs4655581	T	0.08748	1.55E-05	0.0058	0.0013	0.688734
rs12143186	rs4655791	A	0.08748	1.56E-05	0.0058	0.0013	0.688734
rs12143186	rs10493441	A	0.08648	1.25E-05	0.0059	0.0013	0.698571
rs12143186	rs17130631	T	0.08847	1.71E-05	-0.0058	0.0013	0.679102
rs12143186	rs34669611	A	0.08847	1.71E-05	0.0058	0.0013	0.697788
rs12143186	rs34170872	A	0.08748	1.29E-05	-0.0059	0.0013	0.707627
rs12143186	rs55742865	A	0.08748	8.88E-06	0.006	0.0013	0.707627
rs12143186	rs60692352	T	0.08748	8.88E-06	0.006	0.0013	0.707627
rs12143186	rs12117024	A	0.08748	1.28E-05	0.0059	0.0013	0.707627
rs12143186	rs12120445	A	0.08748	1.28E-05	-0.0059	0.0013	0.707627
rs12143186	rs28457654	T	0.08748	1.29E-05	0.0059	0.0013	0.707627
rs12143186	rs17130635	A	0.08748	1.28E-05	0.0059	0.0013	0.707627
rs12143186	rs34491972	TA	0.09344	NA	NA	NA	0.656544
rs12143186	rs35720079	A	0.08748	7.73E-06	0.006	0.0013	0.707627
rs12143186	rs34255463	A	0.08748	7.73E-06	0.006	0.0013	0.707627
rs12143186	rs34387644	T	0.08748	1.53E-05	0.0058	0.0013	0.707627
rs12143186	rs12725309	T	0.08748	1.12E-05	0.0059	0.0013	0.707627
rs12143186	rs10493442	C	0.1004	1.39E-05	-0.0055	0.0013	0.889318
rs12143186	rs12143186	T	0.1113	3.20E-07	-0.0062	0.0012	1
rs186819635	rs142303115	T	0.001988	0.001695	0.0164	0.0052	0.665336
rs186819635	rs536137153	A	0.001988	1.51E-04	-0.0171	0.0045	0.665336
rs145636748	rs147124890	T	0.00994	0.004596	0.0091	0.0032	0.606495
rs145636748	rs182093297	T	0.00497	7.57E-07	-0.0202	0.0041	0.621235
rs145636748	rs145636748	A	0.007952	5.46E-07	0.0197	0.0039	1
rs186819635	rs182467414	T	0.002982	2.93E-06	-0.0216	0.0046	1
rs186819635	rs546009554	A	0.002982	3.53E-06	0.0214	0.0046	1
rs186819635	rs186819635	A	0.002982	2.70E-07	0.0252	0.0049	1
rs184832856	rs184832856	A	0.005964	1.64E-07	-0.0256	0.0049	1
rs184832856	rs148079195	T	0.005964	4.58E-06	-0.023	0.005	1
rs184832856	rs146350867	A	0.005964	1.05E-05	-0.0218	0.005	1
rs10032594	rs70947457	TA	0.3429	NA	NA	NA	0.855209
rs17298280	rs35051447	C	0.1918	NA	NA	NA	0.890094
rs17298280	rs17298280	C	0.2068	2.17E-09	0.0059	0.001	1
rs17298280	rs144351495	A	0.2068	NA	NA	NA	0.988094

NearestGene	Func	CADD	RDB	Promoter	Enhancer	DNAse	Proteins
RPS7P4	intergenic	5.066	7	NA	BLD, GI	NA	NA
RPS7P4	intergenic	0.413	6	BLD	BLD, GI	NA	NA
RPS7P4	intergenic	3.033	7	BLD	BLD, GI	NA	NA
RPS7P4	intergenic	1.273	7	BLD	BLD, GI	NA	NA
RPS7P4	intergenic	2.282	5	NA	NA	NA	NA
RPS7P4	intergenic	8.519	3a	BLD	BLD, GI	BLD,BRST,BLD	P300,STAT3
RPS7P4	intergenic	1.352	4	BLD	BLD, GI	NA	NA
RPS7P4	intergenic	12.76	6	NA	NA	NA	NA
RPS7P4	intergenic	7.774	6	NA	NA	NA	NA
RPS7P4	intergenic	6.82	6	NA	NA	NA	NA
RPS7P4	intergenic	14.19	6	NA	NA	NA	NA
RPS7P4	intergenic	0.681	6	NA	NA	NA	NA
RPS7P4	intergenic	2.883	7	NA	BLD	NA	NA
RPS7P4	intergenic	1.22	7	NA	BLD	NA	NA
RPS7P4	intergenic	0.967	6	NA	NA	NA	NA
RPS7P4	intergenic	3.074	6	NA	NA	NA	NA
RPS7P4	intergenic	3.265	6	NA	NA	NA	NA
RPS7P4	intergenic	0.221	7	NA	NA	NA	NA
RPS7P4	intergenic	1.753	6	NA	NA	NA	NA
COX6B1P7	intergenic	0.815	5	NA	NA	NA	NA
COX6B1P7	intergenic	1.174	7	NA	BLD	NA	NA
ARHGAP29	intronic	1.511	5	NA	NA	NA	NA
ABCD3	intronic	7.496	4	NA	NA	NA	NA
F3	intergenic	1.705	5	NA	NA	SKIN	NA
RP11-86H7.6	ncRNA_intronic	9.827	2b	ESC	15 tissues	ESDR,OVRY	NA
RP11-86H7.6	intergenic	2.026	3a	NA	4 tissues	NA	NA
RP11-86H7.6	intergenic	0.358	5	NA	SKIN, GI	NA	NA
RP11-86H7.6	intergenic	0.684	5	NA	NA	NA	NA
RP11-86H7.6	intergenic	1.012	5	NA	SKIN, GI	NA	NA
NXPH2	intergenic	7.573	6	NA	NA	NA	NA
NXPH2	intergenic	1.19	6	NA	NA	NA	NA
NXPH2	intergenic	3.493	6	NA	NA	NA	NA
GLRA3	intronic	0.243	6	NA	NA	NA	NA
GLRA3	intronic	0.618	6	NA	SKIN	NA	NA
GLRA3	intronic	3.882	3a	BRN, LNG	9 tissues	SKIN	NA
GLRA3	intronic	7.959	NA	NA	5 tissues	PLCNT,BLD	CTCF

Table S6. Continued

IndSigSNP	rsID	Effect_allele	MAF	GWASP	BETA	SE	r2
rs10032594	rs10032594	C	0.335	4.08E-07	-0.0042	8.00E-04	1
rs17298280	rs35319327	A	0.2087	2.55E-09	0.0059	0.001	0.976285
rs17298280	rs13116864	T	0.2097	4.05E-09	0.0058	0.001	0.982355
rs17298280	rs11133052	A	0.2087	3.28E-09	-0.0059	0.001	0.976285
rs17298280	rs11133053	T	0.2097	3.97E-09	0.0058	0.001	0.982355
rs138470454	rs138470454	C	0.006958	9.75E-07	0.0247	0.005	1
rs2160419	rs17207547	T	0.01789	5.26E-06	0.0141	0.0031	0.797985
rs2160419	rs150957438	T	0.0159	2.63E-06	0.0163	0.0035	0.871421
rs2160419	rs2160419	A	0.01392	7.70E-07	-0.0182	0.0037	1
rs140330443	rs140330443	A	0.00497	1.75E-07	0.024	0.0046	1
rs140330443	rs145589789	A	0.00497	NA	NA	NA	0.636791
rs140330443	rs145332950	T	0.003976	NA	NA	NA	0.798397
rs117130005	rs117130005	T	0.0169	6.26E-07	0.0173	0.0035	1
rs117130005	rs149038159	A	0.02087	2.60E-06	0.0166	0.0035	0.802861
rs117130005	rs62066262	A	0.02187	4.76E-05	-0.0125	0.0031	0.698384
rs117130005	rs80212717	A	0.0159	3.88E-06	-0.0174	0.0038	0.727709

NearestGene	Func	CADD	RDB	Promoter	Enhancer	DNAse	Proteins
GLRA3	intronic	1.601	7	NA	NA	NA	NA
GLRA3	intronic	1.16	5	NA	NA	NA	NA
GLRA3	intronic	8.57	6	NA	NA	NA	NA
GLRA3	intronic	1.888	7	NA	NA	NA	NA
GLRA3	intronic	11.41	7	NA	NA	NA	NA
FRMD4A	intronic	5.175	7	NA	NA	BLD	NA
DACH1	intronic	1.659	3a	NA	5 tissues	SKIN,VAS	NA
DACH1	intronic	10.28	5	NA	NA	NA	NA
DACH1	intronic	17.2	3a	NA	NA	MUS	NA
CRAMP1L	intronic	0.488	7	BLD	BLD	NA	NA
FAHD1:MEIOB	intronic	1.546	5	NA	NA	IPSC	NA
MEIOB	intronic	0.893	3a	NA	NA	NA	NA
SLC47A2	intronic	0.786	5	NA	NA	NA	NA
SLC47A2	intronic	0.7	5	NA	4 tissues	HRT	NA
ULK2	intergenic	3.382	3a	BLD	4 tissues	MUS,MUS	NA
ULK2	intergenic	4.022	5	NA	BLD	BLD	NA

Table S7. Pleiotropic effects of the meta-analysis GWAS lead SNPs in GWAS Atlas.

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs140330443	3738	31427789	2019	Psychiatric	Depression - Lifetime number of depressed periods	4.26E-08	57986	A	G
rs17298280	14	28439101	2017	Psychiatric	Posttraumatic stress disorder	2.13E-05	9537	G	C
rs17298280	16	28439101	2017	Psychiatric	Posttraumatic stress disorder	5.55E-05	19884	G	C
rs138470454	3408	31427789	2019	Psychiatric	Average weekly intake of other alcoholic drinks	1.62E-04	91288	C	G
rs10032594	4074	30239722	2018	Metabolic	Body Mass Index	2.03E-04	806834	G	C
rs12143186	3412	31427789	2019	Skeletal	Sitting height	2.27E-04	385393	T	C
rs17298280	3634	31427789	2019	Environment	Illnesses of father: Diabetes	2.43E-04	355137	G	C
rs10032594	4158	30531953	2018	Neurological	Generalised epilepsy	2.78E-04	33446	G	C
rs12143186	3338	31427789	2019	Activities	Years since last cervical smear test (female)	4.64E-04	182552	C	T
rs17298280	3774	31427789	2019	Psychiatric	Traumatic events - Been in aconfiding relationship as an adult	4.75E-04	123584	G	C
rs2160419	201	20383146	2010	Metabolic	Estimated glomerular filtration rate based on serum creatinine	5.20E-04	67093	G	A
rs10032594	3530	31427789	2019	Nutritional	Never eat eggs, dairy, wheat, sugar: Sugar or foods/drinks containing sugar	5.54E-04	384986	G	C
rs145636748	3743	31427789	2019	Psychiatric	Depression - Feelings of worthlessness during worst period of depression	5.78E-04	67012	G	A
rs10032594	4075	30239722	2018	Metabolic	Body Mass Index (male)	6.17E-04	374756	G	C
rs10032594	4165	30531953	2018	Neurological	Juvenile Myoclonic Epilepsy (JME)	6.30E-04	25395	G	C
rs12143186	3691	31427789	2019	Cardiovascular	Diagnoses - secondary ICD10: I10 Essential (primary) hypertension	6.93E-04	244890	C	T
rs10032594	4044	30124842	2018	Metabolic	Body Mass Index	7.00E-04	681275	G	C

Table S7. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs138470454	3782	31427789	2019	Psychiatric	Depression - Sleeping too much	7.56E-04	48926	C	G
rs10032594	619	24816252	2014	Metabolic	Xenobiotics::Chemical::glycerol 2-phosphate	8.28E-04	5912	C	G
rs10032594	3661	31427789	2019	Neoplasms	Cancer register - Histology of cancer tumour: Adenocarcinoma, NOS	8.69E-04	60655	C	G
rs10032594	3939	28892062	2017	Metabolic	Body Mass Index	8.69E-04	158284	C	G
rs12143186	3931	28878392	2017	Metabolic	Monogalactosylation	8.81E-04	2078	NA	NA
rs140330443	3432	31427789	2019	Cognitive	Symbol digit substitution test - Number of symbol digit matches attempted	9.56E-04	95669	A	G
rs140330443	3490	31427789	2019	Nutritional	Cereal type: Oat cereal (e.g. Ready Brek, porridge)	9.66E-04	319477	A	G
rs140330443	3254	31427789	2019	Nutritional	Salt added to food	1.03E-03	386322	G	A
rs10032594	3305	31427789	2019	Reproduction	Lifetime number of sexual partners	1.05E-03	316569	C	G
rs10032594	3068	28240269	2017	Cell	KIR3DS1 - Killer cell immunoglobulin-like receptor 3DS1	1.07E-03	1000	NA	NA
rs12143186	3704	31427789	2019	Reproduction	Diagnoses - secondary ICD10: Z37 Outcome of delivery	1.13E-03	244890	C	T
rs138470454	3577	31427789	2019	Social Interactions	Social support - Leisure/social activities: Pub or social club	1.33E-03	385280	C	G
rs140330443	3424	31427789	2019	Cognitive	Symbol digit substitution test - Number of symbol digit matches made correctly	1.63E-03	95669	A	G
rs145636748	3686	31427789	2019	Endocrine	Diagnoses - secondary ICD10: E11 Type 2 diabetes mellitus	1.76E-03	244890	A	G
rs117130005	2032	28067912	2017	Gastrointestinal	Prognosis in Crohn's Disease	1.78E-03	2734	C	T

Table S7. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs10032594	990	27989323	2017	Immunological	Interleukin-2 receptor, alpha subunit	2.08E-03	3677	C	G
rs12143186	3597	31427789	2019	Cardiovascular	Non-cancer illness code, self-reported: hypertension	2.20E-03	289307	C	T
rs117130005	4094	30617256	2018	Neurological	Proxy and clinically diagnosed Alzheimer's disease	2.21E-03	455258	T	C
rs10032594	2411	28240269	2017	Cell	ASAH2 - Neutral ceramidase	2.45E-03	1000	NA	NA
rs12143186	4555	31676860	2019	Neurological	Left lateral orbitofrontal	2.53E-03	21821	T	C
rs117130005	867	27005778	2016	Metabolic	OmegaL3 fatty acids	2.59E-03	13544	C	T
rs10032594	708	24816252	2014	Metabolic	Metabolite X-11452	2.67E-03	6519	C	G
rs117130005	3639	31427789	2019	Environment	Illnesses of mother: Stroke	2.70E-03	367939	T	C
rs184832856	997	27989323	2017	Immunological	Interferon gamma-induced protein 10 (CXCL10)	2.70E-03	3685	A	C
rs10032594	793	24816252	2014	Metabolic	Metabolite X-12717	2.72E-03	1632	G	C
rs10032594	4365	29899525	2018	Activities	Moderate to vigorous physical activity levels	2.80E-03	377234	G	C
rs138470454	3492	31427789	2019	Nutritional	Coffee type: Decaffeinated coffee (any type)	2.87E-03	303811	G	C
rs10032594	99	23449627	2013	Skeletal	Pubertal growth (female)	2.91E-03	5756	C	G
rs17298280	4004	29513936	2018	Connective Tissue	Juvenile idiopathic arthritis with vs without uveitis	2.92E-03	192	C	G
rs10032594	3704	31427789	2019	Reproduction	Diagnoses - secondary ICD10: Z37 Outcome of delivery	2.95E-03	244890	C	G
rs10032594	2175	28240269	2017	Cell	MICA - MHC class I polypeptide-related sequence A	2.97E-03	1000	NA	NA
rs12143186	1201	25087078	2014	Neurological	Focal epilepsy	2.97E-03	31467	NA	NA

Table S7. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs138470454	3381	31427789	2019	Cognitive	Prospective memory test - Time to answer	3.10E-03	128912	C	G
rs138470454	3985	29497042	2018	Cardiovascular	Heart rate recovery at 10 secnds	3.10E-03	58818	C	G
rs184832856	3274	31427789	2019	Dermatological	Ease of skin tanning	3.11E-03	378364	A	C
rs117130005	3587	31427789	2019	Activities	Types of physical activity in last 4 weeks: Strenuous sports	3.14E-03	384450	C	T
rs12143186	3971	29304378	2018	Skeletal	Total body BMD	3.26E-03	66628	T	C
rs2160419	202	20383146	2010	Metabolic	Estimated glomerular filtration rate based on cystain C	3.30E-03	20957	G	A
rs10032594	3941	28892062	2017	Metabolic	Body Mass Index (female)	3.38E-03	72390	C	G
rs17298280	3341	31427789	2019	Reproduction	Number of live births (female)	3.38E-03	208434	G	C
rs17298280	3426	31427789	2019	Cognitive	Fluid intelligence test - FI3 : word interpolation	3.39E-03	99362	G	C
rs117130005	3594	31427789	2019	Activities	Mineral and other dietary supplements: Calcium	3.58E-03	385261	T	C
rs10032594	2224	28240269	2017	Cell	COMMD7 - COMM domain-containing protein 7	3.65E-03	1000	NA	NA
rs17298280	4553	31676860	2019	Neurological	Left isthmus cingulate	3.66E-03	21821	G	C
rs138470454	3308	31427789	2019	Mortality	Long-standing illness, disability or infirmity	3.72E-03	377498	G	C
rs117130005	3241	31427789	2019	Nutritional	Salad / raw vegetable intake	3.83E-03	363780	C	T
rs17298280	2036	28039263	2017	Ear, Nose, Throat	Cisplatin-associated ototoxicity	3.83E-03	511	G	C
rs10032594	3275	31427789	2019	Activities	Childhood sunburn occasions	3.92E-03	289412	C	G
rs12143186	3314	31427789	2019	Ear, Nose, Throat	Hearing difficulty/problems with background noise	3.93E-03	378722	C	T

Table S7. Continued

SNP	atlas ID	PMID	Year	Domain	Trait	P-value	N	Effect allele	Non-effect allele
rs140330443	4186	31217584	2019	Nutritional	Coffee cups per day	3.93E-03	35902	A	G
rs17298280	3776	31427789	2019	Psychiatric	Traumatic events - Been in serious accident believed to be life-threatening	3.97E-03	126665	G	C
rs145636748	4334	30552067	2018	Respiratory	Asthma	3.99E-03	30810	A	G
rs12143186	1002	27989323	2017	Immunological	Monokine induced by interferon-gamma (CXCL9)	4.13E-03	3685	C	T
rs117130005	4090	29777097	2018	Environment	Family history of Alzheimer's disease	4.31E-03	314278	T	C
rs117130005	4091	29777097	2018	Neurological	Proxy and clinically diagnosed Alzheimer's disease	4.31E-03	388324	T	C
rs12143186	3769	31427789	2019	Psychiatric	Anxiety - Recent restlessness	4.48E-03	126513	C	T
rs10032594	3476	31427789	2019	Activities	Own or rent accommodation lived in: Own outright (by you or someone in your household)	4.60E-03	383032	G	C
rs10032594	3531	31427789	2019	Nutritional	Never eat eggs, dairy, wheat, sugar: I eat all of the above	4.74E-03	384986	C	G
rs12143186	3933	28878392	2017	Metabolic	Galactosylation	4.74E-03	2078	NA	NA
rs12143186	3937	28878392	2017	Metabolic	Fucosylation	4.83E-03	2078	NA	NA
rs117130005	3477	31427789	2019	Activities	Own or rent accommodation lived in: Own with a mortgage	4.88E-03	383032	T	C
rs184832856	4328	30598549	2018	Skeletal	Estimated bone mineral density from heel ultrasounds	4.90E-03	426824	A	C

PMID: Pubmed ID. atlas ID: study ID in GWAS atlas. N: sample size.

Table S8. Pleiotropic effects of the meta-analysis GWAS lead SNPs in GWAS catalog.

IndSigSNP	GWAS catalog SNP	PMID	Trait	MappedGene	Effect allele	Effect allele frequency	P
rs12143186	rs4655791	30595370	Red blood cell count	*RPS7P4 - COX6B1P7*	NR	NR	2.00E-12
rs12143186	rs10493441	32888493	Hemoglobin concentration	*RPS7P4 - COX6B1P7*	A	0.12189	2.00E-10
rs12143186	rs17130631	32888493	Hematocrit	*RPS7P4 - COX6B1P7*	C	0.094406	2.00E-11
rs12143186	rs34387644	32888493	Hematocrit	*RPS7P4 - COX6B1P7*	T	0.122868	6.00E-13
rs12143186	rs10493442	32888493	Red blood cell count	*RPS7P4 - COX6B1P7*	G	0.134858	2.00E-14
rs12143186	rs4655791	32888494	Hematocrit	*RPS7P4 - COX6B1P7*	A	0.093011	4.00E-10
rs12143186	rs4655791	32888494	Red blood cell count	*RPS7P4 - COX6B1P7*	A	0.093012	2.00E-10
rs12143186	rs17130635	33109212	HDL cholesterol levels in HIV infection	*RPS7P4 - COX6B1P7*	NR	NR	6.00E-06
rs12143186	rs4655581	34594039	Red blood cell count	*RPS7P4 - COX6B1P7*	T	NR	1.00E-14
rs12143186	rs4655581	34594039	Hematocrit	*RPS7P4 - COX6B1P7*	T	NR	2.00E-10
rs12143186	rs10493441	34594039	Hemoglobin	*RPS7P4 - COX6B1P7*	A	NR	4.00E-08

PMID: Pubmed ID. NR: not reported

Table S9. Genes identified by MAGMA gene-based analysis in the meta-analysis of GWAS on CPSP development after major and minor surgeries. (Here lists top 20 candidate genes. The full list can be found online)

GENE	CHR	START	STOP	NSNPS	NPARAM	N	ZSTAT	P	SYMBOL
ENSG00000159398	16	55830066	56039943	764	56	95931	3.7417	9.14E-05	*CES5A*
ENSG00000175155	17	57359050	57529090	440	52	95931	3.6767	1.18E-04	*YPEL2*
ENSG00000163629	4	87465468	87786324	939	21	95931	3.6477	1.32E-04	*PTPN13*
ENSG00000179751	19	39643471	39744906	320	40	95931	3.6074	1.55E-04	*SYCN*
ENSG00000188505	19	39637601	39742524	327	39	95931	3.5663	1.81E-04	*NCCRP1*
ENSG00000120436	6	167519759	167621817	464	54	95931	3.5295	2.08E-04	*GPR31*
ENSG00000109339	4	86886276	87565284	2185	73	95931	3.4659	2.64E-04	*MAPK10*
ENSG00000111801	6	26390700	26503643	477	32	95931	3.3032	4.78E-04	*BTN3A3*
ENSG00000185988	19	1474073	1585455	351	53	95931	3.3012	4.81E-04	*PLK5*
ENSG00000116729	1	68514142	68748803	991	105	95931	3.2978	4.87E-04	*WLS*
ENSG00000112486	6	167475295	167603184	491	50	95931	3.2846	5.11E-04	*CCR6*
ENSG00000175309	5	177585498	177709792	560	75	95931	3.2701	5.37E-04	*PHYKPL*
ENSG00000135436	12	49926668	50049422	208	23	95931	3.245	5.87E-04	*FAM186B*
ENSG00000164327	5	38888021	39124510	642	34	95931	3.23	6.19E-04	*RICTOR*
ENSG00000145451	4	175508065	175800465	1246	76	95931	3.1967	6.95E-04	*GLRA3*
ENSG00000130669	19	39566410	39723456	558	42	95931	3.1874	7.18E-04	*PAK4*
ENSG00000185761	19	1455017	1563603	355	53	95931	3.1677	7.68E-04	*ADAMTSL5*
ENSG00000061492	5	137369581	137478054	260	28	95931	3.1615	7.85E-04	*WNT8A*
ENSG00000187778	12	49900327	50011936	209	24	95931	3.1434	8.35E-04	*MCRS1*
ENSG00000181588	19	1504668	1618057	320	50	95931	3.1409	8.42E-04	*MEX3D*

Table S10. Robustness analysis of lead SNPs. The columns headers are the round of iterations. The value in the cell are the P-values in each iteration.

SNP	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
rs10032594	3.14E-03	3.92E-05	6.18E-04	2.81E-04	8.33E-03	7.27E-06	3.68E-07	4.58E-02	6.44E-04	2.10E-04
rs117130005	1.19E-03	1.06E-04	2.52E-02	1.13E-06	2.96E-03	3.68E-05	2.52E-06	2.03E-02	4.37E-05	2.80E-03
rs12143186	5.93E-06	2.05E-03	5.79E-07	1.13E-02	4.11E-03	3.12E-06	2.13E-04	1.16E-04	3.75E-05	6.62E-04
rs138470454	3.82E-03	5.68E-05	4.71E-04	4.96E-04	1.16E-01	6.79E-08	8.43E-03	1.42E-05	1.12E-02	1.18E-05
rs140330443	5.90E-07	4.56E-03	2.66E-05	2.45E-04	6.76E-07	3.84E-03	2.29E-04	3.66E-05	1.58E-04	4.79E-05
rs145636748	8.29E-07	2.37E-02	7.30E-05	1.96E-03	7.67E-04	2.09E-04	3.86E-04	4.29E-04	1.09E-03	1.05E-04
rs17298280	6.25E-03	1.09E-08	9.91E-06	5.54E-05	3.03E-05	1.32E-05	4.51E-06	8.63E-05	7.74E-06	4.90E-05
rs184832856	9.66E-04	4.60E-05	1.69E-02	4.92E-07	1.71E-01	5.29E-09	3.68E-04	1.51E-04	1.99E-08	8.65E-02
rs186819635	5.93E-06	7.44E-03	1.71E-05	4.75E-03	1.76E-04	7.85E-04	5.66E-04	2.63E-04	5.77E-03	1.01E-05
rs2160419	2.31E-03	7.43E-05	1.37E-05	8.40E-03	8.88E-04	2.32E-04	4.94E-03	2.50E-05	1.05E-05	1.09E-02

Table S11. Candidate SNPs from the main GWAS examined in the published CPSP Meta-Analysis. We queried 52 candidate SNPs identified from the main GWAS in the published CPSP study. However, some SNPs were not genotyped in any subcohorts of the published CPSP study. In this table, we have excluded SNPs that were not genotyped in any CPSP subcohorts from the published CPSP study.

Reported Marker	Allele1	Allele2	Freq1	FreqSE	Effect	StdErr	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
rs62066262	A	G	0.98	0	1.528	0.7031	0.02975	??+????	0	0	0	1
rs149038159	A	G	0.0178	0	-1.351	0.7395	0.06772	??-????	0	0	0	1
rs12143186	T	C	0.8937	0.0144	0.2074	0.1436	0.1487	-+++-+-	0	4.128	6	0.6594
rs10032594	C	G	0.3647	0.0368	0.1141	0.0884	0.1968	+-++++-	12.3	6.843	6	0.3356
rs10493442	C	G	0.8996	0.0133	0.1741	0.1467	0.2353	++-+-+-	0	3.189	6	0.7848
rs12754452	A	G	0.9053	0.019	0.1495	0.1509	0.322	-++++++	0	3.419	6	0.7547
rs4655580	T	G	0.0872	0.0174	-0.1216	0.1563	0.4367	+------	0	2.774	6	0.8366
rs4655791	A	T	0.0871	0.0175	-0.1168	0.1565	0.4555	+------	0	2.8	6	0.8335
rs4655581	T	C	0.0871	0.0175	-0.1168	0.1565	0.4555	+------	0	2.8	6	0.8335
rs17130631	T	C	0.915	0.0163	0.1158	0.158	0.4636	-+++-++	0	2.892	6	0.8223
rs34669611	A	C	0.0851	0.0163	-0.1151	0.1581	0.4665	+---+--	0	2.894	6	0.822
rs12725309	T	C	0.0852	0.0162	-0.1143	0.158	0.4696	+---+--	0	2.897	6	0.8216
rs10493441	A	G	0.0856	0.0166	-0.1108	0.1574	0.4817	+---+--	0	2.991	6	0.81
rs12120445	A	G	0.915	0.0164	0.1079	0.1586	0.4961	-+++-++	0	2.931	6	0.8174
rs17130635	A	G	0.085	0.0164	-0.1079	0.1586	0.4961	+---+--	0	2.931	6	0.8174
rs34170872	A	C	0.915	0.0164	0.1079	0.1586	0.4961	-+++-++	0	2.931	6	0.8174
rs28457654	T	C	0.085	0.0164	-0.1079	0.1586	0.4961	+---+--	0	2.931	6	0.8174
rs12117024	A	T	0.085	0.0164	-0.1079	0.1586	0.4961	+---+--	0	2.931	6	0.8174
rs34387644	T	C	0.085	0.0164	-0.1079	0.1586	0.4961	+---+--	0	2.931	6	0.8174
rs17298280	C	G	0.7801	0.0299	-0.0635	0.1057	0.5481	-+---+-	0	2.495	6	0.869

Table S11. Continued

Reported Marker	Allele1	Allele2	Freq1	FreqSE	Effect	StdErr	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
rs13116864	T	C	0.7794	0.0292	-0.0604	0.1056	0.567	-+--+-	0	2.756	6	0.8388
rs35319327	A	T	0.7795	0.0293	-0.0563	0.1057	0.5944	-+--+-	0	2.698	6	0.8457
rs11133053	T	C	0.7794	0.0288	-0.0545	0.1053	0.6044	-+--+-	0	3.307	6	0.7695
rs11133052	A	G	0.2203	0.0294	0.0508	0.1057	0.6309	+-+++-+	0	3.071	6	0.7999
rs150957438	T	C	0.0144	0	0.3604	0.8837	0.6834	??+????	0	0	0	1
rs2160419	A	G	0.9856	0	-0.3604	0.8837	0.6834	??-????	0	0	0	1
rs17207547	T	C	0.0145	0	0.3494	0.8841	0.6927	??+????	0	0	0	1
rs55742865	A	T	0.073	0	-0.0242	0.4036	0.9522	??-????	0	0	0	1
rs35720079	A	T	0.073	0	-0.0242	0.4036	0.9522	??-????	0	0	0	1
rs34255463	A	T	0.073	0	-0.0242	0.4036	0.9522	??-????	0	0	0	1
rs60692352	T	C	0.073	0	-0.0242	0.4036	0.9522	??-????	0	0	0	1

Allele1: Effect allele. Allele2: Non-effect allele. Freq1: allele frequency of effect allele. FreqSE: standard error of allele frequency. Effect: effect size. StdErr: standard error. P-value: meta-analysis P-value. Direction: direction of associations. HetISq:I^2 statistic which measures heterogeneity on scale of 0-100%. HetChiSq: chi-squared statistic in simple test of heterogeneity. HetDf: degrees of freedom for heterogeneity statistic. HetPVal: P-value for heterogeneity statistic.

Table S12. Validation of candidate SNPs reported in the published systematic review.

Gene	SNP	EA	Outcome	Source
KCNS1	rs734784	T	Baseline, 2, 6 and 12 months after surgery; area-under-the-curve score for every pain variable was converted these to a z-score by comparing the patient with the rest of the cohort. CPSP outcome: mean of the four z-scores.	Meta-analysis from systematic review
			Latent classes of pain 6 months after surgery (No Pain, Mild Pain, Moderate Pain, Severe Pain)	
			Analgesic requirement more than 3 months after surgery	CPSP in our study
OPRM1	rs1799971	G	Opioid use < 48 h	Meta-analysis from systematic review
			Pain score < 48 h	
			Analgesic requirement more than 3 months after surgery	CPSP in our study
COMT	rs4680	A	Opioid use < 48 h	Meta-analysis from systematic review
			Analgesic requirement more than 3 months after surgery	CPSP in our study

For each SNP results of previous publications and p-values obtained in this analysis are presented in separate rows.

* Originally reported A > G (G is the effect allele). To align the results, the effect allele is changed to the corresponding allele on the other strand. The OR and CI values are also tranformed after allele alignment

† Effect size and stand error were converted to OR and CI

‡ Reported Standard Mean Difference converted to OR

EA: effect allele. NR: Not reported

	No.studies	No. Subjects	OR	P	Heterogeneity I2 (%)	Heterogeneity Pval
	2	1377	0.662 *	0.05	0	0.344
	N/A	95931	1.001 †	0.1642	0	0.6397
	33	8,227	1.574 ‡	< 0.00001	73	NR
	18	4,619	1.437 ‡	0.0004	59	NR
	N/A	95,931	1.001 †	0.629	0	0.6912
	12	2,259	0.865 ‡	0.19	30	NR
	N/A	95931	1.001 †	0.1063	81.9	0.0188

Table S13. Validation of candidate SNPs reported in previously published CPSP GWASes.

Genes	Lead SNP	EA	EAF	Outcome
NRXN1 | *LOC730100* | *LINC01867*	rs138190025	A	0.017	All primary outcome measures were assessed on a continuous scale based on the Numeric Rating Scale (NRS, 0-10 scale),
			0.0179	Analgesic requirement more than 3 months after surgery
MAP9 | *GUCY1A1* | *GUCY1B1*	rs114837251	T	0.021	All primary outcome measures were assessed on a continuous scale based on the Numeric Rating Scale (NRS, 0-10 scale),
			0.0321	Analgesic requirement more than 3 months after surgery
LOC100130950 | *RABEP1* | *NUP88*	rs3026120	A	0.037	All primary outcome measures were assessed on a continuous scale based on the Numeric Rating Scale (NRS, 0-10 scale),
			0.0522	Analgesic requirement more than 3 months after surgery
PRKCA	rs887797	A	0.335	Neuropathic pain after total joint replacement. Individuals were assigned a phenotype their scores on the painDETECT questionnaire. Scores of >12 were classified as 'possible neuropathic pain'
			0.3217	Analgesic requirement more than 3 months after surgery

For each SNP results of previous publications and p-values obtained in this analysis are presented in separate rows.
* OR was converted to effect size for comparasion

Table S14. Lead SNPs passing the suggestive significance level in the subtype GWAS based on surgery complexity.

Subtype	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
CPSP after major surgery	rs577296200	1:154692176	G	0.001	-0.0453 (0.0089)
CPSP after major surgery	rs139043249	3:22197869	G	0.013	-0.0433 (0.0087)
CPSP after major surgery	rs541492989	3:75974736	C	0.001	-0.0515 (0.0102)
CPSP after major surgery	rs142092081	6:151287775	C	0.015	-0.0307 (0.0062)
CPSP after major surgery	rs140417076	7:150892899	C	0.007	-0.0454 (0.0093)
CPSP after major surgery	rs186260626	10:71262642	T	0.004	-0.0538 (0.0100)
CPSP after major surgery	rs546609386	11:71238231	T	0.004	-0.0543 (0.0111)
CPSP after major surgery	rs140330443	16:1672582	G	0.005	-0.0458 (0.0090)
CPSP after major surgery	rs72801520	16:84971669	A	0.18	-0.0121 (0.0023)
CPSP after minor surgery	rs186819635	1:95071179	C	0.003	-0.0283 (0.0056)
CPSP after minor surgery	rs143392914	2:142344613	C	0.007	-0.0303 (0.0061)
CPSP after minor surgery	rs575459128	3:153401826	C	0.026	-0.0153 (0.0030)
CPSP after minor surgery	rs117873395	8:13280832	A	0.022	-0.0196 (0.0040)
CPSP after minor surgery	rs111506920	11:15881806	A	0.014	0.0151 (0.0030)
CPSP after minor surgery	rs374048856	12:31209580	A	0.017	-0.0227 (0.0046)
CPSP after minor surgery	rs117696131	18:73134903	T	0.014	-0.0156 (0.0031)
CPSP after minor surgery	rs142540066	20:2172320	C	0.007	-0.0254 (0.0051)

CHR_POS: chromosome and position. MAF, minor allele frequency. BETA_SE: effect size and standard error.

	Source	Effect size / BETA	P-value
	Published CPSP meta-analysis. PubmedID: 38862382	2.766	1.07E-08
	CPSP in our study	0.0037	0.2127
	Published CPSP meta-analysis. PubmedID: 38862382	2.638	3.87E-09
	CPSP in our study	0.0043	0.05059
	Published CPSP meta-analysis. PubmedID: 38862382	1.625	6.18E-09
	CPSP in our study	0.001	0.5836
	Previous CPSP GWAS. Pubmed ID: 28051079	0.392 *	1.65E-05
	CPSP in our study	5.00E-04	0.5815

Location	Nearest_Gene	P
intronic	*KCNN3*	3.75E-07
intronic	*ZNF385D*	6.67E-07
intronic	*ROBO2*	4.27E-07
intronic	*MTHFD1L*	8.10E-07
ncRNA_intronic	*IQCA1P1*	9.31E-07
intronic	*TSPAN15*	6.97E-08
upstream	*KRTAP5-7*	9.46E-07
intronic	*CRAMP1L*	3.49E-07
ncRNA_intronic	*RP11-254F19.3*	9.32E-08
intergenic	*RP11-86H7.6*	4.63E-07
intronic	*LRP1B*	5.54E-07
ncRNA_intronic	*RP11-23D24.2*	2.54E-07
intronic	*DLC1*	9.97E-07
intergenic	*RP11-222N13.1*	7.64E-07
ncRNA_intronic	*DDX11-AS1*	8.24E-07
intronic	*SMIM21*	7.49E-07
intergenic	*STK35*	7.48E-07

Table S15. Lead SNPs passing the suggestive significance level in the subtype GWAS based on surgery sites.

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Musculoskeletal	rs192574236	1:196251909	A	0.007	-0.0688 (0.0139)
Musculoskeletal	rs150358364	3:178061231	T	0.007	-0.0632 (0.0120)
Musculoskeletal	rs79160542	3:194564604	T	0.005	-0.0923 (0.0168)
Musculoskeletal	rs142961246	4:152283993	G	0.013	-0.0549 (0.0105)
Musculoskeletal	rs140596867	6:151281848	C	0.015	-0.0464 (0.0090)
Musculoskeletal	rs142333948	7:81569995	C	0.008	-0.0866 (0.0148)
Musculoskeletal	rs149325266	7:152662995	G	0.014	-0.0414 (0.0082)
Musculoskeletal	rs113857436	7:157565408	G	0.005	-0.0894 (0.0146)
Musculoskeletal	rs113962796	8:23582328	G	0.014	-0.0575 (0.0116)
Musculoskeletal	rs140970257	10:30219941	A	0.01	-0.0482 (0.0096)
Musculoskeletal	rs150827447	11:11468879	T	0.007	-0.0648 (0.0128)
Musculoskeletal	rs145942081	12:71693650	C	0.007	-0.0554 (0.0103)
Musculoskeletal	rs143286186	12:133123552	G	0.005	-0.0792 (0.0156)
Musculoskeletal	rs140543530	13:113938559	G	0.008	-0.0672 (0.0129)
Musculoskeletal	rs548148772	13:113964412	C	0.003	-0.0718 (0.0139)
Musculoskeletal	rs148932710	14:28348509	C	0.015	-0.0512 (0.0096)
Musculoskeletal	rs78285271	15:50581906	G	0.013	-0.0461 (0.0090)
Musculoskeletal	rs56387581	15:76707279	T	0.008	-0.0749 (0.0130)
Musculoskeletal	rs142432350	15:77237270	T	0.017	-0.0534 (0.0092)
Musculoskeletal	rs9912298	17:29735752	A	0.228	-0.0133 (0.0026)
Musculoskeletal	rs6115581	20:26248230	C	0.012	-0.0873 (0.0164)
Nervous	rs183456596	1:35557273	A	0.009	-0.1833 (0.0365)
Nervous	rs61809518	1:163390485	C	0.078	-0.0552 (0.0112)
Nervous	rs78006260	1:191072885	G	0.007	-0.1334 (0.0269)
Nervous	rs12409613	1:210093031	G	0.009	-0.1769 (0.0338)
Nervous	rs77492707	2:44900234	T	0.015	-0.1322 (0.0250)
Nervous	rs191668513	2:50215453	A	0.011	-0.1955 (0.0320)
Nervous	rs75727482	2:50509501	A	0.015	-0.1443 (0.0253)
Nervous	rs77900240	2:53466773	T	0.007	-0.2115 (0.0423)
Nervous	rs76986418	3:8549950	A	0.007	-0.1571 (0.0320)
Nervous	rs192284648	3:22750855	T	0.005	-0.2154 (0.0395)
Nervous	rs544052611	3:43106023	C	0.002	-0.2591 (0.0416)
Nervous	rs186797066	4:115344201	A	0.015	-0.1324 (0.0251)
Nervous	rs114585001	4:129724843	C	0.007	-0.2170 (0.0394)
Nervous	rs141533658	4:129906178	A	0.01	-0.1203 (0.0235)

	Location	Nearest_Gene	P
	intronic	*KCNT2*	6.96E-07
	ncRNA_intronic	*RP11-33A14.1:KCNMB2*	1.39E-07
	intergenic	*AC090505.1*	3.97E-08
	intergenic	*RP11-731D1.4*	1.89E-07
	intronic	*MTHFD1L*	2.16E-07
	intergenic	*CACNA2D1*	4.47E-09
	intergenic	*AF104455.1*	4.09E-07
	intronic	*PTPRN2*	8.69E-10
	ncRNA_intronic	*RP11-175E9.1*	7.56E-07
	intergenic	*RP11-224P11.1*	4.87E-07
	intronic	*GALNT18*	3.78E-07
	intronic	*TSPAN8*	6.65E-08
	intronic	*FBRSL1*	3.78E-07
	intergenic	*LDHBP1*	1.75E-07
	intronic	*LAMP1*	2.30E-07
	intergenic	*CTD-3006G17.2*	1.07E-07
	intronic	*GABPB1*	3.21E-07
	intronic	*SCAPER*	9.17E-09
	intronic	*RCN2*	7.34E-09
	intronic	*RAB11FIP4*	4.52E-07
	intergenic	*MIR663A*	1.01E-07
	intronic	*ZMYM1*	5.19E-07
	intergenic	*RP11-408E1.1*	8.96E-07
	intergenic	*HNRNPA1P46*	6.72E-07
	intergenic	*SYT14*	1.59E-07
	intronic	*CAMKMT*	1.24E-07
	intronic	*NRXN1*	9.49E-10
	intronic	*NRXN1*	1.19E-08
	intergenic	*SCARNA16*	5.64E-07
	ncRNA_intronic	*LMCD1-AS1:LMCD1*	9.19E-07
	intergenic	*AC092421.1*	4.98E-08
	intergenic	*FAM198A*	4.55E-10
	intergenic	*UGT8*	1.35E-07
	intergenic	*JADE1*	3.68E-08
	intronic	*SCLT1*	3.11E-07

Table S15. Continued

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Nervous	rs188595506	4:155816051	G	0.012	-0.2128 (0.0422)
Nervous	rs139907974	5:62167520	A	0.012	-0.1660 (0.0336)
Nervous	rs72775352	5:94191720	A	0.011	-0.1519 (0.0296)
Nervous	rs143515443	5:115981755	C	0.013	-0.1659 (0.0309)
Nervous	rs72792312	5:140959817	T	0.003	-0.1898 (0.0388)
Nervous	rs66670765	5:141111764	C	0.007	-0.1731 (0.0331)
Nervous	rs534617780	6:9971891	T	0.01	-0.1468 (0.0300)
Nervous	rs9368350	6:21807964	T	0.025	-0.1242 (0.0246)
Nervous	rs184282756	6:90423766	A	0.007	-0.1792 (0.0350)
Nervous	rs115885479	6:159168547	G	0.01	-0.2233 (0.0372)
Nervous	rs192902761	7:16812793	G	0.015	-0.2498 (0.0429)
Nervous	rs188863706	7:81162580	T	0.007	-0.2048 (0.0408)
Nervous	rs725438	8:6262831	A	0.207	0.0399 (0.0072)
Nervous	rs376535849	8:31425869	G	0.012	-0.1486 (0.0274)
Nervous	rs143310214	9:3011149	T	0.008	-0.1609 (0.0288)
Nervous	rs147784907	9:8532336	C	0.007	-0.1857 (0.0362)
Nervous	rs143343874	9:36807208	G	0.011	-0.1258 (0.0253)
Nervous	rs143029485	9:115979781	C	0.003	-0.2133 (0.0414)
Nervous	rs182236962	9:116195605	G	0.01	-0.2586 (0.0445)
Nervous	rs181486723	10:4374215	C	0.006	-0.2033 (0.0410)
Nervous	rs11258040	10:12913754	G	0.144	0.0419 (0.0082)
Nervous	rs568404902	10:14976896	A	0.009	-0.1135 (0.0231)
Nervous	rs77993488	10:91390586	A	0.013	-0.1769 (0.0312)
Nervous	rs117059362	10:95516264	T	0.004	-0.1724 (0.0348)
Nervous	rs142761018	10:110556342	G	0.004	-0.1591 (0.0321)
Nervous	rs138973335	12:39603780	A	0.005	-0.1751 (0.0347)
Nervous	rs148949678	12:90645666	T	0.01	-0.1718 (0.0342)
Nervous	rs3138299	12:91555133	G	0.003	-0.1690 (0.0335)
Nervous	rs150859461	13:28070512	G	0.005	-0.1984 (0.0392)
Nervous	rs75696744	14:91861224	G	0.003	-0.1823 (0.0340)
Nervous	rs77122242	15:92082382	C	0.006	-0.1894 (0.0376)
Nervous	rs78401878	15:96520812	G	0.016	-0.1801 (0.0325)
Nervous	rs62044134	16:18177335	T	0.055	-0.0675 (0.0125)
Nervous	rs77265023	16:52773162	G	0.029	-0.0956 (0.0194)
Nervous	rs117618811	18:48897291	A	0.018	-0.1514 (0.0300)
Nervous	rs146774953	20:2166138	C	0.006	-0.2104 (0.0391)

	Location	Nearest_Gene	P
	intergenic	*RBM46*	4.53E-07
	intergenic	*ISCA1P1*	8.06E-07
	intronic	*MCTP1*	2.99E-07
	intergenic	*CTB-118N6.2*	8.14E-08
	intronic	*DIAPH1*	9.87E-07
	intergenic	*ARAP3*	1.69E-07
	intronic	*OFCC1*	9.96E-07
	ncRNA_intronic	*CASC15*	4.51E-07
	intronic	*MDN1*	3.16E-07
	intronic	*SYTL3*	1.94E-09
	intronic	*TSPAN13*	5.96E-09
	ncRNA_intronic	*AC008163.4*	5.07E-07
	ncRNA_intronic	*RP11-115C21.2*	3.07E-08
	intergenic	*RNA5SP261*	5.62E-08
	ncRNA_intronic	*CARM1P1*	2.21E-08
	intronic	*PTPRD*	2.96E-07
	intergenic	*MIR4475*	6.65E-07
	intronic	*FKBP15*	2.55E-07
	intergenic	*C9orf43*	6.30E-09
	intergenic	*LINC00703*	6.93E-07
	intergenic	*CCDC3*	3.03E-07
	intronic	*DCLRE1C*	8.73E-07
	intronic	*PANK1*	1.42E-08
	intergenic	*LGI1*	7.26E-07
	ncRNA_intronic	*RP11-655H13.2*	7.27E-07
	intergenic	*RP11-421H10.2*	4.70E-07
	intergenic	*RP11-753N8.1*	4.94E-07
	intronic	*DCN*	4.39E-07
	intergenic	*RNU6-63P*	4.09E-07
	intronic	*CCDC88C*	8.60E-08
	ncRNA_intronic	*RP11-661P17.1*	4.91E-07
	intergenic	*RP11-4G2.1*	3.16E-08
	ncRNA_intronic	*CTA-481E9.4:CTA-481E9.3*	7.10E-08
	intergenic	*RP11-297L17.6*	7.93E-07
	ncRNA_intronic	*RP11-267C16.1*	4.44E-07
	intergenic	*STK35*	7.47E-08

Table S15. Continued

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Nervous	rs4813372	20:19772734	G	0.188	-0.0382 (0.0076)
Otorhinolaryngology and eye	rs144109064	1:117790362	G	0.004	-0.0656 (0.0133)
Otorhinolaryngology and eye	rs140477955	1:177186132	G	0.006	-0.0797 (0.0149)
Otorhinolaryngology and eye	rs114025835	1:243725223	C	0.013	-0.0567 (0.0097)
Otorhinolaryngology and eye	rs567543360	2:40262057	C	0.005	-0.0724 (0.0148)
Otorhinolaryngology and eye	rs183668952	2:40306985	T	0.006	-0.0646 (0.0129)
Otorhinolaryngology and eye	rs192096859	2:40451332	A	0.005	-0.0719 (0.0140)
Otorhinolaryngology and eye	rs182836894	2:73493487	G	0.005	-0.0791 (0.0142)
Otorhinolaryngology and eye	rs142421984	2:131604170	G	0.007	-0.0727 (0.0140)
Otorhinolaryngology and eye	rs148986963	2:235140514	C	0.016	-0.0618 (0.0110)
Otorhinolaryngology and eye	rs112689096	3:41675475	T	0.003	-0.0760 (0.0154)
Otorhinolaryngology and eye	rs183601200	3:47157990	T	0.007	-0.0698 (0.0132)
Otorhinolaryngology and eye	rs182679612	3:48470361	G	0.014	-0.0456 (0.0093)
Otorhinolaryngology and eye	rs186414503	3:79188378	C	0.005	-0.0785 (0.0142)
Otorhinolaryngology and eye	rs560014471	3:79958614	C	0.003	-0.0806 (0.0148)
Otorhinolaryngology and eye	rs2705523	3:112268654	A	0.03	-0.0634 (0.0113)
Otorhinolaryngology and eye	rs138022171	4:4989566	G	0.007	-0.0774 (0.0151)
Otorhinolaryngology and eye	rs115917570	4:31104218	C	0.012	-0.0731 (0.0147)
Otorhinolaryngology and eye	rs150614626	4:40544008	T	0.013	-0.0399 (0.0081)
Otorhinolaryngology and eye	rs28375678	4:42460202	G	0.007	-0.0797 (0.0148)
Otorhinolaryngology and eye	rs72684647	4:124345714	T	0.014	-0.0449 (0.0088)
Otorhinolaryngology and eye	rs34335674	4:174109837	C	0.009	-0.0447 (0.0089)
Otorhinolaryngology and eye	rs112392689	4:178018865	G	0.036	-0.0310 (0.0063)
Otorhinolaryngology and eye	rs116186726	5:10046438	T	0.015	-0.0510 (0.0099)
Otorhinolaryngology and eye	rs189467180	5:65286755	A	0.013	-0.0467 (0.0091)
Otorhinolaryngology and eye	rs148431350	5:132572084	G	0.009	-0.0794 (0.0139)
Otorhinolaryngology and eye	rs141820427	5:164013156	G	0.004	-0.0634 (0.0117)
Otorhinolaryngology and eye	rs141639052	5:175558122	T	0.023	-0.0385 (0.0071)
Otorhinolaryngology and eye	rs150460128	5:175563274	C	0.037	-0.0311 (0.0059)
Otorhinolaryngology and eye	rs186213237	5:180651880	A	0.01	-0.0533 (0.0105)
Otorhinolaryngology and eye	rs10946583	6:22918146	T	0.005	-0.0824 (0.0158)
Otorhinolaryngology and eye	rs144090505	6:98042118	A	0.031	-0.0351 (0.0061)
Otorhinolaryngology and eye	rs112286671	6:98101460	A	0.001	-0.0711 (0.0144)
Otorhinolaryngology and eye	rs17057953	6:98170442	C	0.02	-0.0403 (0.0069)
Otorhinolaryngology and eye	rs142066183	6:166096487	C	0.008	-0.0757 (0.0154)
Otorhinolaryngology and eye	rs187684394	7:14674813	G	0.001	-0.0683 (0.0137)

	Location	Nearest_Gene	P
	ncRNA_intronic	*RP1-122P22.2*	5.18E-07
	intergenic	*VTCN1*	8.50E-07
	intronic	*BRINP2*	8.17E-08
	intronic	*AKT3*	4.42E-09
	ncRNA_intronic	*SLC8A1-AS1*	9.53E-07
	ncRNA_intronic	*SLC8A1-AS1*	4.95E-07
	ncRNA_intronic	*SLC8A1-AS1:SLC8A1*	2.74E-07
	intronic	*FBXO41*	2.40E-08
	intronic	*ARHGEF4*	2.07E-07
	intergenic	*RP11-309M7.1*	1.90E-08
	intronic	*ULK4*	8.03E-07
	intronic	*SETD2*	1.34E-07
	intronic	*PLXNB1*	9.04E-07
	intronic	*ROBO1*	3.35E-08
	intergenic	*HMGB1P38*	4.87E-08
	intronic	*ATG3*	1.95E-08
	intergenic	*CYTL1*	3.04E-07
	intronic	*PCDH7*	6.49E-07
	intronic	*RBM47*	8.71E-07
	intronic	*ATP8A1*	6.58E-08
	intergenic	*SPRY1*	3.32E-07
	intronic	*GALNT7*	5.18E-07
	intergenic	*RN7SKP136*	8.93E-07
	intergenic	*CTD-2199O4.1*	2.61E-07
	intronic	*ERBB2IP*	3.00E-07
	intronic	*FSTL4*	1.22E-08
	ncRNA_intronic	*CTC-340A15.2*	6.52E-08
	ncRNA_intronic	*FAM153B:RP11-844P9.1*	5.78E-08
	upstream	*RP11-826N14.4*	1.57E-07
	ncRNA_exonic	*TRIM41:CTC-338M12.7*	3.72E-07
	ncRNA_intronic	*RP1-209A6.1*	1.93E-07
	intergenic	*RP1-104O17.1*	9.87E-09
	intergenic	*RP1-104O17.1*	8.64E-07
	ncRNA_intronic	*RP1-104O17.2*	4.18E-09
	intergenic	*PDE10A*	9.38E-07
	intronic	*DGKB*	6.11E-07

Table S15. Continued

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Otorhinolaryngology and eye	rs112098945	7:79203581	C	0.006	-0.0567 (0.0096)
Otorhinolaryngology and eye	rs147399128	7:79450226	A	0.011	-0.0572 (0.0095)
Otorhinolaryngology and eye	rs139575987	7:81861280	T	0.004	-0.0749 (0.0153)
Otorhinolaryngology and eye	rs183060999	7:82767040	C	0.003	-0.0502 (0.0102)
Otorhinolaryngology and eye	rs181003467	7:89728898	G	0.005	-0.0801 (0.0140)
Otorhinolaryngology and eye	rs113615998	7:127483087	C	0.005	-0.0690 (0.0114)
Otorhinolaryngology and eye	rs189245591	8:6050306	A	0.012	-0.0664 (0.0127)
Otorhinolaryngology and eye	rs117036368	8:27269743	T	0.017	-0.0386 (0.0076)
Otorhinolaryngology and eye	rs1591019	9:12995720	C	0.013	-0.0777 (0.0152)
Otorhinolaryngology and eye	rs72737678	9:87339787	C	0.009	-0.0755 (0.0150)
Otorhinolaryngology and eye	rs117342970	9:93503742	G	0.014	-0.0780 (0.0139)
Otorhinolaryngology and eye	rs139854432	9:113386600	A	0.012	-0.0679 (0.0136)
Otorhinolaryngology and eye	rs17433234	9:131856026	G	0.042	-0.0300 (0.0055)
Otorhinolaryngology and eye	rs181363645	10:25049065	T	0.008	-0.0823 (0.0165)
Otorhinolaryngology and eye	rs149407040	10:31030346	T	0.011	-0.0581 (0.0114)
Otorhinolaryngology and eye	rs370536036	10:121186712	C	0.011	-0.0589 (0.0119)
Otorhinolaryngology and eye	rs112929014	10:131210795	G	0.005	-0.0559 (0.0108)
Otorhinolaryngology and eye	rs142754737	11:43865714	C	0.01	-0.0406 (0.0082)
Otorhinolaryngology and eye	rs115787633	11:63677976	G	0.008	-0.0797 (0.0150)
Otorhinolaryngology and eye	rs36086368	11:117741853	T	0.029	-0.0311 (0.0063)
Otorhinolaryngology and eye	rs141510766	12:4000987	C	0.008	-0.0590 (0.0111)
Otorhinolaryngology and eye	rs183255888	12:18279966	C	0.006	-0.0853 (0.0135)
Otorhinolaryngology and eye	rs17460652	12:38991604	C	0.013	-0.0739 (0.0139)
Otorhinolaryngology and eye	rs142327036	12:97917584	A	0.02	-0.0419 (0.0080)
Otorhinolaryngology and eye	rs185195574	12:102646166	A	0.002	-0.0797 (0.0141)
Otorhinolaryngology and eye	rs183726235	12:121062438	G	0.007	-0.0810 (0.0140)
Otorhinolaryngology and eye	rs530824349	13:24552053	A	0.007	-0.0640 (0.0126)
Otorhinolaryngology and eye	rs117552520	13:58463589	C	0.011	-0.0444 (0.0086)
Otorhinolaryngology and eye	rs58670107	14:34848146	T	0.032	-0.0366 (0.0073)
Otorhinolaryngology and eye	rs78944161	14:55385847	C	0.004	-0.0700 (0.0142)
Otorhinolaryngology and eye	rs143823069	15:78433624	G	0.003	-0.0806 (0.0130)
Otorhinolaryngology and eye	rs140652220	15:79536216	G	0.009	-0.0591 (0.0106)
Otorhinolaryngology and eye	rs180879818	16:8588224	T	0.004	-0.0798 (0.0156)
Otorhinolaryngology and eye	rs77009457	16:52821580	T	0.006	-0.0668 (0.0117)
Otorhinolaryngology and eye	rs142362723	16:53296689	T	0.014	-0.0554 (0.0107)
Otorhinolaryngology and eye	rs140296693	17:30785492	T	0.005	-0.0577 (0.0115)

	Location	Nearest_Gene	P
	intergenic	*AC091813.2*	3.56E-09
	intergenic	*RNU6-849P*	1.92E-09
	intronic	*CACNA2D1*	9.53E-07
	intronic	*PCLO*	9.12E-07
	ncRNA_intronic	*STEAP2-AS1*	9.94E-09
	intronic	*SND1*	1.54E-09
	ncRNA_intronic	*RP11-124B13.1*	1.65E-07
	intronic	*PTK2B*	4.30E-07
	intergenic	*TDPX2*	3.44E-07
	intronic	*NTRK2*	5.21E-07
	intergenic	*OR7E109P*	2.05E-08
	intergenic	*RP11-410K21.2*	6.21E-07
	intergenic	*CRAT*	5.52E-08
	intergenic	*ARHGAP21*	6.18E-07
	intergenic	*RP11-14C22.6*	3.18E-07
	intronic	*GRK5*	6.67E-07
	intergenic	*MGMT*	2.26E-07
	ncRNA_intronic	*HSD17B12:RP11-613D13.5*	7.21E-07
	UTR3	*MARK2*	1.09E-07
	intronic	*FXYD6-FXYD2:FXYD6*	8.53E-07
	ncRNA_intronic	*RP11-664D1.1*	1.03E-07
	intergenic	*RERGL*	2.42E-10
	intergenic	*RP11-804F13.1*	1.08E-07
	ncRNA_intronic	*RMST*	1.53E-07
	intergenic	*RP11-18O15.1*	1.72E-08
	intergenic	*RP11-728G15.1*	6.68E-09
	intergenic	*RP11-307N16.6*	3.67E-07
	intergenic	*RNA5SP30*	2.38E-07
	intergenic	*EGLN3*	5.41E-07
	intergenic	*GCH1*	7.82E-07
	ncRNA_exonic	*IDH3A:RP11-285A1.1*	6.26E-10
	ncRNA_intronic	*RP11-17L5.4*	2.83E-08
	intergenic	*RP11-483K5.3*	3.09E-07
	intergenic	*RP11-297L17.6*	1.25E-08
	intronic	*CHD9*	2.24E-07
	intronic	*PSMD11*	5.09E-07

Table S15. Continued

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Otorhinolaryngology and eye	rs148372107	17:60128232	G	0.01	-0.0635 (0.0112)
Otorhinolaryngology and eye	rs180905197	17:60792325	C	0.01	-0.0815 (0.0160)
Otorhinolaryngology and eye	rs140692279	18:77436593	A	0.009	-0.0831 (0.0146)
Otorhinolaryngology and eye	rs188987627	19:2821190	T	0.007	-0.0715 (0.0124)
Otorhinolaryngology and eye	rs58546514	19:6012782	A	0.007	-0.0605 (0.0119)
Otorhinolaryngology and eye	rs10423785	19:6122667	C	0.009	-0.0494 (0.0098)
Otorhinolaryngology and eye	rs141278022	19:6134399	G	0.01	-0.0481 (0.0097)
Otorhinolaryngology and eye	rs6119406	20:32403459	A	0.021	-0.0550 (0.0104)
Otorhinolaryngology and eye	rs181509690	20:41249767	A	0.008	-0.0699 (0.0140)
Vascular	rs183439493	1:6336577	C	0.007	-0.1867 (0.0377)
Vascular	rs56027618	1:19992708	C	0.009	-0.2045 (0.0355)
Vascular	rs141533450	1:23071144	G	0.005	-0.2147 (0.0390)
Vascular	rs186956366	1:89310489	C	0.005	-0.1835 (0.0360)
Vascular	rs192976456	1:115631128	G	0.009	-0.1956 (0.0399)
Vascular	rs143702759	1:215705263	T	0.011	-0.1308 (0.0249)
Vascular	rs181347218	2:78321676	A	0.01	-0.1346 (0.0239)
Vascular	rs72646816	2:179469252	T	0.002	-0.1527 (0.0307)
Vascular	rs140160158	2:207506872	C	0.009	-0.1648 (0.0310)
Vascular	rs78463690	3:61112510	G	0.007	-0.2653 (0.0417)
Vascular	rs75436416	3:62115280	C	0.009	-0.1809 (0.0311)
Vascular	rs142953390	3:141345291	A	0.006	-0.2123 (0.0393)
Vascular	rs182123115	3:151776286	T	0.003	-0.2041 (0.0356)
Vascular	rs114690408	3:156140697	G	0.006	-0.1751 (0.0344)
Vascular	rs191868075	4:119131235	A	0.001	-0.1525 (0.0311)
Vascular	rs139010916	5:7796705	G	0.007	-0.1629 (0.0293)
Vascular	rs183705493	5:27400465	A	0.003	-0.2032 (0.0369)
Vascular	rs189537860	5:97504885	A	0.008	-0.1862 (0.0378)
Vascular	rs142496645	5:136999942	G	0.006	-0.1892 (0.0386)
Vascular	rs143243129	5:137566499	C	0.014	-0.1368 (0.0260)
Vascular	rs192660913	5:173454893	A	0.004	-0.2135 (0.0402)
Vascular	rs139258100	6:68487539	C	0.008	-0.1890 (0.0385)
Vascular	rs9918416	6:137874827	G	0.005	-0.2602 (0.0392)
Vascular	rs7778151	7:37983762	A	0.031	-0.0797 (0.0162)
Vascular	rs201876806	7:141366203	A	0.002	-0.1782 (0.0356)
Vascular	rs142828077	8:129716168	C	0.006	-0.2037 (0.0384)
Vascular	rs145866362	9:34845420	G	0.005	-0.1913 (0.0389)

	Location	Nearest_Gene	P
	intronic	*MED13*	1.22E-08
	ncRNA_intronic	*RP11-156L14.1:MARCH10*	3.38E-07
	ncRNA_intronic	*RP11-567M16.3*	1.28E-08
	intronic	*ZNF554*	7.69E-09
	ncRNA_intronic	*CTC-232P5.1:RFX2*	3.83E-07
	ncRNA_intronic	*RFX2:CTB-66B24.1*	4.24E-07
	intronic	*RFX2*	7.05E-07
	intronic	*CHMP4B*	1.14E-07
	intronic	*PTPRT*	5.87E-07
	intronic	*ACOT7*	7.07E-07
	exonic	*HTR6*	8.09E-09
	intronic	*EPHB2*	3.63E-08
	intergenic	*GTF2B*	3.42E-07
	intronic	*TSPAN2*	9.73E-07
	intergenic	*KCTD3*	1.47E-07
	ncRNA_intronic	*AC012494.1*	1.88E-08
	ncRNA_intronic	*TTN-AS1:TTN*	6.70E-07
	upstream:downstream	*AC010731.4*	1.07E-07
	intronic	*FHIT*	1.94E-10
	intronic	*PTPRG*	5.87E-09
	intergenic	*RASA2*	6.46E-08
	intergenic	*RP11-246A10.1*	9.98E-09
	intronic	*KCNAB1*	3.54E-07
	intronic	*NDST3*	9.12E-07
	intronic	*ADCY2*	2.85E-08
	intergenic	*CTD-3007L5.1*	3.59E-08
	intergenic	*AC008834.1*	8.36E-07
	intronic	*KLHL3*	9.84E-07
	intergenic	*CDC23*	1.47E-07
	intergenic	*RP11-619L12.1*	1.06E-07
	intergenic	*RP11-301G19.1*	9.11E-07
	intergenic	*BTF3L4P3*	3.17E-11
	intronic	*EPDR1:SFRP4*	9.01E-07
	exonic	*KIAA1147*	5.42E-07
	intergenic	*AC068570.1*	1.16E-07
	intergenic	*FAM205B*	8.94E-07

Table S15. Continued

SUBTYPE	SNP	CHR_POS	Effect_allele	MAF	BETA_SE
Vascular	rs529184264	9:106304580	C	0.01	-0.1910 (0.0328)
Vascular	rs2499074	10:19548616	G	0.003	0.1916 (0.0366)
Vascular	rs140288403	12:26578011	A	0.011	-0.2067 (0.0394)
Vascular	rs192150237	12:117083614	T	0.013	-0.0885 (0.0180)
Vascular	rs977655	13:26304569	T	0.428	0.0267 (0.0054)
Vascular	rs183714483	13:59785211	T	0.005	-0.1528 (0.0292)
Vascular	rs118004027	13:60921520	A	0.02	-0.1228 (0.0243)
Vascular	rs137934155	16:7647041	T	0.005	-0.1402 (0.0287)
Vascular	rs561675364	16:71769017	A	0.011	-0.1305 (0.0260)
Vascular	rs537106596	18:62161477	T	0.004	-0.2103 (0.0388)
Vascular	rs542144583	19:7531151	C	0.009	-0.1495 (0.0289)
Vascular	rs571517049	19:48112003	C	0.008	-0.1420 (0.0277)
Vascular	rs143395477	20:44348859	G	0.022	-0.0964 (0.0178)
Vascular	rs77346510	21:41595557	G	0.01	-0.1181 (0.0239)
Vascular	rs113239628	22:23045501	G	0.007	-0.2000 (0.0350)
Vascular	rs138436163	22:44967113	T	0.009	-0.2286 (0.0387)
Visceral	rs116064503	1:157483563	C	0.014	-0.0215 (0.0036)
Visceral	rs116574040	1:213969148	A	0.008	-0.0239 (0.0046)
Visceral	rs11120239	1:214196849	A	0.026	-0.0153 (0.0029)
Visceral	rs184832856	2:139555831	A	0.006	-0.0311 (0.0056)
Visceral	rs150286579	7:123550997	C	0.009	-0.0331 (0.0061)
Visceral	rs148043468	10:10519756	A	0.007	-0.0263 (0.0054)
Visceral	rs568548387	11:23021186	C	0.002	-0.0282 (0.0057)
Visceral	rs576814352	11:23026712	C	0.001	-0.0292 (0.0057)
Visceral	rs560964605	12:15592550	C	0.003	-0.0313 (0.0063)
Visceral	rs2160419	13:72314346	A	0.014	-0.0201 (0.0041)
Visceral	rs117088052	16:87023365	C	0.003	-0.0274 (0.0053)
Visceral	rs7275118	20:18010447	T	0.341	0.0047 (0.0009)
Visceral	rs567912123	20:60816289	C	0.011	-0.0245 (0.0049)

CHR_POS: chromosome and position. MAF, minor allele frequency. BETA_SE: effect size and standard error.

	Location	Nearest_Gene	P
	intergenic	*RP11-436F21.1*	5.57E-09
	intronic	*MALRD1*	1.59E-07
	intronic	*ITPR2*	1.56E-07
	intergenic	*RP11-497G19.1*	8.77E-07
	intronic	*ATP8A2*	8.53E-07
	intergenic	*RPP40P2*	1.69E-07
	intergenic	*TDRD3*	4.42E-07
	intronic	*RBFOX1*	9.95E-07
	intronic	*AP1G1*	4.97E-07
	intergenic	*RNU7-146P*	5.78E-08
	intronic	*CTD-2207O23.3:ARHGEF18*	2.24E-07
	ncRNA_intronic	*CTD-2571L23.8:GLTSCR1*	3.10E-07
	intergenic	*SPINT4*	6.07E-08
	intronic	*DSCAM*	7.61E-07
	intergenic	*IGLV3-22*	1.14E-08
	ncRNA_intronic	*LINC00207*	3.56E-09
	UTR3	*FCRL5*	3.34E-09
	intergenic	*RP11-323I1.1*	2.01E-07
	intronic	*PROX1*	1.68E-07
	intergenic	*NXPH2*	2.18E-08
	intergenic	*SPAM1*	6.46E-08
	intergenic	*RP11-271F18.4*	9.18E-07
	intergenic	*RP11-17A1.3*	6.85E-07
	intergenic	*RP11-17A1.3*	2.64E-07
	ncRNA_exonic	*PTPRO:RP11-6K23.1*	5.55E-07
	intronic	*DACH1*	9.82E-07
	intergenic	*RP11-134D3.1*	1.95E-07
	intronic	*OVOL2*	2.38E-07
	intronic	*OSBPL2*	6.38E-07

Table S16. Genetic correlations between GWAS on CPSP development after major surgeries and other traits.

p1	p2	rg	se	z	p
CPSP major	CPSP minor	NA	NA	NA	NA
CPSP major	Published CPSP study	0.6812	0.7900	0.8622	0.3886
CPSP major	Chronic headaches pain	-0.2722	0.2843	-0.9574	0.3384
CPSP major	Chronic knee pain	0.1159	0.3336	0.3475	0.7282
CPSP major	Chronic neck shoulder pain	-0.0953	0.3443	-0.2767	0.7820
CPSP major	Ever smoke	-0.1804	0.1477	-1.2220	0.2218
CPSP major	ICD abdominal pain main	0.3049	0.3239	0.9415	0.3464
CPSP major	ICD abdominal pain secondary	-0.2927	0.4291	-0.6821	0.4952
CPSP major	Self-reported abdominal pain	-0.0080	0.2134	-0.0377	0.9699
CPSP major	Self-reported headache	0.1280	0.1670	0.7665	0.4434
CPSP major	Self-reported hip pain	0.5682	0.3477	1.6340	0.1023
CPSP major	Self-reported knee pain	0.5157	0.3168	1.6280	0.1035
CPSP major	Self-reported neck shoulder pain	0.1896	0.1837	1.0320	0.3020
CPSP major	Back pain	0.2897	0.1952	1.4840	0.1379
CPSP major	Depression	0.3943	0.2665	1.4800	0.1390
CPSP major	BMI	0.2191	0.1370	1.5990	0.1097

p1 = trait 1. p2 = trait 2. rg = genetic correlation. se = standard error of rg. p = p-value for rg. h2_obs, h2_obs_se = observed scale h2 for trait 2 and standard error. h2_int, h2_int_se = single-trait LD Score regression intercept for trait 2 and standard error. gcov_int, gcov_int_se = cross-trait LD Score regression intercept and standard error.

h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
NA	NA	NA	NA	NA	NA
0.3478	0.3020	0.9950	0.0057	-0.0030	0.0037
0.0620	0.0073	1.0080	0.0069	-0.0006	0.0047
0.0244	0.0055	0.9974	0.0063	0.0036	0.0043
0.0246	0.0053	1.0010	0.0063	0.0108	0.0045
0.0896	0.0035	1.0630	0.0099	-0.0075	0.0051
0.0101	0.0017	1.0040	0.0068	0.0064	0.0048
0.0055	0.0018	1.0030	0.0063	0.0073	0.0048
0.0177	0.0015	1.0120	0.0072	0.0056	0.0044
0.0424	0.0026	1.0310	0.0094	0.0053	0.0049
0.0222	0.0016	1.0150	0.0067	0.0079	0.0053
0.0375	0.0022	1.0100	0.0087	0.0028	0.0049
0.0318	0.0018	1.0210	0.0076	0.0157	0.0051
0.0418	0.0019	1.0350	0.0096	0.0103	0.0054
0.0608	0.0024	0.9959	0.0101	0.0066	0.0051
0.2050	0.0065	1.0480	0.0238	0.0177	0.0066

Table S17. Genetic correlations between subtype GWASes.

p1	p2	rg	se	z	p
Musculoskeletal	Musculoskeletal	1.0000	0.0000	599000.0000	0.0000
Musculoskeletal	Nerves	-0.5862	1.2390	-0.4731	0.6361
Musculoskeletal	Otorhinolaryngology eye	-0.4083	1.0380	-0.3934	0.6941
Musculoskeletal	Vascular	0.4229	0.8848	0.4779	0.6327
Nerves	Musculoskeletal	-0.5862	1.2390	-0.4731	0.6361
Nerves	Nerves	1.0000	0.0000	335000.0000	0.0000
Nerves	Otorhinolaryngology eye	-0.7503	1.8850	-0.3981	0.6905
Nerves	Vascular	0.2118	1.3410	0.1579	0.8745
Otorhinolaryngology eye	Musculoskeletal	-0.4083	1.0380	-0.3934	0.6941
Otorhinolaryngology eye	Nerves	-0.7503	1.8850	-0.3981	0.6905
Otorhinolaryngology eye	Otorhinolaryngology eye	1.0000	0.0004	2646.0000	0.0000
Otorhinolaryngology eye	Vascular	-0.8999	1.8390	-0.4894	0.6246
Vascular	Musculoskeletal	0.4229	0.8848	0.4779	0.6327
Vascular	Nerves	0.2118	1.3410	0.1579	0.8745
Vascular	Otorhinolaryngology eye	-0.8999	1.8390	-0.4894	0.6246
Vascular	Vascular	1.0000	0.0000	2140000.0000	0.0000

p1 = trait 1. p2 = trait 2. rg = genetic correlation. se = standard error of rg. p = p-value for rg. h2_obs, h2_obs_se = observed scale h2 for trait 2 and standard error. h2_int, h2_int_se = single-trait LD Score regression intercept for trait 2 and standard error. gcov_int, gcov_int_se = cross-trait LD Score regression intercept and standard error.

Table S18. Cross check between our CPSP definition with self-reported CPSP in the UKB.

		Self-reported CPSP		
		controls	*cases*	Total
CPSP phenotype in our study	*controls*	29769	1370	
	cases	658	52	
				31849

h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
0.038710	0.027830	0.990200	0.006166	0.990200	0.006166
0.070890	0.145300	1.001000	0.006623	-0.005340	0.004570
0.024570	0.046060	0.998000	0.005912	-0.002601	0.004446
0.086020	0.130700	0.996400	0.006333	-0.003233	0.004652
0.036860	0.027920	0.990800	0.006192	-0.005340	0.004570
0.073510	0.144200	1.001000	0.006625	1.001000	0.006625
0.022120	0.046180	0.998500	0.005889	0.003732	0.004334
0.082540	0.132300	0.996600	0.006357	0.005406	0.005147
0.037880	0.027770	0.990500	0.006158	-0.002601	0.004446
0.071520	0.144800	1.001000	0.006635	0.003732	0.004334
0.026240	0.046310	0.997800	0.005920	0.997800	0.005920
0.079040	0.130000	0.996800	0.006301	-0.004139	0.004405
0.038750	0.027780	0.990200	0.006158	-0.003233	0.004652
0.070470	0.145400	1.001000	0.006609	0.005406	0.005147
0.023240	0.045940	0.998200	0.005928	-0.004139	0.004405
0.088770	0.129400	0.996100	0.006333	0.996100	0.006333

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Chapter 6

Pain Predict Genetics: Protocol for a prospective observational study of clinical and genetic factors to predict the development of postoperative pain

Song Li [1], Regina L.M. van Boekel [2], Sandra A.S. van den Heuvel [2], Marieke J.H. Coenen [1], Kris C.P. Vissers [2]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands.

[2] Department of Anesthesiology, Pain and Palliative Medicine, Radboud university medical center, Nijmegen, The Netherlands.

Corresponding author:
Regina L.M. van Boekel, Radboud University Medical Center, Department of Anesthesiology, Pain and Palliative Medicine, Rianne.vanBoekel@radboudumc.nl

Abstract

Introduction. Postoperative pain remains a challenging medical condition impacting the quality of life of every patient. Although several predictive factors for postoperative pain have been identified, an adequate prediction of postoperative pain in patients at risk has not been achieved yet.

The primary objective of this study is to identify specific genetic risk factors for the development of acute and chronic postoperative pain to construct a prediction model facilitating a more personalized postoperative pain management for each individual. The secondary objectives are to build a databank enabling researchers to identify other risk factors for postoperative pain, for instance, demographic and clinical outcome indicators; provide insight into (genetic) factors that predict pharmacological pain relief; investigate the relationship between acute and chronic postoperative pain.

Methods and analysis. In this prospective, observational study, patients who undergo elective surgery will be recruited to a sample size of approximately 10,000 patients. Postoperative acute and chronic pain outcomes will be collected through questionnaires at different time points after surgery in the follow-up of six months. Potential genetic, demographic, and clinical risk factors for prediction model construction will be collected through blood, questionnaires, and electronic health records, respectively.

Genetic factors associated with acute and/or chronic postoperative pain will be identified using a genome-wide association (GWA) analysis. Clinical risk factors as stated in the secondary objectives will be assessed by multivariable regression. A clinical easy-to-use prediction model will be created for postoperative pain to allow clinical use for the stratification of patients.

Ethics and dissemination. The Institutional Review Board of the Radboud university medical center approved the study (authorization number: 2012/117). The results of this study will be made available through peer-reviewed scientific journals and presentations at relevant conferences, which will finally contribute to personalized postoperative pain management.

Trial registration number NCT02383342

Strengths and limitations of this study.

- This is a large prospective study to identify genetic and other risk factors for postoperative pain.
- We will build a databank with comprehensive interdisciplinary measurements that assess postoperative pain from multiple perspectives.
- Outcome measurements of pain by patient-reported outcomes, rather than evaluated by professionals.
- The investigating biomarkers of postoperative pain are limited to genetic variants.

Keywords
Postoperative pain, Genome-wide association study (GWAS), Risk factor, Prediction model, Pharmacogenetics

Introduction

Pain after surgery remains a challenging medical and societal problem [1]. Pain is one of the most common postsurgical side effects, with moderate to severe acute postoperative pain occurring in about 41% of the patients [2-4]. Severe postoperative pain is associated with an increased incidence of postoperative complications [5], including prolonged hospital stay, readmissions, and significant reduction of patient satisfaction and quality of life [6, 7]. Besides, acute postoperative pain is associated with chronic pain development after surgery [8]. A recent position paper from the International Association for the Study of Pain stated that among the almost 40 million people undergoing surgery globally each year, one out of ten develops chronic postsurgical pain (CPSP), and one out of hundred suffers from severe CPSP, which will negatively affect patients' quality of life [9]. In addition, postoperative pain is a considerable burden on health care service costs, both directly due to patients' increased consumption of medical care and indirectly due to absenteeism, reduced productivity, and increased social welfare payments [10-15].

The management of both acute postoperative pain [2, 16] and CPSP [2, 17] has remained suboptimal. Despite major investments in clinical protocols and guidelines for structural pain management, infrastructure, and acute pain services (APS), no significant outcome improvements in the quality of postoperative pain management for individual patients have been achieved in the last fifteen years [10, 11].

Given the high incidence of postoperative pain, identifying patients at risk for CPSP before the operation is important to apply more personalized pain prevention strategies. The most important demographic and clinical risk factors for postoperative pain are younger age, female sex, smoking, history of depressive symptoms, anxiety symptoms, sleep difficulties, higher body mass index, presence of preoperative pain, and use of preoperative analgesics [18]. Based on these factors, models have been developed to predict severe acute postoperative pain [19, 20] and CPSP [21, 22]. A recent study has evaluated a presurgical risk score for CPSP in a prospective cohort, and it reliably identified about 70% of the patients undergoing surgeries at risk of CPSP [23, 24].

As a multifactorial trait, the incidence variation of CPSP in the population can be explained partly by the demographic and clinical risk factors mentioned above, and partly due to the genetic and epigenetic differences among patients [25, 26]. To

improve the accuracy and power of prediction, efforts have been made to predict CPSP using genetic variants [21, 24]. However, no unequivocal genetic predictors have been found yet. In addition, many exploratory studies investigated the possible role of candidate genes in postoperative pain development. In particular, associations have been found between CPSP and the *μ-opioid receptor (OPRM1)* and *catechol-O-methyl transferase (COMT)* genes [27, 28]. Still, these results have not been confirmed by others. *OPRM1* is also associated with basal pain sensitivity differences [29], which could be caused by the altered opioid binding potential in the central nervous system [30]. More recently, hypothesis-free methods, such as genome-wide association studies, have been applied for CPSP to identify markers across the genome [31, 32]. One of the studies showed that a genetic variant in the *protein-kinase C* gene is linked to neuropathic pain after complete joint replacement. This gene is involved in long-term potentiation, synaptic plasticity, chronic pain, and memory, indicating that this gene may be relevant for neuropathic pain initiation. The disadvantage of this study is that it was small in terms of patient numbers and only focused on one specific surgical procedure.

Besides genetic variants for altered pain sensitivity, gene variants in drug metabolism can also play a role. Understanding the reasons for ineffective treatment can facilitate the early identification of patients at risk and provide more effective and customized postoperative management. Some associated genes with pain treatment outcomes are also involved in pain development, such as *COMT* [33-35]. Genes involved in the action site of active drugs or the drugs' metabolism might play a role in the therapeutic response of this drug. A well-known example is the *cytochrome P450* (*CYP*) family investigated for several drugs (e.g., codeine and tramadol) [36]. However, this area has never been charted in a large population [37].

To date, adequate prediction of patients at risk for postoperative pain in clinical practice has not been achieved for several reasons. First, although many demographic, clinical, and lifestyle factors of postoperative pain have been reported [18], a lack of consensus on the best outcome indicators for postoperative pain management [38, 39] hinders choosing the proper outcome variables for prediction model construction. Second, the potential genetic risk factors of postoperative pain prediction remain obscure. The role of genetic factors in postoperative pain have not been investigated sufficiently, making it challenging to select appropriate genetic risk factors to construct a prediction model. Third, when prediction models are updated, external validation (i.e., in a new population) is important before being implemented in a clinical setting [40-43], which is often difficult due to the lack of validation cohorts. For these reasons, we hypothesize

that a global structural multicenter diagnostic program of postoperative pain in a surgical patient population will be valuable for better identifying patients at risk of CPSP and ultimately preventing postoperative pain using individualized pharmacological and non-pharmacological interventions.

Objectives

The primary objective of the Pain Predict Genetics (PPG) study is to identify genetic risk factors for acute and chronic postoperative pain development and to construct a prediction model for personalized postoperative pain management.

The secondary objectives of the PPG study are to build a databank enabling researchers to 1) identify other risk factors for the development of acute and chronic postoperative pain; 2) provide insights into complications and other clinical outcome indicators after surgery; 3) provide insights into the relationship between acute and chronic postoperative pain; 4) identify (genetic) factors that predict pharmacological pain relief. The databank will be open to the public with access fees, and reasonable requests will be discussed in the research group before approval.

The extensive data collection on (chronic) postoperative pain development of patients undergoing surgery offers many possibilities for additional research questions using conventional statistical methods and artificial intelligence, e.g., machine learning. The cohort could be used to 1) conduct epidemiological studies; 2) investigate other parameters (for example, types of surgery) that are involved in the development of chronic postoperative pain; 3) validate new prediction models for (chronic) postoperative pain; 4) identify factors for the postoperative outcome (for example, death, long-term hospitalization, complications); 5) collaborate with other groups to perform large-scale analysis to identify predictors for the development of (chronic) postoperative pain.

Methods and analyses

Study design

A prospective, observational study of 10,000 patients will undergo elective surgery. This study will run for at least ten years, during which period it must be possible to include the intended number of patients. Patient inclusion after CMO (Human

Research Committee, in Dutch Commissie Mensgebonden Onderzoek) approval was started in March 2015, and patient inclusion was temporarily stopped in 2020 due to COVID restrictions. In the near future, this study will be continued as a multicenter study; hospitals have already been approached and indicated that they intend to participate.

Patient and public involvement

During the design of the study the patients aided in the pilot phase of the questionnaires, during the recruitment the patients are informed concerning the project. In addition, patient reported outcomes will be used. Patients will be informed about the outcome of the study at several moments (depending on the obtained results).

Participants

Patients who undergo elective surgery and are eligible for this study will be approached before their planned surgery during the preoperative consultation. In this way, potential participants will have sufficient time to consider the study information. If any questions arise, it is possible to contact the researchers by telephone or ask the questions during the preoperative consultation. During the preoperative consultation (outpatient clinic or by telephone), the physician (assistant) will ask the patient if they are interested to participating in the study. If the patient is willing to participate, the informed consent form will be signed and dated. If patients have an online preoperative consultation, this procedure will take place digitally, and patients receive the study forms (signed in advance) at home to return if they consent.

Patients are eligible for study inclusion if they 1) are older or equal to 16 years; 2) undergo elective surgery with an incision, including cardiothoracic surgery (e.g., cardiomyotomy), general surgery (e.g., breast resection), neurological surgery (e.g., nerve decompression), oral and maxillofacial surgery (e.g., removal of head and neck benign and malignant tumors), otorhinolaryngology (e.g., tympanoplasty), plastic surgery (e.g., breast reconstruction), trauma and orthopedic surgery (e.g., arthroplasty), urology (e.g., prostatectomy) and vascular surgery (e.g., treatment of varicose veins); 3) can read and understand the patient information; 4) will provide informed consent. Patients will be excluded if they 1) intend to undergo another surgery within six months; 2) do not have enough knowledge of the language in words and understanding to complete questionnaires.

Measurements

Questionnaires

After written informed consent, participants will be asked to complete questionnaires before and after their surgery. An overview of the study workflow and data collection time points can be found in **Figure 1** and **Table 1**. All patient data will be stored in an online digital database, Castor [44]. The reliability and validity of all questionnaires for measurement collection have been validated in the corresponding populations.

The first digital questionnaire must be completed the day before the surgery (no longer than one week before). Before surgery, the following parameters will be collected (**Table 1,** Supplementary File 1): demographic characteristics (such as gender, age, BMI), expected incision size in mm, pain intensity, pain disability, preoperative anxiety and need for information, pain catastrophizing, pain sensitivity, preoperative chronic pain characteristics, and depressive symptoms.

After surgery, the following parameters will be collected: actual incision size in mm on day 1; pain intensity on day 1, 2, 3, week 1 and 6, and month 3 and 6; physical activities on day 1, 2, 3, week 1; pain disability on week 1 and 6, and month 3 and 6; postoperative chronic pain characteristics on month 3 and 6; characteristics of pain on month 3 and 6.

Pain intensity will be measured with an 11-point numerical rating scale (NRS) at rest and during a normal patient action at that time [20]. The endpoints represent the extremes of the pain experience: 0 means "no pain at all", and 10 means "worst possible pain".

Pain disability (disability associated with pain) will be measured by the widely used Pain Disability Index Dutch language version (PDI) [45, 46]. The PDI is a 7-item questionnaire to investigate the magnitude of the self-reported disability in different situations such as work, leisure time, daily life activities, and sports. The questionnaire is constructed on an 11-point NRS in which 0 means "no disability" and 10 means "maximum disability".

Preoperative anxiety and need for information will be evaluated by the Amsterdam Preoperative Anxiety and Information Scale (APAIS) [47]. The APAIS consists of six questions and each score on a 5-point Likert scale from 1 (not at all) to 5 (extremely), with four questions to assess the patient's preoperative anxiety score and two questions to assess the patient's need for information regarding the scheduled surgery and anesthesia [20].

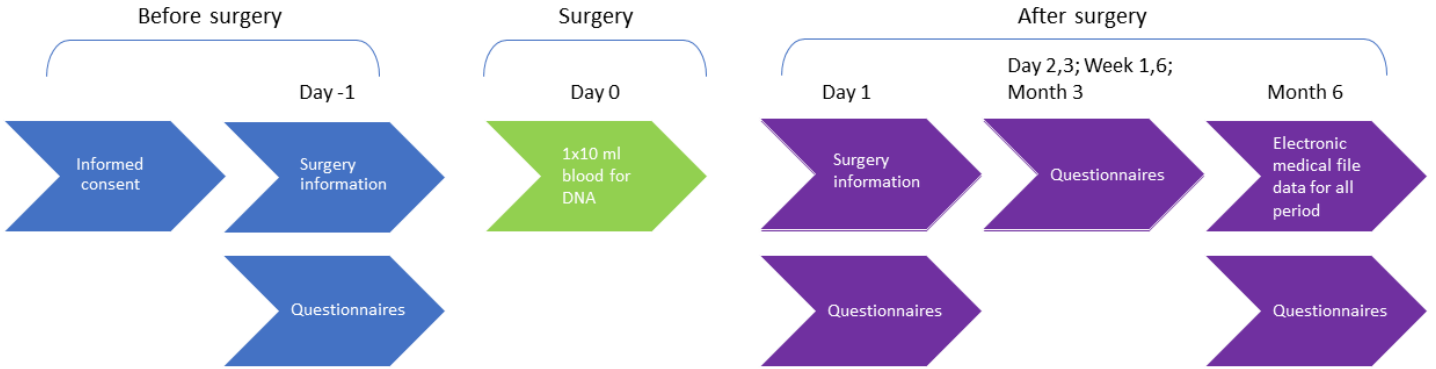

Figure 1. Pain Predict Genetics study design overview. After written informed consent, participants will be asked to complete questionnaires before and after their surgery. One tube of blood will be collected for DNA isolation using the intravenous line in place for surgery. Clinical information will be collected from the electronic patient file after the operation.

Table 1. Overview of data collection.

	T0	Day -1	Surgery	Day 1	Day 2	Day 3	Week 1	Week 6	Month 3	Month 6
Informed consent	x									
Questionnaires										
Demografic data		x								
Incision size		x		x						
Pain scores		x		x	x	x	x	x	x	x
Physical activities				x	x	x	x			
Pain disability index		x					x	x	x	x
APAIS		x								
PCS		x								
PSQ		x								
Chronic pain		x							x	x
IDS depression		x								
Brief pain inventory									x	x
Data electronic medical file										
Physical status by ASA										x
Type of surgery										x
Duration of surgery										x
Type of anesthesia										x
Complications										x
Hospital stay										x
Pain medication use										x

Table 1. Continued

	T0	Day -1	Surgery	Day 1	Day 2	Day 3	Week 1	Week 6	Month 3	Month 6
Incision size										x
Second surgery within 6 months										x
General clinical outcome indicators										x
Body material*										
1x10 ml blood for DNA			x							

*In the event that it is not possible to collect a blood sample during surgery, the subject may be asked to provide a DNA sample via a saliva collection tube.
APAISI, Amsterdam Pre-operative Anxiety and Information Scale. PCS, Pain Catastrophizing Scale. PSQ, Pain Sensitivity questionnaire. IDS, Inventory of Depressive Symptomatology. ASA, American Society of Anesthesiologists classification.

Pain catastrophizing is generally described as an absurd negative orientation towards hurtful stimuli and is important in pain coping [48]. It will be measured by the Pain Catastrophizing Scale (PCS), a self-evaluating questionnaire consisting of 13 questions. People are asked to indicate the degree to which they have thoughts and feelings when experiencing pain using the 0 (not at all) to 4 (all the time) scale, and a total score will be yielded (range from 0 to 52).

The Pain Sensitivity Questionnaire (PSQ) will measure patients' preoperative pain sensitivity [49, 50]. The PSQ consists of 17 questions that describe daily life situations; respondents score their pain intensity for these situations on an NRS by scoring 0 (not painful) to 10 (severest pain imaginable).

The severity of overall depressive symptoms will be assessed by the Inventory of Depressive Symptomatology Self Report (IDS-SR) [51, 52]. IDS-SR is a 30-item questionnaire, and each item has four statements scored on a four-point scale from 0 to 3. There are two items about either increasing or decreasing appetite and two items about increasing or decreasing weight. Only the item with the higher score from both pairs will be chosen. The total score is based on 28 items and ranges from 0 to 84.

Physical activities (ability to perform normal activities) will be measured by questions assessing the degree of physical activities interfered by surgery, including bed activities (such as turning), breathing deeply of coughing, sleeping, and activities out of bed. Each item is scored on an 11-point NRS in which 0 means did not interfere and 10 means completely interfered. These questions are derived from the validated International Pain Outcomes questionnaire and are found responsive to asking patients about their ability to perform normal activities directly after surgery [53].

Characteristics of pain will be measured by the Brief Pain Inventory – Short Form (BPI-SF), which is a shortened version of the Brief Pain Inventory [54]. BPI-SF evaluates pain severity during the past 24 hours and current level, with 0 representing "no pain" and 10 "the worst pain imaginable". Seven items in BPI-SF assess interference with daily functioning (such as general activity, walking, and work) on an 11-point scale, where 0 represents "no interference" and 10 "complete interference".

Collection of body material

One tube of blood will be collected for DNA isolation. The burden for the patient is minimalized as blood will be taken using the intravenous line in place for surgery. If it is impossible to collect blood presurgically or postsurgically, we will collect saliva for DNA isolation (Genefix DNA saliva collectors; GFX-02/50, Isohelix).

Clinical information

The following clinical information will be collected from the electronic patient file six months after operation (**Table 1**): physical status by The American Society of Anesthesiologists classification (ASA-status); type of surgery; duration of surgery; type of anesthesia; postoperative complications within 30 days after surgery, one-time retrospectively, which is defined as any medical adverse outcome occurring between admission and 30 days after operation. Complications occurring in the operation room and complications directly related to anesthesia (e.g., nausea which resolves immediately after medication in the operation room) will not be included [5, 55]. Furthermore, data on pain medication use, before surgery and after surgery; actual incision size in mm; second surgery within 6 months; general clinical outcome indicators, including surgical site infection at 30 days, stroke within 30 days of surgery, death within 30 days of surgery, admission to the intensive care unit within 14 days of surgery, readmission to hospital within 30 days of surgery, and length of hospital stay (with or without in-hospital mortality) will be collected [38].

Outcome measures

The outcome measures are acute postoperative pain and chronic postoperative pain. Acute postoperative pain is defined as pain experienced directly after surgery. Thresholds or cut-off points of the pain intensity are set as none to mild (0-3), moderate (4-7), and severe (8-10) [56, 57]. The definition of CPSP is in agreement with IASP terminology of chronic postsurgical pain, i.e., "*chronic pain that develops or increases in intensity after a surgical procedure persists beyond the healing process, i.e., at least 3 months after the surgery*" [9]. CPSP will be measured by a chronic pain characteristics questionnaire postoperatively at three and six months. Patients will be asked to indicate whether they had a recent pain experience, the site of pain, and whether it lasted more than three months [58, 59]. The intensity of CPSP will also be characterized by the pain scores questionnaire using the same threshold as acute postoperative pain. The influence of pain on functional and mood changes will be measured by the PDI and the BPI-SF.

Sample size calculation

The power of the genetic study is based on the primary research question investigating which genetic factors are associated with postoperative pain. Power is calculated using the Genetic Power Calculator [60], and the estimated number of patients is based on a GWA approach. For chronic postoperative pain, we assume a case-control analysis for discrete traits (2df test), a risk allele frequency of 30%, a linkage disequilibrium (D') of 0.8, a prevalence of chronic postoperative pain of 15%, and the relative risk of chronic

postoperative pain for persons who are heterozygous of 1.5 and for homozygous persons of 2.25. For a power of 80% with a p-value cut off 5×10^{-8} (genome-wide significance threshold), we need 750 patients with chronic postoperative pain and 4,250 people without chronic postoperative pain. For acute pain, the power is even higher. With the same population, we have more than 80% power to detect a relative risk of 1.2 and 1.44 for heterozygous and homozygous patients, respectively. This higher power is due to the higher prevalence of acute (moderate to severe) pain of 55%. Most importantly, results will be replicated in the additional study participants, as the total number of patients included in the study will be 10,000. In addition, we will use cohorts of our collaborators for replication purposes.

Statistical analysis

The key objective is to identify genetic risk factors that can predict development of acute or chronic postoperative pain and validate previously reported SNPs. A GWA approach will be used as the main analysis. Phenotype data and DNA will be used to identify genetic factors. We will use 5,000 patients for the discovery of genetic variants. Samples will be genotyped with the Infinium Global Screening Array (Illumina). Pre-imputation quality control, principal component analyses, and imputation will follow the RICOPILI pipeline [61]. Potential confounding by ethnic origin will be corrected by principal component analyses. The 1000 Genomes reference panel will be used for imputation, followed by post-imputation quality control in PLINK [62]. Associations between SNPs and the presence of acute or chronic pain will be performed using cutting-edge methods when data collection is finished. Results will be replicated. SNPs that can be validated will be included in the prediction model described below.

Secondary objectives include identifying other potential risk factors for acute and chronic postoperative pain. Therefore, a univariate association of each potential predictor will be calculated and tested in a multivariable regression model. We will use a least absolute shrinkage and selection operator (Lasso) regression. Shrinkage is where data values are shrunk towards a central point, like the mean. Lasso is a regression analysis method that performs both variable selection and regularization to enhance the prediction accuracy and interpretability of the statistical model it produces. After identifying these risk factors, a prediction rule will be created for (moderate to severe) acute and chronic postoperative pain. Based on this prediction rule, a simple, clinically easy applicable tool will be developed to allow clinical use for the stratification of patients. The predictive performance will be studied in another cohort of patients to test whether the rule is generalizable across time and place. Because it appears from the literature that acute and chronic

pain are correlated after surgery, additional correlation analysis will be performed to investigate this correlation in the data.

Similar approaches will be followed to identify the clinical and genetic factors that predict pharmacological pain relief. For some pain medicines, genes that impact pain relief are already known (e.g., *CYP2D6* and morphine). We will first investigate those genes to see if these variants indeed contribute to pharmacological pain relief differences.

Ethics and dissemination

The study will be conducted according to the principles of the Declaration of Helsinki version 2013 and in accordance with the Medical Research Involving Human Subjects Act and Good Clinical Practice. The study was approved by the local ethics committee for human research in Nijmegen (Medical Review Ethics Committee Region Arnhem-Nijmegen, authorization number: 2012/117). This study was registered on ClinicalTrials.gov (NCT02383342).

The privacy of the participants is guaranteed by storing encrypted data. Every participant will receive a pseudo-anonymous study number. No identifying data is recorded within the meaning of the law. The key is only accessible to the study team and monitors. Data and material will only be used in coded form within possible collaborations.

The results of this study will be made available through peer-reviewed scientific journals and presentations at relevant conferences. After a thorough evaluation, decisions will be made regarding including the identified risk factors and constructed prediction models into clinical guidelines, thus facilitating personalized postoperative pain management.

Discussion

This cohort will be a large prospective study to identify risk factors for postoperative pain and to build and evaluate dedicated prediction models for postoperative pain in surgical patients. In addition, the comprehensive information collected in this study will also enable us to answer other research questions regarding postoperative pain, such as the relationships between acute and chronic postoperative pain

development. Eventually, these results will be applied in the clinical settings to improve the quality of life for patients who develop postoperative pain.

The strengths of this study are that we will include all elective major operations rather than limiting to one specific operation as in previous studies [32], which allows us to investigate the shared genetic background of postoperative pain in different operations. Furthermore, as there are discrepancies in pain intensity scores understanding [63] and pain management decisions [63, 64] between patients and caregivers, the patient's perspective should be respected and assessed for pain evaluation and management [65, 66]. Therefore, pain assessment will be conducted by patients themselves (patient-reported outcomes) rather than professionals in this study, leading to a more comprehensive outcome assessment and interpretation [67]. Moreover, the single-use of NRS might be inadequate for patients' pain experience evaluation and pain management decisions [66, 68, 69]. Thus, another strength of this cohort is that the experience of pain will be estimated by multidimensional measurements focusing on patients' overall functionality rather than merely a NRS pain score. Besides, the comprehensively collected information for postoperative pain in this cohort also empowers analysis that cannot be performed in large-scale registry data (e.g., UK Biobank) as such phenotype data is not available in those datasets. The data collected in this cohort will also enable additional research using conventional and cutting-edge statistical methods like artificial intelligence.

The possible limitations of this study are that we will only investigate DNA variants as biomarkers for pain prediction as our primary research goal. However, other epigenetic [69] [70], transcriptomic [70], proteomics [71], and metabolic markers [72] are also potentially involved in (postoperative) pain development. For instance, recent studies indicate that methylation patterns might predict opioid treatment outcomes [69, 70]. As the DNA sample of patients is accessible, we will be able to characterize the multi-omics biomarker signatures of postoperative pain in future research, such as investigating the association between epigenetic changes and postoperative pain. In addition, when prediction tools are applied in clinical settings, the sensitivity and specificity of prediction tools are crucial to evaluate their adequacy and usefulness [73]. Although the measurement tools used in prediction models are well-validated and verified (see methods), our findings could still be subject to false positive or negative errors because all measurement tools have limitations. Furthermore, chronic pain assessment is more complex than acute pain [74], and GWAS findings are sometimes incidental [75]. We will consider seeking other available cohorts for validation and applying other statistical methods

to validate our findings in future studies, such as polygenic risk scores [76]. Another potential limitation is that loss of follow-up of patients might result in lower patient numbers than expected. Despite this potential concern, we still expect a sufficient sample size as additional centres will start patient inclusion, and the measurements are mainly from patient-reported outcomes via digital follow-up.

Identifying the genetic background of postoperative pain development may give valuable insights into the mechanisms underlying the relationship between postoperative pain and complications after surgery. This may open the way to identify new targets for treatment and potentially simplify the risk profiling assay for future use, yielding a simpler, more accurate, and cost-efficient assay or product. The contribution of improved prevention and treatment of pain after surgery will benefit many patients undergoing surgery and society by decreasing health care service costs.

Trial status

Patient recruitment is expected to continue until 2025. Recruitment has already started in Radboud university medical center, with more than 500 patients recruited as of October 2021. National and international collaborations will be greatly accepted after careful consideration.

Author contributions

RvB, MC and KV are responsible for overall planning and execution, formulation and evolution of overarching research goals and aims, development and design of the methodology. RvB, MC, and SvdH will be responsible for project management and coordination responsibility. Analyses and data visualization will be conducted by SL, RvB, and MC. SL prepared the draft of the manuscript, and all authors critically revised the manuscript.

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Competing interests statement

The authors have no relevant financial or non-financial interests to disclose.

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Supplementary materials

Appendix a: General data

General data

- What is your year of birth?
- What is your gender? male/female
- What is your length ? ..... cm
- What is your weight ? ..... kg

- What country were you born in?.....

- What country(ies) were your parents born in?.....

- What country(ies) were your grandparents born in? .....

- What human race are you? (black, white, Asian, etc.) .....

Data of the surgery:

- Would you please describe your surgery:

.....
.....
.....
.....
.....

- How much pain do you expect after surgery (0= no pain, 10=worst pain imaginable)
- Will you stay one or more nights in the hospital after surgery? Yes / No

Appendix b: Pain before and after surgery

Circle how much pain you have, expressed as a number. The pain score means a score between 0 and 10, where 0 means no pain and 10 means the worst pain imaginable. For your pain, consider a figure between 0 and 10. You also tick whether you think the pain is acceptable or not.

Pain while being at rest at this moment (0-10)	No pain 0-1-2-3-4-5-6-7-8-9-10 worst pain imaginable
Pain score at this moment if you perform a normal effort (0-10)	No pain 0-1-2-3-4-5-6-7-8-9-10 worst pain imaginable
Do you think pain is acceptable to you at this moment?	☐ Pain acceptable ☐ Pain not acceptable
Only pre-operatively: How much pain do you expect after surgery?	No pain 0-1-2-3-4-5-6-7-8-9-10 worst pain imaginable

Appendix c: Physical activities

Circle the one number below that best describes how much, since your surgery, pain interfered with or prevented you from doing physical activities, expressed by figure. The score means a figure between 0 and 10, where 0 means no interference and 10 means complete interference.

1. How much has pain interfered with or prevented you from doing activities in bed such as turning, sitting up, changing position (0= did not interfere, 10= completely interfered)

   0 1 2 3 4 5 6 7 8 9 10

2. How much has pain interfered with or prevented you from breathing deeply of coughing (0= did not interfere, 10= completely interfered)

   0 1 2 3 4 5 6 7 8 9 10

3. How much has pain interfered with or prevented you from sleeping (0= did not interfere, 10= completely interfered)

   0 1 2 3 4 5 6 7 8 9 10

4. Have you been out of bed since your surgery?

   Yes/no

5. If yes, how much did pain interfere or prevent you from doing activities out of bed such as walking, sitting in a chair, standing at the sink (0= did not interfere, 10= completely interfered)

   0 1 2 3 4 5 6 7 8 9 10

Appendix d: Pain disability index

We would like to know how much pain is preventing you from doing what you would normally do or from doing it as well as you normally would. Respond to each category indicating the overall impact of pain in your life, not just when pain is at its worst. For each of the 7 categories of life activity listed, please circle the number on the scalethat describes the level of disability you typically experience. A score of 0 means no disability at all, and a score of 10 signifies that all of the activities in which you would normally be involved have been totally disrupted or prevented by your pain. In case of no pain, please circle "0".

1. Family/Home Responsibilities This category refers to activities of the home or family. It includes chores or duties performed around the house (e.g. yard work) and errands or favors for other family members (e.g. driving the children to school).	No disability Worst disability 0-1-2-3-4-5-6-7-8-9-10
2. Recreation This disability includes hobbies, sports, and other similar leisure time activities.	No disability Worst disability 0-1-2-3-4-5-6-7-8-9-10
3. Social activity This category refers to activities, which involve participation with friends and acquaintances other than family members. It includes parties, theater, concerts, dining out, and other social functions.	No disability Worst disability 0-1-2-3-4-5-6-7-8-9-10
4. Occupation This category refers to activities that are part of or directly related to one's job. This includes non-paying jobs as well, such as that of a housewife or volunteer.	No disability Worst disability 0-1-2-3-4-5-6-7-8-9-10
5. Sexual behavior This category refers to the frequency and quality of one's sex life.	No disability Worst disability 0-1-2-3-4-5-6-7-8-9-10

Appendix d: Continued

6. Self care This category includes activities, which involve personal maintenance and independent daily living (e.g. taking a shower, driving, getting dressed, etc.)	No disability 0-1-2-3-4-5-6-7-8-9-10	Worst disability
7. Life-support activities This category refers to basic life supporting behaviors such as eating, sleeping and breathing.	No disability 0-1-2-3-4-5-6-7-8-9-10	Worst disability

Appendix e: Anxiety and need for information

Please circle the number on the scale that describes your experience:

The Amsterdam Preoperative Anxiety and Information Scale (APAIS):

	Not at all				**Extremely**
I am worried about the anesthetic	1	2	3	4	5
The anesthetic is on my mind continually	1	2	3	4	5
I am worried about the procedure	1	2	3	4	5
The procedure is on my mind continually	1	2	3	4	5
I would like to know as much as possible about the anesthetic	1	2	3	4	5
I would like to know as much as possible about the procedure	1	2	3	4	5

Appendix f: Pain Catastrophizing Scale (PCS)

Pain Catastrophizing Scale (PCS)

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0=not at all 1=to a slight degree 2=to a moderate degree 3=to a great degree 4=all the time

When I'm in pain …….

1.	I worry all the time about whether the pain will end	0	1	2	3	4
2.	I feel I can't go on	0	1	2	3	4
3.	It's terrible and I think that it's never going to get any better	0	1	2	3	4
4.	It's awful and I feel that it overwhelms me	0	1	2	3	4
5.	I feel that I can't stand it any more	0	1	2	3	4
6.	I become afraid that the pain will get worse	0	1	2	3	4
7.	I keep thinking of other painful events	0	1	2	3	4
8.	I anxiously want the pain to go away	0	1	2	3	4
9.	I can't seem to keep it out of my mind	0	1	2	3	4
10.	I keep thinking about how much it hurts	0	1	2	3	4
11.	I keep thinking about how badly I want the pain to stop	0	1	2	3	4
12.	There's nothing I can do to reduce the intensity of the pain	0	1	2	3	4
13.	I wonder whether something serious may happen	0	1	2	3	4

Appendix g: Pain Sensitivity Questionnaire

Pain Sensitivity Questionnaire

This questionnaire contains a series of questions in which you should imagine yourself in certainsituations. You should then decide if these situations would be painful for you and if yes, how painful they would be.

Let 0 stand for no pain; 1 is an only just noticeable pain arid.I0 the most severe painthat you can imagine or consider possible.

Please mark the scale with a cross on the number that is most true for you. Keep in mind that there are no "right" or "wrong" answers; only your personal assessment of the situation counts.

Please try as much as possible not to allow your fear or aversion of the imagined situations affect your assessment of painfulness.

1. Imagine you bump your shin badly on a hard edge, for example, on the edge of a glass coffee table. How painful would that be for you?
   0 = not at all painful, 10= most severe pain imaginable

   0 1 2 3 4 5 6 7 8 9 10

2. Imagine you burn your tongue on a very hot drink.
   0 1 2 3 4 5 6 7 8 9 10

3. Imagine your muscles are slightly sore as the result of physical activity.
   0 1 2 3 4 5 6 7 8 9 10

4. Imagine you trap your finger in a drawer.
   0 1 2 3 4 5 6 7 8 9 10

5. Imagine you take a shower with lukewarm water.
   0 1 2 3 4 5 6 7 8 9 10

6. Imagine you have mild sunburn on your shoulders.
   0 1 2 3 4 5 6 7 8 9 10

7. Imagine you grazed your knee falling off your bicycle.
   0 1 2 3 4 5 6 7 8 9 10

8. Imagine you accidentally bite your tongue or cheek badly while eating.
   0 1 2 3 4 5 6 7 8 9 10

9. Imagine walking across a cool tiled floor with bare feet.
   0 1 2 3 4 5 6 7 8 9 10

10. Imagine you have a minor cut on your finger and inadvertently get lemon juice in the wound.
    0 1 2 3 4 5 6 7 8 9 10

11. Imagine you prick your fingertip on the thorn of a rose.
    0 1 2 3 4 5 6 7 8 9 10

12. Imagine you stick your bare hands in the snow for a couple of minutes or bring your hands in contact with snow for some time, for example, while making snowballs.
    0 1 2 3 4 5 6 7 8 9 10

13. Imagine you shake hands with someone who has a normal grip.
    0 1 2 3 4 5 6 7 8 9 10

14. Imagine you shake hands with someone who has a very strong grip.
    0 1 2 3 4 5 6 7 8 9 10

15. Imagine you pick up a hot pot by inadvertently grabbing its equally hot handles.
    0 1 2 3 4 5 6 7 8 9 10

16. Imagine you are wearing sandals and someone with heavy boots steps on your foot.
    0 1 2 3 4 5 6 7 8 9 10

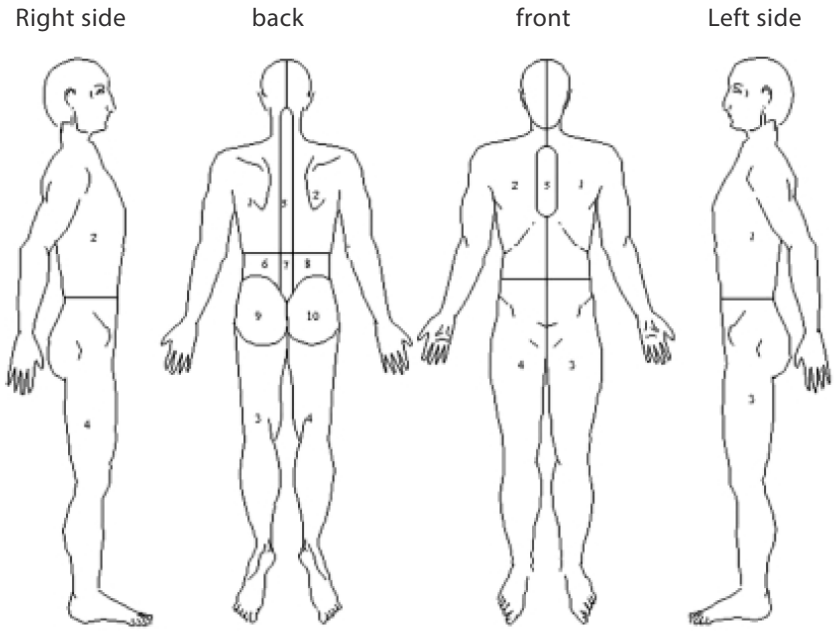
17. Imagine you bump your elbow on the edge of a table ("funny bone").
    0 1 2 3 4 5 6 7 8 9 10

Appendix h: Chronic pain

Did you experience any pain in the last month that lasted for a day or more?

- ☐ Yes, next question
- ☐ No

Can you indicate in the drawings below where you suffer (have suffered) from pain?



Is this the same spot as the spot you are operated on? Yes/no

Does the pain differ from the pain before surgery? Yes/no

How long have you been affected by the above-mentioned pain?

- ☐ Less than three months
- ☐ More than three months

Appendix i: Inventory of depressive symptomatology (self-report) (IDS-SR)

INVENTORY OF DEPRESSIVE SYMPTOMATOLOGY (SELF-REPORT)
(IDS-SR)

NAME: _____ TODAY'S DATE _____

Please circle the one response to each item that best describes you for the past seven days.

1. Falling Asleep:

   0 I never take longer than 30 minutes to fall asleep.
   1 I take at least 30 minutes to fall asleep, less than half the time.
   2 I take at least 30 minutes to fall asleep, more than half the time.
   3 I take more than 60 minutes to fall asleep, more than half the time.

2. Sleep During the Night:

   0 I do not wake up at night.
   1 I have a restless, light sleep with a few brief awakenings each night.
   2 I wake up at least once a night, but I go back to sleep easily.
   3 I awaken more than once a night and stay awake for 20 minutes or more, more than half the time.

3. Waking Up Too Early:

   0 Most of the time, I awaken no more than 30 minutes before I need to get up.
   1 More than half the time, I awaken more than 30 minutes before I need to get up.
   2 I almost always awaken at least one hour or so before I need to, but I go back to sleep eventually.
   3 I awaken at least one hour before I need to, and can't go back to sleep.

4. Sleeping Too Much:

   0 I sleep no longer than 7-8 hours/night, without napping during the day.
   1 I sleep no longer than 10 hours in a 24-hour period including naps.
   2 I sleep no longer than 12 hours in a 24-hour period including naps.
   3 I sleep longer than 12 hours in a 24-hour period including naps.

5. Feeling Sad:

   0 I do not feel sad
   1 I feel sad less than half the time.
   2 I feel sad more than half the time.
   3 I feel sad nearly all of the time.

6. Feeling Irritable:

   0 I do not feel irritable.
   1 I feel irritable less than half the time.
   2 I feel irritable more than half the time.
   3 I feel extremely irritable nearly all of the time.

7. Feeling Anxious or Tense:

   0 I do not feel anxious or tense.
   1 I feel anxious (tense) less than half the time.
   2 I feel anxious (tense) more than half the time.
   3 I feel extremely anxious (tense) nearly all of the time.

8. Response of Your Mood to Good or Desired Events:

   0 My mood brightens to a normal level which lasts for several hours when good events occur.
   1 My mood brightens but I do not feel like my normal self when good events occur.
   2 My mood brightens only somewhat to a rather limited range of desired events.
   3 My mood does not brighten at all, even when very good or desired events occur in my life.

9. Mood in Relation to the Time of Day:

   0 There is no regular relationship between my mood and the time of day.
   1 My mood often relates to the time of day because of environmental events (e.g., being alone, working).
   2 In general, my mood is more related to the time of day than to environmental events.
   3 My mood is clearly and predictably better or worse at a particular time each day.

   9A. Is your mood typically worse in the morning, afternoon or night? (circle one)

   9B. Is your mood variation attributed to the environment? (yes or no) (circle one)

10. The Quality of Your Mood:

    0 The mood (internal feelings) that I experience is very much a normal mood.
    1 My mood is sad, but this sadness is pretty much like the sad mood I would feel if someone close to me died or left.
    2 My mood is sad, but this sadness has a rather different quality to it than the sadness I would feel if someone close to me died or left.
    3 My mood is sad, but this sadness is different from the type of sadness associated with grief or loss.

Appendix i: Continued

Please complete either 11 or 12 (not both)

11. Decreased Appetite:

    0 There is no change in my usual appetite.
    1 I eat somewhat less often or lesser amounts of food than usual.
    2 I eat much less than usual and only with personal effort.
    3 I rarely eat within a 24-hour period, and only with extreme personal effort or when others persuade me to eat.

12. Increased Appetite:

    0 There is no change from my usual appetite.
    1 I feel a need to eat more frequently than usual.
    2 I regularly eat more often and/or greater amounts of food than usual.
    3 I feel driven to overeat both at mealtime and between meals.

Please complete either 13 or 14 (not both)

13. Decreased Weight (Within the Last Two Weeks):

    0 I have not had a change in my weight.
    1 I feel as if I've had a slight weight loss.
    2 I have lost 2 pounds or more.
    3 I have lost 5 pounds or more.

14. Increased Weight (Within the Last Two Weeks):

    0 I have not had a change in my weight.
    1 I feel as if I've had a slight weight gain.
    2 I have gained 2 pounds or more.
    3 I have gained 5 pounds or more.

15. Concentration/Decision Making:

    0 There is no change in my usual capacity to concentrate or make decisions.
    1 I occasionally feel indecisive or find that my attention wanders.
    2 Most of the time, I struggle to focus my attention or to make decisions.
    3 I cannot concentrate well enough to read or cannot make even minor decisions.

16. View of Myself:

    0 I see myself as equally worthwhile and deserving as other people.
    1 I am more self-blaming than usual.
    2 I largely believe that I cause problems for others.
    3 I think almost constantly about major and minor defects in myself.

17. View of My Future:

0 I have an optimistic view of my future.
    1 I am occasionally pessimistic about my future, but for the most part I believe things will get better.
    2 I'm pretty certain that my immediate future (1-2 months) does not hold much promise of good things for me.
    3 I see no hope of anything good happening to me anytime in the future.

18. Thoughts of Death or Suicide:

    0 I do not think of suicide or death.
    1 I feel that life is empty or wonder if it's worth living.
    2 I think of suicide or death several times a week for several minutes.
    3 I think of suicide or death several times a day in some detail, or I have made specific plans for suicide or have actually tried to take my life.

19. General Interest:

    0 There is no change from usual in how interested I am in other people or activities.
    1 I notice that I am less interested in people or activities.
    2 I find I have interest in only one or two of my formerly pursued activities.
    3 I have virtually no interest in formerly pursued activities.

20. Energy Level:

    0 There is no change in my usual level of energy.
    1 I get tired more easily than usual.
    2 I have to make a big effort to start or finish my usual daily activities (for example, shopping, homework, cooking or going to work).
    3 I really cannot carry out most of my usual daily activities because I just don't have the energy.

21. Capacity for Pleasure or Enjoyment (excluding sex):

    0 I enjoy pleasurable activities just as much as usual.
    1 I do not feel my usual sense of enjoyment from pleasurable activities.
    2 I rarely get a feeling of pleasure from any activity.
    3 I am unable to get any pleasure or enjoyment from anything.

22. Interest in Sex (Please Rate Interest, not Activity):

    0 I'm just as interested in sex as usual.
    1 My interest in sex is somewhat less than usual or I do not get the same pleasure from sex as I used to.
    2 I have little desire for or rarely derive pleasure from sex.
    3 I have absolutely no interest in or derive no pleasure from sex.

23. Feeling slowed down:

    0 I think, speak, and move at my usual rate of speed.
    1 I find that my thinking is slowed down or my voice sounds dull or flat.
    2 It takes me several seconds to respond to most questions and I'm sure my thinking is slowed.
    3 I am often unable to respond to questions without extreme effort.

24. Feeling restless:

    0 I do not feel restless.
    1 I'm often fidgety, wring my hands, or need to shift how I am sitting.
    2 I have impulses to move about and am quite restless.
    3 At times, I am unable to stay seated and need to pace around.

25. Aches and pains:

    0 I don't have any feeling of heaviness in my arms or legs and don't have any aches or pains.
    1 Sometimes I get headaches or pains in my stomach, back or joints but these pains are only sometime present and they don't stop me from doing what I need to do.
    2 I have these sorts of pains most of the time.
    3 These pains are so bad they force me to stop what I am doing.

26. Other bodily symptoms:

    0 I don't have any of these symptoms: heart pounding fast, blurred vision, sweating, hot and cold flashes, chest pain, heart turning over in my chest, ringing in my ears, or shaking.
    1 I have some of these symptoms but they are mild and are present only sometimes.
    2 I have several of these symptoms and they bother me quite a bit.
    3 I have several of these symptoms and when they occur I have to stop doing whatever I am doing.

27. Panic/Phobic symptoms:

    0 I have no spells of panic or specific fears (phobia) (such as animals or heights).
    1 I have mild panic episodes or fears that do not usually change my behavior or stop me from functioning.
    2 I have significant panic episodes or fears that force me to change my behavior but do not stop me from functioning.
    3 I have panic episodes at least once a week or severe fears that stop me from carrying on my daily activities.

28. Constipation/diarrhea:

    0 There is no change in my usual bowel habits.
    1 I have intermittent constipation or diarrhea which is mild.
    2 I have diarrhea or constipation most of the time but it does not interfere with my day-to-day functioning.
    3 I have constipation or diarrhea for which I take medicine or which interferes with my day-to-day activities.

29. Interpersonal Sensitivity:

    0 I have not felt easily rejected, slighted, criticized or hurt by others at all.
    1 I have occasionally felt rejected, slighted, criticized or hurt by others.
    2 I have often felt rejected, slighted, criticized or hurt by others, but these feelings have had only slight effects on my relationships or work.
    3 I have often felt rejected, slighted, criticized or hurt by others and these feelings have impaired my relationships and work.

30. Leaden Paralysis/Physical Energy:

    0 I have not experienced the physical sensation of feeling weighted down and without physical energy.
    1 I have occasionally experienced periods of feeling physically weighted down and without physical energy, but without a negative effect on work, school, or activity level.
    2 I feel physically weighted down (without physical energy) more than half the time.
    3 I feel physically weighted down (without physical energy) most of the time, several hours per day, several days per week.

Which 3 items (questions) were the easiest to understand? _____

Thank you

Range 0-84 Score: _____

Appendix j: Brief Pain Inventory

Date: _____/_____/_____ Time: _____

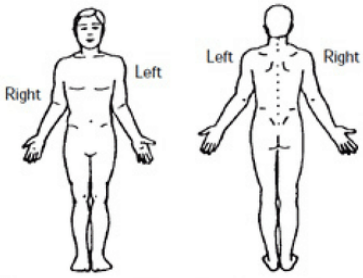
Name: _____ _____ _____

Last First Middle intial

1) Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

1. Yes 2. No

2) On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3) Please rate your pain by circling the one number that best describes your pain at its **worst** in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

4) Please rate your pain by circling the one number that best describes your pain at its **least** in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

5) Please rate your pain by circling the one number that best describes your pain on **average**.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

6) Please rate your pain by circling the one number that tells how much pain you have **right now**.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

7) What treatments or medications are you receiving for your pain?

_____

_____

8) In the past 24 hours, how much **relief** have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10	20	30	40	50	60	70	80	90	100%
No relief										Complete relief

9) Circle the one number that describes how, during the past 24 hours, pain has **interfered** with your:

A. General activity

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

C. Walking ability

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

D. Normal work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

Chapter 7

Genome-wide association study on pharmacological outcomes of musculoskeletal pain in UK Biobank

Song Li[1], Geert Poelmans[2], Regina L.M. van Boekel[3], Marieke J.H. Coenen[1*]

Authors' Affiliations:

[1] Department of Human Genetics, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands.

[2] Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands.

[3] Department of Anesthesiology, Pain and Palliative Medicine, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands.

*Corresponding author

Marieke J.H. Coenen, Ph.D., Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, the Netherlands. M.Coenen@erasmusmc.nl

Abstract

The pharmacological management of musculoskeletal pain starts with NSAIDs, followed by weak or strong opioids until the pain is under control. However, the treatment outcome is usually unsatisfying due to inter-individual differences. To investigate the genetic component of treatment outcome differences, we performed a genome-wide association study (GWAS) in ~ 23 000 participants with musculoskeletal pain from the UK Biobank. NSAID vs. opioid users were compared as a reflection of the treatment outcome of NSAIDs. We identified one genome-wide significant hit in chromosome 4 (rs549224715, $P = 3.88 \times 10^{-8}$). Suggestive significant ($P < 1 \times 10^{-6}$) loci were functionally annotated to 18 target genes, including four genes linked to neuropathic pain processes or musculoskeletal development. Pathway and network analyses identified immunity-related processes and a (putative) central role of *EGFR*. However, this study should be viewed as a first step to elucidate the genetic background of musculoskeletal pain treatment.

Keywords
Musculoskeletal pain, Analgesic ladder, Genome-wide association study, UK Biobank, Non-steroidal anti-inflammatory drugs (NSAIDs)

Introduction

Chronic musculoskeletal pain is one of the most frequent causes of suffering and disability [1]. The nature of musculoskeletal pain can be nociceptive or neuropathic, for which the corresponding pain management differs. The treatment of nociceptive musculoskeletal pain follows the WHO three-step analgesic ladder [2]: the first treatment step is non-opioid analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs); the second step is weak opioids for mild to moderate pain, such as tramadol; the third step is strong opioids for moderate to severe pain, such as morphine.

Unfortunately, effective pain management is challenged by inter-individual differences, with unsatisfied pain treatment rates ranging from 34 to 79% [3]. The underlying factors of ineffective pain treatment are multifactorial, including demographic characteristics (age, sex, socioeconomic status) [4, 5], lifestyle (smoking and alcohol intake) [6], comorbidities (psychological status) [7], and genetic factors. The genetic background of pain treatment outcomes has been investigated using candidate gene approaches. Some drug-metabolizing genes are associated with treatment outcomes for specific drugs, e.g., *CYP2D6* and codeine [8]. In addition, genes implicated in pain (sensitivity) may contribute to pain treatment outcomes because greater pain sensitivity is associated with increased opioid use [9] and poorer chronic pain treatment outcomes [10].

However, none of these findings predict pain treatment outcomes sufficiently to optimize pain treatment in a clinical setting. Furthermore, these studies are limited by small gene panels and sample size and report contradictory results [11]. The most investigated genetic variant is the 118A to G basepair change in the *OPRM1* gene (rs1799971). Genetic variants in *OPRM1* are thought to influence the opioid response by altering the μ-opioid receptor binding affinity of exogenous opioid ligands, such as morphine [12]. The G allele was associated with higher opioid dosing [13, 14] but shown to be protective against pain in other studies [15, 16]. Therefore, definitive conclusions on these genetic associations cannot be drawn yet, and a non-hypothesis driven approach in a large population is needed. Except for several recent, successful large-scale GWASs of chronic pain phenotypes [17-19], the number of GWASs focusing on pain treatment outcomes is still limited. Moreover, the most frequently used phenotype in GWASes investigating pain treatment is the response to certain drugs for acute pain (e.g., analgesic requirement or pain relief score after surgery [20, 21]), but long-term pain treatment outcomes are less investigated.

This study sought to identify genetic variants associated with switching to a higher analgesic ladder in people with musculoskeletal pain from the UK Biobank. A GWAS was performed including subjects treated according to the WHO analgesic ladders, and comparisons were made between NSAID and opioid users as a reflection of pain treatment outcome.

Method

We conducted a GWAS comparing NSAID users and opioid users using data from the UK Biobank, and post-GWAS analyses were performed for suggestively significant ($P < 1\times10^{-6}$) signals.

Study population

The UK Biobank is a general population cohort with over 0.5 million participants aged 40–69 recruited across the United Kingdom (UK) [22]. Recently released primary care (general practitioners, 'GPs') data provides longitudinal structured diagnosis and prescription data, which were used for phenotype definition. At the time of analysis, the interim release of GP data was available, which contained data on approximately 45% of the UK Biobank participants. UK Biobank obtained informed consent from all participants.

Phenotype definition

Figure 1 describes the phenotype definition. To define patients on NSAID and/or opioid treatment, we first extracted all participants with musculoskeletal pain records and participants with pain prescription records from the GP data (see Table S1 for the diagnosis codes and Table S2 for the pain prescriptions (NSAIDs and opioids) codes included in this study). Then, these two datasets were merged by pseudonymized subject ID, GP data provider and day. As we focus on long-term pain treatment, participants with more than one musculoskeletal pain diagnosis record were included. Patients were eligible for inclusion in the study when they had a pain prescription record occurring on the same day as the diagnosis to ensure that the prescriptions are indeed for musculoskeletal pain treatment. Nociceptive musculoskeletal pain diagnoses were selected in READ code rather than ICD10 codes because the READ code is used throughout the GP data, while the ICD10 code is only available for hospital inpatient records. Therefore, the READ code was used to link diagnosis data with the prescription data in the GP.

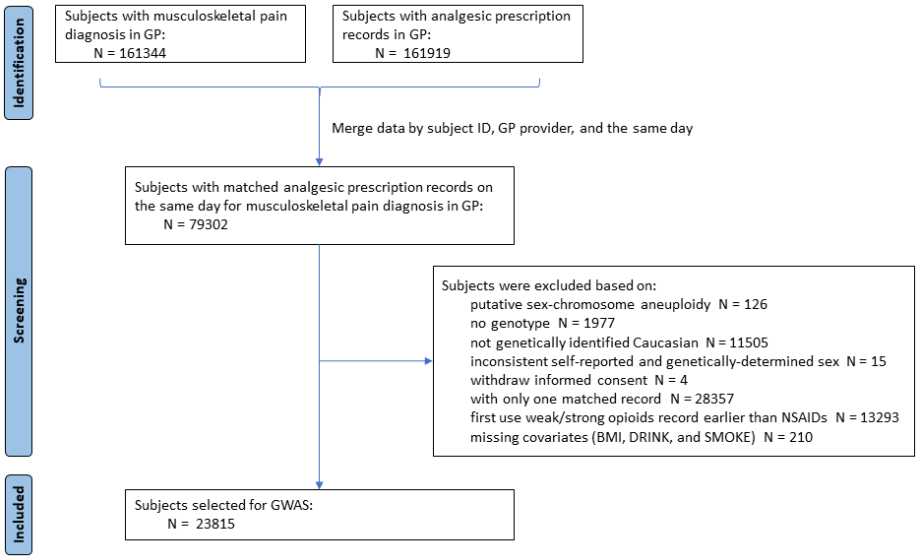


Figure 1. Flowchart of the phenotype definition. GP: general practitioner. Covariates: BMI, body mass index; DRINK: drink frequency; SMOKE: smoking status.

The outcome used for the analysis was defined as a dichotomous score (case/control): NSAID users were defined as controls and opioid users as cases. Opioid users were analyzed as a whole because the strong opioid user group is small (n = 365) and assuming mechanistic similarities between weak and strong opioids. Participants who did not meet the following two quality control (QC) steps were removed. First, participants with only one treatment event were removed to safeguard the inclusion of only participants with relatively long-term treatment. Second, a chronological check was applied for the first prescription of each ladder to ensure that the treatment ladder was correctly followed, i.e., opioids followed initial NSAID use. As the GP data is longitudinal, by using this definition, we could distinguish between patients who stay at NSAID treatment and those switching to the next level of the analgesic ladder. The script for defining analgesic ladder switching phenotype can be found in the GitHub repository (https://github.com/lisongmiller/UKB_GWAS_pain_treatment_outcome).

Genotyping and quality control

Genotyping procedures and PCA-based ancestry inference have been described in detail elsewhere [23]. Routine QC steps for genetic markers on autosomes included removal of single nucleotide polymorphisms (SNPs) with (1) an imputation quality score less than 0.8, (2) a minor allele frequency (MAF) less than 0.005, (3) a Hardy-

Weinberg equilibrium (HWE) test P-value less than $1 \times 10{-}6$, and (4) a genotyping call rate less than 0.95.

QC steps for genetic markers on the X chromosome's pseudo autosome region (PAR) were the same as autosomes. For non-PAR of the X chromosome, additional QC steps included setting heterozygous haploid genotypes as missing for males, excluding multi-allelic SNPs, and excluding variants with significantly different MAFs between males and females in the NSAID user group ($P < 0.05/\#SNPs$), and variants that violated HWE were examined in the NSAID user group using only females.

Routine QC steps for the samples include removal of participants with (1) inconsistent self-reported and genetically determined sex, (2) missing individual genetic data with a frequency of more than 0.1, and (3) putative sex-chromosome aneuploidy. Participants were excluded from the analysis if they were considered outliers due to missing heterozygosity, not white British ancestry based on the genotype, and missing covariate data.

Definition of covariates

The following variables from the UK Biobank data set were used for the covariate definition: (1) depression history, which was defined as "YES" if depression records were found in self-reported, inpatient hospital or GP data, and (2) drinking frequency, which was derived from data field 1558: "Daily or almost daily" or "Three or four times a week" was defined as high drinking frequency, other values except for "Prefer not to answer" were defined as low drinking frequency. Differences in categorical covariates were tested by a χ^2 test. Differences in continuous covariates were compared by t-test in SAS version 9.4 (SAS Institute Inc., Cary, NC).

Genome-wide association study

A GWAS was conducted using binary phenotypes, i.e., NSAID users (controls) versus opioid users (cases), we will refer to this analysis as the primary analysis. For markers on the autosomal chromosomes and PAR region of the X chromosome, GWAS on swithcing to a higher analgesic ladder was conducted using a linear function in GCTA [24], adjusting for age, sex, BMI, depression history, smoking status, drinking frequency, assessment center, genotyping array, and the first ten principal components (PCs). The selection of PCs was based on scree plot (Figure S1). To examine the nature of pain between groups, all nociceptive musculoskeletal pain diagnosis codes were grouped into one of the following categories: inflammatory,

mechanical, and mechanism not specified. The percentage of subjects in each diagnosis category was compared by a χ^2 test.

Markers on the non-PAR region of the X chromosome were analyzed by a sex-stratified analysis in XWAS [25]. A p-value less than 5×10^{-8} was considered genome-wide significant, and P-values between 1×10^{-6} and 5×10^{-8} were defined as suggestively significant. FUMA was used to define the lead SNPs (SNPs with the smallest P-value in each locus) and independent significant signals (SNPs in LD (r2 > 0.6) with lead SNPs and remaining significant after conditioning on the lead SNPs in each locus).

Sensitivity analysis and robustness analysis

To test the effect of adjusting study-specific covariates to the association results, a sensitivity analysis was performed by removing all the study-specific covariates. Therefore, only the following covariates were included in a sensitivity analysis: sex, age, 10PCs, array type, and assessment center.

To validate the GWAS results, a robustness analysis was performed by splitting the sample randomly into two equally sized subsets five times for the lead SNPs (n = 8) as described by Janita Bralten *et al.* [26]. In addition, a more stringent P-value threshold was obtained by a permutation test. In each permutation, genetic associations were performed based on randomly shuffled phenotypes and adjusted for the same covariates as in the primary analysis, and the lowest p-value was recorded. The permutation was run 5000 times, and the permuted threshold is the value at the 5th percentile of the distribution.

Functional annotation

Bayesian fine-mapping of lead loci

As GWAS identified lead variants are not always the causal variants affecting the trait, the true causal variant can be the SNPs correlated to the lead variants through linkage disequilibrium (LD). To identify this, Bayesian fine-mapping in PAINTOR [27] was performed which leverages both the association strength and genomic functional location to prioritize causal variants. Lead SNPs were analyzed to identify the most likely causal SNPs in each locus. PAINTOR calculates the posterior probability (PP) of causality for SNPs in each genomic region by leveraging the strength of association (Z score) and the LD pattern. The 1000 Genomes (Phase 3) were used for LD matrix calculation. The calculated PP for each SNP was sorted from high to low, and variants together reaching a PP of 0.95 were used to define 95% credible sets.

Functional annotation of SNPs present in the 95% credible sets

To evaluate the impact of the non-coding variants in the 95% credible sets, HaploReg v4.1 [28] was used to annotate these SNPs for regulatory functions. Specifically, the analyzed regulatory functions were (1) the presence of exonic, nonsynonymous variants in high LD ($r^2 \geq 0.8$), (2) overlap with epigenetic histone marks of active enhancers (H3K4me1 and H3K27ac) and active promoters (H3K4me3 and H3K9ac), and (3) the sensitivity to DNase. As histone marker overlap is tissue-specific, relevant cell lines were selected from the complete data set (see Table S3). Besides regulatory functions, potential pleiotropy effects (previously reported associations with other phenotypes) of the variants were investigated in Haploreg. For SNPs not available in Haploreg, proxy SNPs were obtained by LD proxy (https://ldlink.nci.nih.gov/). For loci containing more than ten likely causal variants, only the lead SNP and SNPs with the maximum posterior probability (PPmax) of the SNPs in one locus were annotated.

Gene mapping

To annotate suggestively significant GWAS SNPs (P-value $< 1 \times 10^{-6}$) and SNPs in LD (LD> 0.6) with them to genes, a bioinformatic tool FUMA was used. Three strategies were adopted in FUMA: positional mapping, expression quantitative trait loci (eQTL) mapping, and chromatin interaction mapping. For the positional mapping, SNPs were functionally annotated to known protein-coding genes based on physical distance (within a 10 kb window). For eQTL mapping, SNPs were functionally annotated to genes up to 1 Mb away based on known cis-eQTLs. As gene expression is tissue-specific, we selected the following tissues for mapping: brain, muscle, kidney, liver, nerve, skin, and fibroblast. Significant eQTLs were defined as eQTLs with a false discovery rate (FDR) < 0.05. Finally, chromatin interactions for gene mapping were assessed. Chromatin interaction can occur in two genomic regions that are spatially close when DNA folds together, even if the genomic regions are at a long-range physical distance. Genes in regions of chromatin interaction containing candidate SNPs were assessed in the same tissues as the eQTL mapping. An FDR $< 1 \times 10^{-6}$ was defined as a significant interaction. It is noteworthy that FUMA links SNPs to genes by combining information from biological data sources to generate hypotheses for further functional validation experiments. Therefore, annotated genes are not guaranteed to be causally linked to the investigated SNP.

Heritability and power calculation

Narrow-sense heritability was calculated by the GREML method in GCTA, which first calculates the genetic relationship matrix from all the autosomal SNPs, then performs a REML (restricted maximum likelihood) analysis [29, 30]. The same covariates as used in the GWAS analysis were included in the heritability analysis.

The power of heritability was calculated using GCTA-GREML Power Calculator (https://shiny.cnsgenomics.com/gctaPower/) assuming a disease risk in the population of 0.5, trait heritability as 0.08, and α level as 0.05.

The power of GWAS was calculated using the CaTS power calculator (http://csg.sph.umich.edu/abecasis/cats), assuming an additive model with the following input parameters: a significance level of 5×10^{-8}, the prevalence of phenotype (opioid use) of 50%, and a relative genotypic risk of 1.135, based on 11,089 cases and 12,726 controls.

Pathway enrichment analysis

To investigate if the genes identified in the GWAS could be linked to biological pathways and networks involved in pain (treatment), a gene-based functional pathways enrichment was performed using Ingenuity Pathway Analysis software (IPA®, QIAGEN Inc., Redwood City, California, U.S.). IPA is based on prior knowledge of direct and indirect gene relationships from experimentally observed data in mammals and all cell types. A gene-based P-value was computed twice using the gene analysis function in MAGMA v1.08. Firstly, a gene-based P-value was calculated using nominally significant SNPs (P-value < 0.05) in the protein-coding region of genes without flanking regions. Secondly, the same analysis was performed, including only nominally significant SNPs in the protein-coding gene regions and 100 kilobases (kb) pair upstream and downstream flanking regions, to take cis-eQTL effects into account [31-33]. Both analyses were combined, and the smallest P-value of each gene was selected for pathway and network analyses with IPA (see gene list in Table S4). Pathways with an FDR < 0.05 were considered statistically significantly enriched. To illustrate the core molecules in the networks, a radial plot was generated of the merged top five networks.

Genetic correlation analysis

Genetic correlation is the proportion of variance that two traits share due to genetic causes, i.e., the correlation between the genetic influences on a trait and the genetic influences on a different trait. Identifying genetic correlations can estimate the degree of pleiotropy or causal overlap between complex traits and diseases [34].

Genetic correlations between switching to a higher analgesic ladder and other complex traits were investigated by linkage disequilibrium score regression through LD Hub v1.9.3. The tested traits were selected from the LD hub, and the following categories were selected: education, anthropometric, sleeping, psychiatric, personality, cognitive, autoimmune, smoking behavior, kidney, neuro-

logical, and UKB phenotypes. Correlations with P-values less than 8.4×10^{-5} (0.05/596) were considered significant. Since the top nominally significant correlations were overrepresented by pain (category 100048, and data field 6154 in the UKB) and socioeconomic status phenotypes, the percentage of nominally significant ($P < 0.05$) correlations in these two categories were compared with all other categories by a χ^2 test. The socioeconomic status phenotypes consisted of qualifications (data-field 6138), employment (category 100064) in the UKB, and all education phenotypes in the LD hub [35-38].

Ordinal GWAS

Besides the binary case/control analysis, an additional GWAS was performed using an ordinal outcome. For the 'ordinal phenotype', an ordinal score of '1', '2', or '3' was assigned to NSAID users (persons only using NSAIDs), weak opioid users (persons using NSAIDs and weak opioids), and strong opioid users (persons using NSAID, weak opioid, and strong opioids), respectively. Also for the ordinal analysis, patients with one treatment event and not following chronological treatment were removed. The ordinal regression analysis was conducted in OrdinalGWAS [39] using the same covariates as the binary analysis.

Subtype GWAS and secondary GWAS

As inflammatory pain is an important subtype of pain, a subtype GWAS was carried out for this phenotype specifically. Only inflammatory nociceptive musculoskeletal pain diagnosis codes were used for participant selection. Some participants with inflammatory nociceptive musculoskeletal pain received pain treatment for other types of pain (e.g., mechanical nociceptive musculoskeletal pain or not specified) over time. These participants were excluded from the analysis.

Moreover, as diagnostic codes were often not repeatedly recorded[40] or reported as repeat prescriptions, a secondary GWAS was performed for pain medication users with less strict diagnosis criteria than the primary analysis. To ensure a relatively homogenous population, we included participants with at least one nociceptive musculoskeletal pain treatment event but without any other limitations on their pain treatment purposes. To focus on long-term treatment and remove outliers, participants were removed if they only had one prescription record or prescription record numbers on a log scale outside the 1.5 inter-quantile range. In addition, participants already included in the primary GWAS analysis were not included in this analysis.

Both the subtype and secondary GWASs were performed using the binary phenotype and following the same procedure as the primary analysis, but only

autosomal markers were examined. To investigate whether the identified loci in these two analyses overlapped with the primary analysis, lead SNPs with a suggestive threshold ($P < 1 \times 10^{-6}$) were compared with the primary GWAS signals for LD correlation (r2 > 0.6) in LDpair (https://ldlink.nci.nih.gov).

Results

GWAS

After quality control, we identified 12 726 NSAID users (control) and 11 089 opioid users (cases) in the UK Biobank dataset. **Table 1** summarizes the demographics of the cases and controls, and all tested covariates were found to be significantly different (P < 0.0001).

Table 1. Demographics of NSAID users (control) and opioid users (cases) in the UK Biobank. Categorical covariates are represented as frequency (percentage) and compared by the χ2 test. Continuous covariates are presented as mean (standard deviation) and compared by an independent t-test. Depression history was defined as "YES" if depression records were found in the self-reported, inpatient hospital, or GP data. Drinking frequency was defined from data field 1558, "Daily or almost daily" or "Three or four times a week" defined as high drinking frequency, other values except for "Prefer not to answer" defined as low drinking frequency. a Percentage of subjects within a certain category of pain in each group. Footnote a: one subject could have more than one type of diagnosis, so the percentage sum is not equal to 1.

	NSAID user	Opioid user	P value
Gender			P < 0.0001
Male	6092 (47.87%)	4625 (41.71%)	
Female	6634 (52.13%)	6464 (58.29%)	
Age (years)	58.34 (±7.49)	59.07 (±7.37)	P < 0.0001
BMI (kg × m-2)	28.13 (±4.66)	29.34 (±5.28)	P < 0.0001
Depression history			P < 0.0001
Yes	2308 (18.14%)	2694 (24.29%)	
No	10418 (81.86%)	8395 (75.71%)	
Alcohol intake frequency			P < 0.0001
High frequency	5900 (46.36%)	4256 (38.38%)	
Low frequency	6826 (53.64%)	6833 (61.62%)	
Smoking status			P < 0.0001
Never	6640 (52.18%)	5274 (47.56%)	
Previous	4940 (38.82%)	4391(39.60%)	
Current	1146 (9.01%)	1424 (12.84%)	

Table 1. Continued

	NSAID user	Opioid user	P value
Type of pain *			
Inflammatory	8236 (64.71%)	7491 (67.55%)	P < 0.0001
Mechanical	863 (6.78%)	1093 (9.86%)	P < 0.0001
Not specified	9923 (77.97%)	10209 (92.06%)	P < 0.0001
Total analgesic prescription records Mean (SD)			
	3.79 (3.14)	10.84 (17.00)	P < 0.0001
NSAID Prescription records Mean (SD)			
	3.79 (3.14)	6.72 (9.67)	P < 0.0001
NSAID prescription duration (days) Mean (SD)			
	1963.87 (1992.97)	2223.45 (2319.20)	P < 0.0001
Total	12726	11089	

There were 9 435 994 SNPs available for GWAS analysis after quality control. The genomic control value (lambda) was 1.008. One intergenic locus located at chromosome 4 reached genome-wide significance, in which the most significant SNP was rs549224715 ($P = 3.92 \times 10^{-8}$) **(Figure 2, Table 2)**. Seven loci surpassed the suggestive P-value threshold ($P < 1 \times 10^{-6}$), and no other independent SNPs (SNPs remaining significant after conditioning on lead SNPs in the locus) were identified in each locus. In addition, the ordinal GWAS results were consistent with the GWAS using binary outcomes (Figure S2). However, we did not find any genome-wide significant loci or overlapped suggestively significant loci with the primary GWAS (Figure S3, Figure S4, Table S5, Table S6).

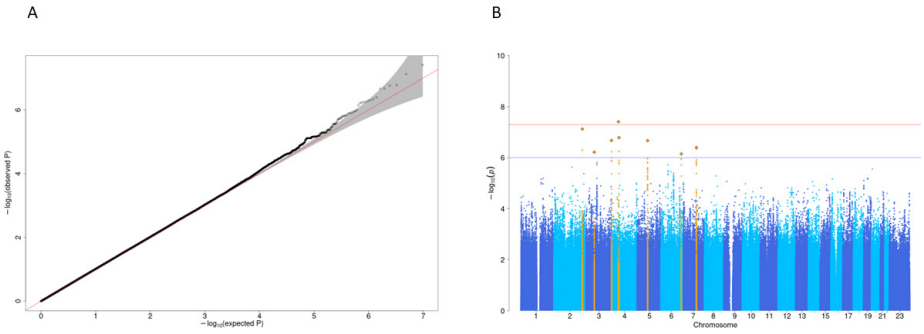


Figure 2. Q-Q plot and Manhattan plot of primary analysis for comparing NSAID versus opiod users. (a) Q-Q plot of the GWAS results. The red line indicates the distribution under the null hypothesis, and the shaded area indicates the 95% confidence band. (b) Manhattan plot of the GWAS results. The red line corresponds to the genome-wide significance threshold of 5×10^{-8}, whereas the blue indicates the suggestive threshold of 1×10^{-6}. Lead variants are highlighted as orange diamonds. Variants in one locus (within 400 Kb) are highlighted in orange.

Table 2. Overview of the lead SNPs passing suggestive significance in the primary GWAS for NSAID versus opioid users. Bold font indicates the SNP that passed the genome-wide significant threshold (5 × 10-8). CHR:POS physical position of the SNP, A1 effect allele, AF1 effect allele frequency, BETA (SE) effect size of SNP, and standard error (SE). # Identified SNP in the sensitivity analysis is rs34147893, which is in complete linkage disequilibrium with rs13133042.

					Primary GWAS		Sensitivity analysis		Secondary analysis	
SNP	**CHR:POS**	**A1**	**AF1**	**Location**	**BETA (SE)**	**P**	**BETA (SE)**	**P**	**BETA (SE)**	**P**
rs549224715	**4:49088326**	**C**	**0.012**	**intergenic**	**0.111 (0.020)**	**3.92E-08**	0.118 (0.021)	9.90E-09	-0.012(0.019)	5.27E-01
rs12694371	2:216597632	A	0.331	ncRNA_intronic	0.026 (0.005)	7.57E-08	0.026 (0.005)	1.07E-07	0.006(0.005)	2.07E-01
rs13133042 #	4:53043381	G	0.011	intergenic	0.111 (0.021)	1.65E-07	0.109 (0.020)	2.68E-08	-0.011(0.021)	6.05E-01
rs73062440	3:194712150	G	0.169	intergenic	-0.031 (0.006)	2.12E-07	-0.031 (0.006)	3.69E-07	-0.010(0.006)	7.70E-02
rs143781228	5:79586466	A	0.023	intergenic	-0.077 (0.015)	2.16E-07	-0.081 (0.015)	7.20E-08	0.001(0.014)	9.46E-01
rs10264795	7:98299788	G	0.455	intergenic	0.023 (0.004)	4.04E-07	0.023 (0.005)	5.57E-07	-0.002(0.004)	5.99E-01
rs73126966	3:63744617	T	0.011	intergenic	0.108 (0.022)	6.12E-07	0.108 (0.022)	1.05E-06	0.040(0.020)	5.10E-02
rs13218801	6:154712589	G	0.280	intergenic	0.025 (0.005)	7.14E-07	0.024 (0.005)	1.33E-06	0.003(0.005)	6.01E-01

Our study had 80% power to identify SNPs with a risk allele frequency of 5% and genotypic relative risk of 1.135. The SNP heritability was 0.16 (P-value = 0.16), and the power for this analysis was 96%.

Sensitivity analysis and robustness analysis

In the sensitivity analysis, the lead SNP rs549224715 in the primary GWAS remained the strongest signal, and two more lead SNPs reached genome-wide significance (rs143781228 and rs34147893 (in complete LD with SNP rs13133042 identified in the primary analysis)). Three other lead SNPs in the primary GWAS (rs12694371, rs73062440, and rs10264795) remained suggestively significant, except for the SNPs rs73126966 and rs13218801 (**Table 2**).

In the robustness analysis, six of eight suggestively loci passed at least the nominal significance threshold ($P < 0.05$) for all ten iterations. Two lead SNPs (rs13218801 and rs73062440) failed one out of ten iterations (Table S7).

In the permutation analysis, the permutated P-value at 5^{th} percentile is 1.61×10^{-8} (Figure S5). None of the lead SNPs passed this threshold.

Functional annotation

Statistical fine-mapping of loci and functional annotation of SNPs

In five out of eight loci, the lead SNPs in the locus had the maximum PP (PP_{max}) (Figure S6). The regulatory function annotation results suggested that most genetic variants were potentially involved in transcriptional regulatory modulation (Figure S6).

We assessed whether the SNPs in the 95% credible sets were previously reported to be associated with other traits. However, no pleiotropic effects were identified.

Gene mapping

A total of 18 unique annotated genes were identified by SNPs in the 95% credible sets (**Table 3**). Five genes were annotated by genomic location, seven genes were identified by cis-eQTL mapping, ten genes were annotated as SNPs in regions where 3D chromatin interactions occurred, and four genes were identified by at least two mapping strategies.

Table 3. SNPs mapped to candidate genes using FUMA. SNPs in LD (r2 > 0.6) with lead SNPs were mapped to genes. posMapSNPs, the number of SNPs mapped to this gene based on positional mapping; eqtlMapSNPs, the number of SNPs mapped to this gene based on eQTL mapping; eqtlMapminP, the minimum eQTL P-value of mapped SNPs; eqtlMapminQ, the minimum eQTL FDR of mapped SNPs; ciMap, "Yes" if the gene is mapped by chromatin interaction mapping; minGwasP, the minimum P-value of mapped SNPs.

Lead SNPs	Gene Symbol	chr	Methods used for gene mapping					minGwasP
			posMapSNPs	eqtlMapSNPs	eqtlMapminP	eqtlMapminQ	ciMap	
rs12694371	*FN1*	2	0	0	—	—	Yes	7.57E-08
rs73062440	*LSG1*	3	0	0	—	—	Yes	2.12E-07
	FAM43A	3	0	0	—	—	Yes	2.12E-07
	XXYLT1	3	0	0	—	—	Yes	2.12E-07
	PPP1R2	3	0	0	—	—	Yes	2.12E-07
rs549224715	*TXK*	4	0	2	2.41E-04	4.56E-14	No	3.92E-08
rs13133042	*DCUN1D4*	4	2	0	—	—	No	1.73E-07
	LRRC66	4	0	0	—	—	Yes	1.73E-07
	SGCB	4	1	0	—	—	Yes	1.73E-07
	SPATA18	4	1	0	—	—	Yes	1.73E-07
rs143781228	*PAPD4*	5	0	0	—	—	Yes	2.16E-07
	SERINC5	5	5	1	1.26E-04	6.95E-29	No	1.10E-06
	FAM151B	5	0	10	4.35E-12	0	No	2.16E-07
	ANKRD34B	5	0	10	1.83E-57	0	No	2.16E-07
	DHFR	5	0	10	2.16E-32	0	No	2.16E-07
rs13218801	*IPCEF1*	6	0	1	1.01E-07	1.87E-15	Yes	7.14E-07
	CNKSR3	6	7	0	—	—	No	7.14E-07
rs10264795	*NPTX2*	7	0	31	4.21E-07	1.47E-13	No	4.04E-07

Pathway enrichment

Pathway enrichment analysis in IPA prioritized 15 significant pathways with an FDR < 0.05, in which the top prioritized pathways were mainly implicated in the immunological response. (Table S8).

The network analysis yielded a total of 25 prioritized networks. The top network contained 33 genes with the EGFR protein in the center. EGFR remained in the center after merging the five networks with the lowest P-value (Table S9, Figure S7).

Genetic correlation with other traits

The genetic correlation analysis did not yield significant correlations (Bonferroni corrected P-value < 8.39×10^{-5}). The top correlated trait was overall health rating (rg = 0.5316, P = 0.0087), followed by years of schooling [36] (Rg = -0.5431, P = 0.0102) (Table S10). However, among the nominally significant correlations (P < 0.05), we found an overrepresentation of pain and socioeconomic status traits compared to the other traits (43.48% vs. 8.55%, P = 3.35×10^{-12}, Table S11).

Discussion

In this study, we identified one genome-wide significant hit and seven loci with suggestive significance associated with analgesic ladder switching from NSAID to opioid. Although pain or pain treatment is characterized by sex differences, i.e., females are more vulnerable to pain and opioid use [5], no significant signals were found on the X chromosome.

The functional link between the genome-wide significant SNP (rs549224715) on chromosome 4 and switching to a higher pain treatment ladder remains unclear. The nearest gene of this locus, *CWH43,* is associated with Seckel Syndrome, characterized by growth delays before birth. Another gene, *TXK,* was annotated by eQTL to this SNP which has previously been linked to rheumatoid arthritis [41]. Considering that our investigated phenotype is switching from NSAID to opioid use, this association is worth further validation and investigation.

Two genes identified from other loci are prioritized as they are involved in neuropathic pain. *NPTX2* was identified by both eQTL mapping and gene-based analysis with the lowest P-value (2.71×10^{-5}) (Table S2). NPTX2 is down regulated in the brain in induced chronic neuropathic pain [42] and induced endometriosis [43] mouse models. The other gene is *IPCEF1*, is previously related to neuropathic pain

conditions [44]. In addition, we identified two genes linked to muscular or skeletal dystrophy: *SGCB* and *FN1*. These genes are of interest as musculoskeletal dystrophy is characterized by pain.

None of identified genes were implicated in the metabolism and working mechanisms of specific NSAIDs, which is perhaps not unexpected. As subcategories in NSAIDs, such as non-selective NSAIDs and selective COX-2 inhibitors, were analyzed as a whole, this may have diluted signals related to a specific NSAID. However, stratified analyses per drug were not practical as the groups would become too small to obtain sufficient power.

The narrow-sense heritability result in our study is in agreement with similar phenotypes, opioid response (60% in cold pressor-induced pain and 12% in heat pressor) in a twin study [45] and chronic pain (0.08 to 0.31) [7]. Since we have sufficient power for the heritability analysis, the insignificant narrow-sense heritability result suggests that genetic factors might not play an important role in switching to a higher analgesic ladder compared with other well-known environmental, psychological, and socioeconomic factors influencing pain and pain treatment [46]. However, our results should be further validated in a larger sample size considering the fact that a large number of common variants with very small effect sizes contribute to the complex traits. Take height as an example, a close heritability estimation of height to the pedigree-based heritability estimation was made possible with larger sample sizes with n > 100,000 for height in a recent GWAS meta-analysis [47].

Our study might add to the current evidence of the biological mechanisms of pain treatment. Most variants in the 95% credible sets showed potential transcription regulatory functions, which aligns with research indicating that epigenetic changes are involved in chronic pain [48] and pain treatment [49]. For instance, epigenetic restructuring in a candidate gene (*OPRM1)* and global DNA methylation was observed after opioid use [50, 51]. Furthermore, the network analysis identified EGFR, preliminary studies suggest a role of EGFR in pain modulation in preclinical studies [52] and analgesic effects in clinical settings [53]. However, the results from this analysis should be interpreted carefully as the input consisted of nominally significant genes from the GWAS analysis. In addition, the overrepresentation of pain phenotypes in genetic correlation analysis also matched reports that opioid users tend to have more chronic and severe pain conditions [54].

Although six lead SNPs passed the robustness analysis, only the lead SNP passed the permutation threshold in the sensitivity analysis, and none of the SNPs survived

permutation analysis which might suggest these results can still be false positive findings. In addition, we failed to replicate our findings in the secondary analysis. Unfortunately, replicating the results in other independent cohorts is difficult due to the limited number of publicly available large-scale data similar to UK Biobank and the lack of cohorts measuring similar outcomes. It might still be worth validating our results in a large cohort with a clear outcomes definition, such as the ongoing Pain Predict Genetics cohort in our center (NCT02383342).

The merit of our study is that we have a large sample size investigating analgesic ladder switching from NSAIDs to opioids. However, the limitation is that we utilized a derived phenotype, so we cannot distinguish whether switching ladders is because of pain progress, poor treatment response to certain drugs, or a combination of both factors. However, it does not matter whether the genes might reflect pain severity or pain treatment outcome, as they still have the potential to predict analgesic ladder switching. Despite the limitations in phenotype definition, the group characteristics are similar to previous publications, with a roughly even share of NSAID users and opioid users in the population [55], and the reported risk factors for using opioids are also in line with previous literature [54, 55].

In conclusion, we identified one locus achieving genome-wide significance for a derived pain treatment outcome phenotype. Some identified genes could be linked to neuropathic pain and musculoskeletal development. This study should be viewed as an initial stepping stone for future research. We show a small genetic contribution to analgesic ladder switching. For future research, it might be better to focus on a specific disease or outcome for a specific treatment.

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Data availability

Summary statistics of the primary analysis are available at DANS archive (https://doi.org/10.17026/dans-xns-un6c).

Gene mapping results are available at FUMA (https://fuma.ctglab.nl/browse/378).

Author contributions

Song Li analyzed the data and prepared the manuscript. Geert Poelmans contributed to the pathway and network analysis and revised the manuscript. Regina L.M. van Boekel contributed to the phenotype definition and revised the manuscript. Marieke J.H. Coenen conceptualized the study, supervised the overall project and revised the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest disclosures

The authors declare that they have no conflicts of interest.

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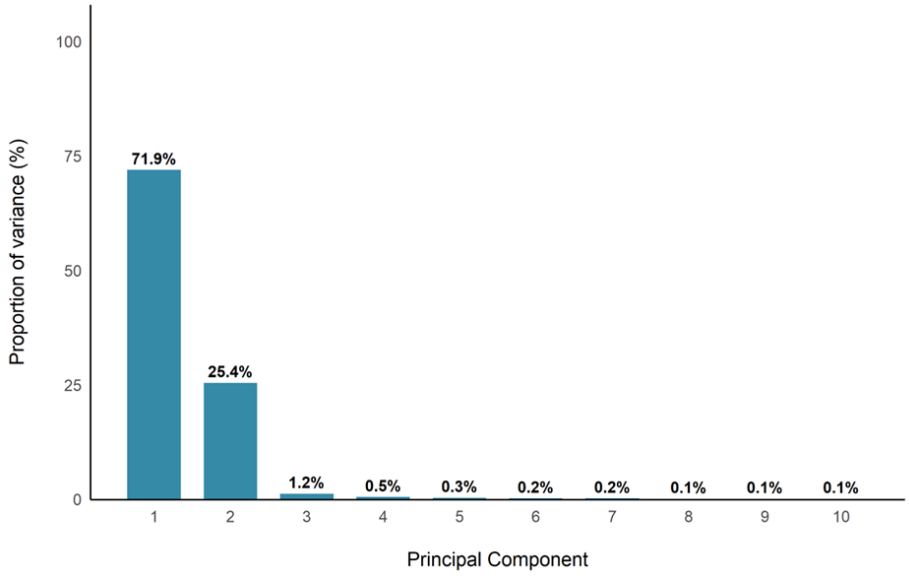
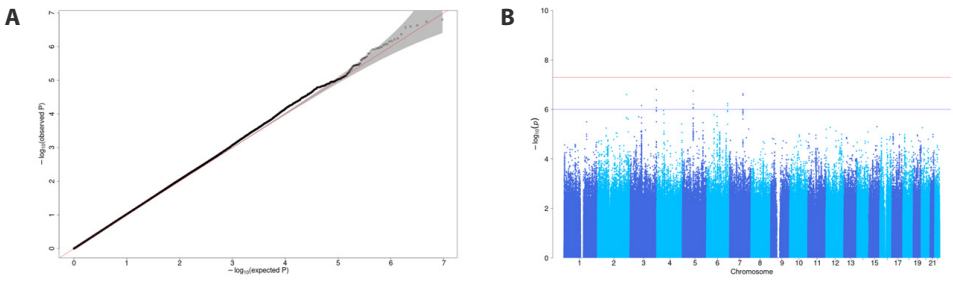
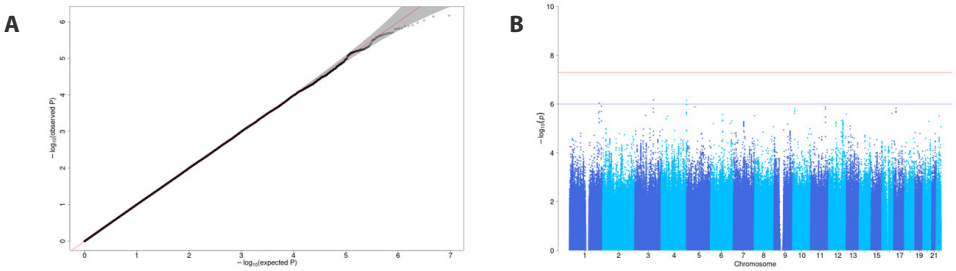
Supplementary materials



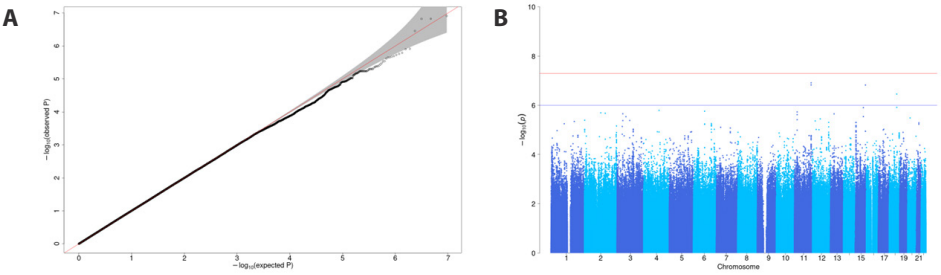
Supplementary Figure 1. Scree plot of principle components. X-axis represents principle components, y-axis represents variance explained by each component.



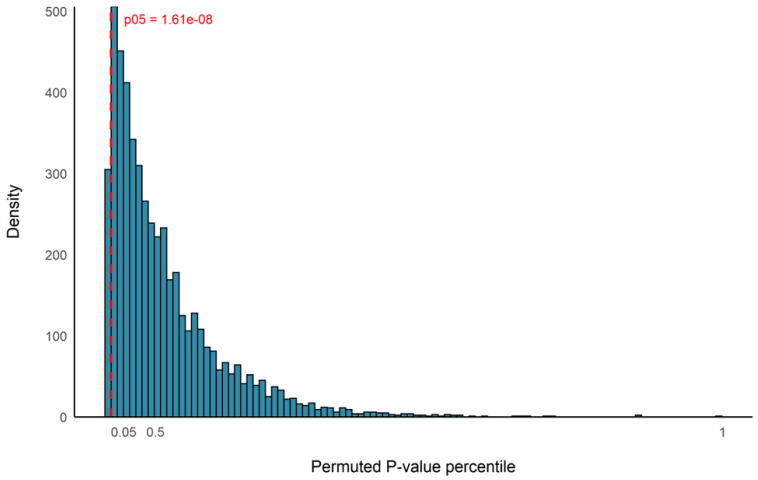
Supplementary Figure 2. GWAS results of for nociceptive musculoskeletal pain using the ordinal phenotype. A) Q-Q plot; B) Manhattan plot.



Supplementary Figure 3. Subtype GWAS results of NSAID versus opioid users for inflammatory nociceptive musculoskeletal pain. A) Q-Q plot; B) Manhattan plot.



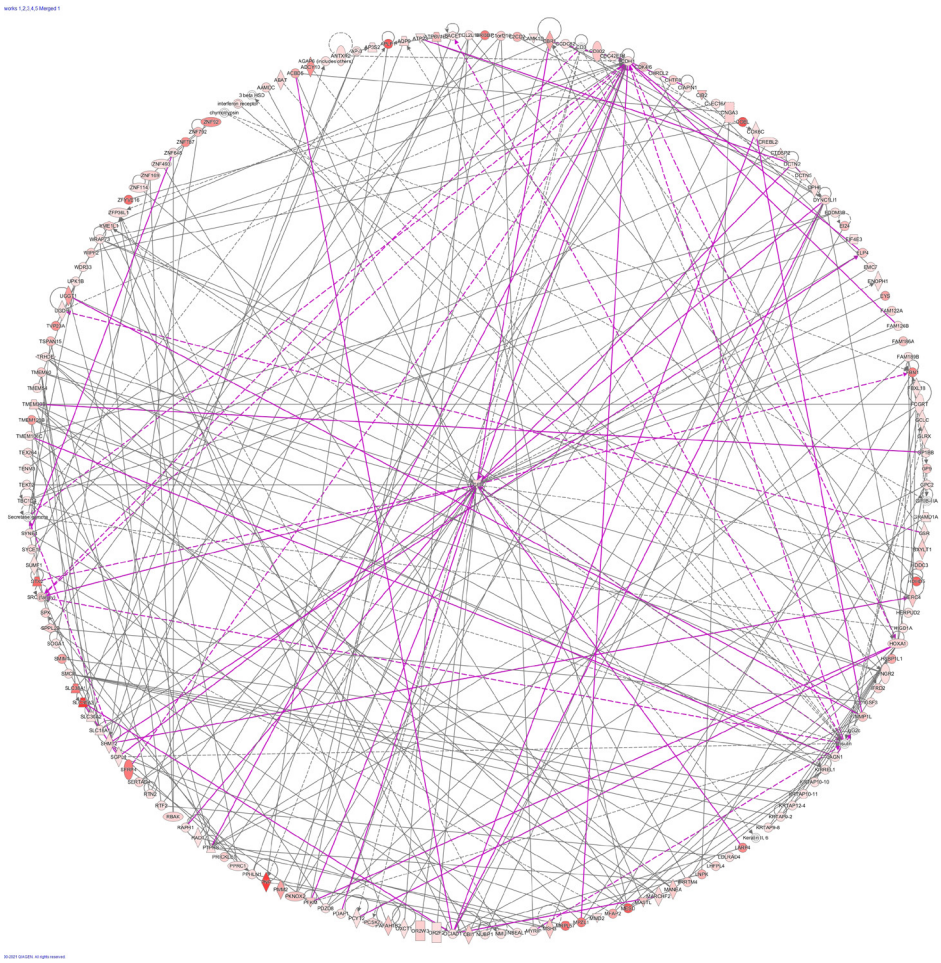
Supplementary Figure 4. Secondary GWAS results of NSAID versus opioid users for nociceptive musculoskeletal pain using less stringent phenotype definition. A) Q-Q plot; B) Manhattan plot.



Supplementary Figure 5. Distribution of permutation P-values. The number on X-axis indicates the percentile of permuted P-values. The value of 5th percentile (p05) is added in the figure.

lead SNP in locus	Chr	Nr. SNPs Cred. Set	Cred. set SNP [a]	SNP function	Gene.refGene	Exonic nonsynonymous	eQTL	H3K4me1_Enh	H3K4me3_Pro	H3K27ac_Enh	H3K9ac_Pro	Dnase
rs12694371	2	3	**rs12694371**	intronic	*LINC00607*							
			rs13026392	intronic	*AC012668.2*							
			rs11677106	intronic	*AC012668.2*							
rs73062440	3	15	rs73062440	intergenic	*XXYLT1*							
			rs59524354	intergenic	*XXYLT1*							
rs73126966	3	3	**rs73126966**	intergenic	*C3orf49*							
			rs138930166	intergenic	*RP11-129B22.1*							
			rs144992688	intronic	*C3orf49*							
rs13133042	4	3	rs13106592	intergenic	*SPATA18*							
			rs146777523	intergenic	*USP46*							
			rs34147893	intronic	*DCUN1D4*							
rs549224715	4	2	**rs549224715**	intergenic	*CWH43*							
			rs114874144	intergenic	*OCIAD1*							
rs143781228	5	8	**rs143781228**	intergenic	*SNORA31*							
			rs58869205	intergenic	*SERINC5*							
			rs57149560	intergenic	*SERINC5*							
			rs10075519	intergenic	*SNORA31*							
			rs9293814	intergenic	*SNORA31*							
			rs28401747	intergenic	*SNORA31*							
			rs10038569	intergenic	*SNORA31*							
			rs79747351	intergenic	*SERINC5*							
rs13218801	6	3	**rs13218801**	intergenic	*CNKSR3*							
			rs9322462	intergenic	*CNKSR3*							
			rs72995428	intronic	*CNKSR3*							
rs10264795	7	14	rs10264795	intergenic	*NPTX2*							
			rs118127734	intergenic	*NPTX2*							

Supplementary Figure 6. Functional annotation results of 95% credible set SNPs. Different colors indicate credible SNPs in LD with exonic nonsynonymous variants, overlap with active chromatin marks of active enhancer (H3K4me1, H3K27ac) or promoters (H3K4me3, H3K9ac), and sensitive to DNAse. The selected cell types for annotating overlap with chromatin marks and DNAse are provided in supplementary Table 1. eQTL overlap is obtained from FUMA. Footnote a: When the 95% credible set included more than 10 likely causal variants, only lead SNP and SNP with maximum posterior probability (PP_{max}) were selected for annotation. SNPs in bold indicate that the lead SNP remains maximum posterior probability.



Supplementary Figure 7. Network analysis of the main GWAS genes with ingenuity pathway analysis (IPA). The proteins encoded by the 1457 genes with nominal significance (P-value < 0.05 ; the list of these genes are provided in Supplementary Table 2) were used for the network analysis. After merging the top 5 networks and generating a 'radial'plot, EGFR is at the center of molecular interactions. All proteins in red/pink are encoded by genes present in the input dataset. The more red a protein is, the more significant the P-value for the input gene that encodes this protein is.

Supplementary Table 1. Selected nociceptive musculoskeletal diagnosis from GP diagnosis data.
Supplementary Table 2. Selected pain medications in GP prescriptions data.

(Supplementary Table 1 and Table 2 can be found online: https://static-content.springer.com/esm/art%3A10.1038%2Fs41397-023-00314-x/MediaObjects/41397_2023_314_MOESM1_ESM.pdf)

Supplementary Table 3. Selected cells and tissues for SNP functional annotation in Haploreg.

Epigenome ID (EID)	Group	Mnemonic	Description
E007	ES-deriv	ESDR.H1.NEUR.PROG	H1 Derived Neuronal Progenitor Cultured Cells
E009	ES-deriv	ESDR.H9.NEUR.PROG	H9 Derived Neuronal Progenitor Cultured Cells
E010	ES-deriv	ESDR.H9.NEUR	H9 Derived Neuron Cultured Cells
E052	Myosat	MUS.SAT	Muscle Satellite Cultured Cells
E053	Neurosph	BRN.CRTX.DR.NRSPHR	Cortex derived primary cultured neurospheres
E054	Neurosph	BRN.GANGEM.DR.NRSPHR	Ganglion Eminence derived primary cultured neurospheres
E055	Epithelial	SKIN.PEN.FRSK.FIB.01	Foreskin Fibroblast Primary Cells skin01
E056	Epithelial	SKIN.PEN.FRSK.FIB.02	Foreskin Fibroblast Primary Cells skin02
E057	Epithelial	SKIN.PEN.FRSK.KER.02	Foreskin Keratinocyte Primary Cells skin02
E058	Epithelial	SKIN.PEN.FRSK.KER.03	Foreskin Keratinocyte Primary Cells skin03
E059	Epithelial	SKIN.PEN.FRSK.MEL.01	Foreskin Melanocyte Primary Cells skin01
E061	Epithelial	SKIN.PEN.FRSK.MEL.03	Foreskin Melanocyte Primary Cells skin03
E066	Other	LIV.ADLT	Liver
E067	Brain	BRN.ANG.GYR	Brain Angular Gyrus
E068	Brain	BRN.ANT.CAUD	Brain Anterior Caudate
E069	Brain	BRN.CING.GYR	Brain Cingulate Gyrus
E070	Brain	BRN.GRM.MTRX	Brain Germinal Matrix
E071	Brain	BRN.HIPP.MID	Brain Hippocampus Middle
E072	Brain	BRN.INF.TMP	Brain Inferior Temporal Lobe
E073	Brain	BRN.DL.PRFRNTL.CRTX	Brain_Dorsolateral_Prefrontal_Cortex
E074	Brain	BRN.SUB.NIG	Brain Substantia Nigra
E081	Brain	BRN.FET.M	Fetal Brain Male
E082	Brain	BRN.FET.F	Fetal Brain Female
E086	Other	KID.FET	Fetal Kidney
E089	Muscle	MUS.TRNK.FET	Fetal Muscle Trunk
E090	Muscle	MUS.LEG.FET	Fetal Muscle Leg
E100	Muscle	MUS.PSOAS	Psoas Muscle
E107	Muscle	MUS.SKLT.M	Skeletal Muscle Male
E108	Muscle	MUS.SKLT.F	Skeletal Muscle Female
E120	ENCODE2012	MUS.HSMM	HSMM Skeletal Muscle Myoblasts Cells
E121	ENCODE2012	MUS.HSMMT	HSMM cell derived Skeletal Muscle Myotubes Cells
E125	ENCODE2012	BRN.NHA	NH-A Astrocytes Primary Cells
E126	ENCODE2012	SKIN.NHDFAD	NHDF-Ad Adult Dermal Fibroblast Primary Cells
E127	ENCODE2012	SKIN.NHEK	NHEK-Epidermal Keratinocyte Primary Cells

Supplementary Table 4. P-value of genes calculated by MAGMA.
(Here lists top 20 candidate genes. The complete Table 4 can be found online. https://static-content.springer.com/esm/art%3A10.1038%2Fs41397-023-00314-x/MediaObjects/41397_2023_314_MOESM1_ESM.pdf)

Gene	Chromosome	Gene-wide P	SNPs used to calculate gene-wide P	Number of SNPs used
NPTX2	7	2.71E-05	SNPs in gene +/- 100 kb of flanking regions	796
OSGEP	14	1.29E-04	SNPs in gene	35
UCN	2	1.64E-04	SNPs in gene +/- 100 kb of flanking regions	414
MPV17	2	2.27E-04	SNPs in gene +/- 100 kb of flanking regions	440
TRIM54	2	2.71E-04	SNPs in gene +/- 100 kb of flanking regions	477
SLC30A3	2	2.75E-04	SNPs in gene +/- 100 kb of flanking regions	484
DNAJC5G	2	2.97E-04	SNPs in gene +/- 100 kb of flanking regions	445
GTF3C2	2	3.55E-04	SNPs in gene +/- 100 kb of flanking regions	443
TMEM55B	14	3.86E-04	SNPs in gene +/- 100 kb of flanking regions	831
PRR23A	3	4.01E-04	SNPs in gene	6
HSP90B1	12	4.40E-04	SNPs in gene +/- 100 kb of flanking regions	1066
PNP	14	4.41E-04	SNPs in gene +/- 100 kb of flanking regions	866
HCST	19	5.11E-04	SNPs in gene +/- 100 kb of flanking regions	557
CD300C	17	5.28E-04	SNPs in gene	24
APEX1	14	5.34E-04	SNPs in gene +/- 100 kb of flanking regions	830
SLC5A6	2	5.94E-04	SNPs in gene +/- 100 kb of flanking regions	506
ATRAID	2	5.95E-04	SNPs in gene +/- 100 kb of flanking regions	488
TYROBP	19	6.17E-04	SNPs in gene +/- 100 kb of flanking regions	566
MESDC1	15	6.65E-04	SNPs in gene +/- 100 kb of flanking regions	544
CIITA	16	6.96E-04	SNPs in gene	153

Gene with gene-wide P < 0.05, based on all SNPs within the gene itself

Gene with gene-wide P < 0.05, based on all SNPs within the gene itself and 100 kilobases (kb) of up- and downstream flanking genetic regions.

Supplementary Table 5. Lead SNPs identified in subtype GWAS. CHR:POS physical position of the SNP, A1 effect allele, AF1 effect allele frequency, BETA (SE) effect size of SNP and standard error (SE).

SNP	CHR:POS	A1	AF1	BETA (SE)	P
rs370862902	1:223201265	G	0.010	0.193 (0.039)	9.04E-07
rs60716072	3:138957697	G	0.019	0.142 (0.028)	6.67E-07
rs62341133	4:189891160	G	0.282	0.044 (0.009)	7.10E-07

Supplementary Table 6. Lead SNPs identified in secondary GWAS. CHR:POS physical position of the SNP, A1 effect allele, AF1 effect allele frequency, BETA (SE) effect size of SNP and standard error (SE).

				Secondary GWAS		Primary GWAS	
SNP	**CHR:POS**	**A1**	**AF1**	**BETA (SE)**	**P-value**	**BETA (SE)**	**P-value**
rs61907224	11:123814742	G	0.096	0.039 (0.007)	1.23E-07	-0.001(0.007)	0.938
rs141754034	15:95929570	G	0.014	0.095 (0.018)	1.50E-07	0.028(0.018)	0.13
rs8089653	18:55694228	T	0.903	0.037 (0.007)	3.50E-07	0.001(0.008)	0.901

Supplementary Table 7. Robustness analysis of lead SNPs.

	Iteration round									
SNP	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**
rs10264795	3.90E-05	2.64E-03	8.47E-05	1.17E-03	4.86E-05	1.93E-03	8.50E-05	1.42E-03	2.99E-04	4.52E-04
rs12694371	9.34E-04	2.58E-05	2.45E-02	1.15E-07	1.47E-02	2.25E-07	7.03E-06	1.94E-03	2.37E-06	3.62E-03
rs13133042	3.95E-04	1.02E-04	1.64E-05	2.14E-03	8.47E-05	5.80E-04	8.72E-04	3.23E-05	9.20E-03	1.67E-06
rs13218801	3.15E-04	7.97E-04	1.08E-02	1.12E-05	2.44E-04	1.20E-03	5.61E-08	1.09E-01	1.01E-04	1.69E-03
rs143781228	3.64E-04	2.05E-04	8.31E-03	3.10E-06	7.34E-05	5.65E-04	1.47E-04	4.23E-04	1.05E-04	4.33E-04
rs549224715	1.19E-04	7.83E-05	3.34E-06	2.24E-03	1.43E-05	6.38E-04	2.92E-04	2.36E-05	3.08E-03	1.18E-06
rs73062440	1.02E-01	7.89E-09	2.75E-04	1.89E-04	9.20E-06	3.96E-03	7.71E-04	6.37E-05	5.69E-04	8.49E-05
rs73126966	7.79E-03	1.38E-05	2.51E-04	6.52E-04	3.25E-05	4.67E-03	1.51E-06	2.25E-02	3.44E-05	5.29E-03

Supplementary Table 8. Significant associated pathways in pain treatment outcome from IPA. -log(p-value), negative logarithm of pathway enrichment P-value. Ratio, the ratio of genes from input file in this pathway. Molecules, genes from input file in this pathway.

Ingenuity Canonical Pathways	-log (p-value)	Ratio	Molecules
Role of Lipids/Lipid Rafts in the Pathogenesis of Influenza	7.37	0.458	IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNAR1
Retinoic acid Mediated Apoptosis Signaling	6.95	0.27	CASP8, DAP3, IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNAR1, PARP2, RARA, RARB, TNFSF10
Role of RIG1-like Receptors in Antiviral Innate Immunity	6.54	0.304	CASP8, IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IKBKE, IRF3, NFKBID
Role of PI3K/AKT Signaling in the Pathogenesis of Influenza	5.02	0.221	GNAI2, IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNAR1, IRF3, NFKBID, PIK3CD
Role of Cytokines in Mediating Communication between Immune Cells	4.87	0.241	IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IL17F, IL20, IL24
Role of PKR in Interferon Induction and Antiviral Response	4.65	0.161	CASP1, CASP8, E2F1, HSP90AB1, HSP90B1, HSPA14, IFNA1/IFNA13, IFNA14, IFNA21, IFNA4, IFNAR1, IFNGR2, IKBKE, IL24, IRF3, NFKBID, NLRP11, NLRP13, NLRP4, NLRP5, NLRP8, NPM1
Activation of IRF by Cytosolic Pattern Recognition Receptors	4.6	0.215	IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNAR1, IKBKE, IRF3, NFKBID
SPINK1 General Cancer Pathway	4.29	0.203	EGFR, MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1M, MT1X, MT2A, MT3, MT4, PIK3CD, RRAS
Role of Wnt/GSK-3β Signaling in the Pathogenesis of Influenza	4.27	0.192	CSNK1A1, IFNA1/IFNA13, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, IFNAR1, WNT4, WNT7A, WNT7B
cAMP-mediated signaling	3.79	0.124	ADCY10, ADORA1, ADRA2C, AGTR1, APEX1, CAMK1D, CAMK2D, CHRM5, CNGA3, DRD1, DUSP1, GABBR2, GDPD1, GNAI2, GNAO1, GRM2, HTR1B, HTR1E, HTR5A, LPAR1, OPRL1, P2RY12, PDE4A, PKIA, PTGER4, PTH1R, RGS10, RXFP4, TDP2
MSP-RON Signaling Pathway	3.77	0.203	ACTB, KLK1, KLK15, KLK2, KLK3, KLK4, KLK5, KLK6, KLK7, MST1, MST1R, PIK3CD
Intrinsic Prothrombin Activation Pathway	3.74	0.233	COL2A1, COL5A3, KLK1, KLK15, KLK2, KLK3, KLK4, KLK5, KLK6, KLK7
CREB Signaling in Neurons	3.67	0.0967	ACKR1, ADCY10, ADGRF3, ADGRG1, ADGRG3, ADGRG5, ADORA1, ADRA2C, AGTR1, CACNB1, CACNG4, CACNG5, CAMK2D, CCKAR, CHRM5, DRD1, EGFR, FPR3, GABBR2, GHSR, GNAI2, GNAO1, GNAT1, GPR27, GPR37, GPRC5A, GRIN2D, GRM2, HTR1B, HTR1E, HTR5A, LPAR1, MCHR1, NMBR, NOTUM, NTSR2, OPRL1, P2RY11, P2RY12, PIK3CD, PLCD3, PLCZ1, POLR2C, POLR2D, POLR2E, POLR2F, POLR2J, POLR2J2/POLR2J3, PTGER4, PTGFR, PTH1R, RRAS, RXFP4, SSTR4, TACR1, TACR2, TAS1R3, VN1R2, VN1R4
Nucleotide Excision Repair Pathway	3.04	0.229	ERCC1, POLR2C, POLR2D, POLR2E, POLR2F, POLR2J, POLR2J2/POLR2J3, XPC
Hereditary Breast Cancer Signaling	2.99	0.132	ACTB, CDK1, CDK4, CHEK1, E2F1, FANCL, NPM1, PIK3CD, POLR2C, POLR2D, POLR2E, POLR2F, POLR2J, POLR2J2/POLR2J3, RRAS, SMARCC1, SMARCE1, UBC, XPC

Supplementary Table 9. Enriched networks in pain treatment outcome from IPA. Score, negative logarithm of network enrichment P-value. Focus molecules, genes with the most interactions to other genes which are connected together to form a network.

Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
AAMDC, ATP6V1E2, EGFR, ELP4, ENOPH1, EYS, FAM122A, FAM126B, FAM186A, FCGRT, GPC2, GXYLT1, HSBP1L1, IgG2c, Keratin II, 6, KIRREL1, LRRTM4, MANEA, MESD, MPZL1, MRPL57, OR2F2, OR2W3, PMM2, PRICKLE1, PTPRS, SERTAD4, SUMF1, TEKT2, TMEM30B, TRHDE, UGGT1, ZNF114, ZNF493, ZNF787	42	33	[Cancer, Dermatological Diseases and Conditions, Organismal Injury and Abnormalities]
ANTXR2, AP-3, AP3S2, C1orf216, CAMK1D, CD302, CDC42EP1, CDH1, CIB2, COBL, CREBL2, DPH6, EMC7, HERPUD2, IFNGR2, interferon receptor, JAGN1, MYRIP, PDAP1, PKNOX2, RAPH1, SLC18A1, SLC30A2, SLC30A3, SLC38A1, SMIM1, STX2, SYCE1L, TENM3, TMEM120B, TMEM60, TVP23A, UGDH, WIPF2, ZFYVE16	42	33	[Auditory Disease, Cellular Movement, Molecular Transport]
3 beta HSD, ABAT, ADCY10, AGAP6 (includes others), AQP9, BCL2L14, CBR1, CTDSP2, EI24, EIF4E3, GCLC, GLRX, GSR, HDHD5, IMMP1L, Insulin, LARP4, MMD2, NBEAL1, OCIAD1, OXCT1, PFKM, PPHLN1, RBAK, SFRP4, SGPL1, SHMT2, SOGA1, TBC1D4, WDR33, YME1L1, ZNF169, ZNF648, ZNF792, ZNF92	42	33	[Cell Morphology, Hematological System Development and Function, Inflammatory Response]
ACBD5, ATP23, CHRDL2, chymotrypsin, CIAPIN1, CLEC16A, CNGA3, DCTN5, FAM189B, FBN1, FBXL18, GP1BB, GP9, GPIIB-IIIA, GRAMD1A, HDDC3, HERC4, HOXA1, KRTAP10-10, KRTAP10-11, KRTAP12-4, KRTAP9-2, KRTAP9-8, LDLRAD4, LHFPL4, LNPK, MASTL, MFAP2, OBI1, PDZD8, RTF2, SMCP, SRC (family), TEX264, WRAP73	40	32	[Cell Morphology, Embryonic Development, Hair and Skin Development and Function]
APLP1, BACE1, BRI3BP, C2CD2, CCDC82, CD3, CDK4/6, CHTF8, COX6C, DCTN2, DYNC1LI1, EDDM3B, HIGD1A, IFRD2, IGSF3, MARCHF2, MSH3, NMU, NUBP1, PAFAH1B2, PCSK7, PCYT2, PNP, PPRC1, RAD1, RTN2, Secretase gamma, SPPL2B, SPX, SYNE4, TMEM106C, TMEM54, TSPAN15, UPK1B, ZFP36L1	40	32	[Auditory Disease, Hereditary Disorder, Neurological Disease]
14-3-3, ARHGAP9, C1orf35, CAD, DHX30, DRC7, FMNL1, GRWD1, HEXIM1, KSR1, MARS1, MTREX, Nfat (family), NOX4, NUDT21, OR10G4, peroxidase (miscellaneous), PPAN, PPM1G, RAB43, RBBP6, RPL12, RPL24, RPL27, RPL36, RPL9, S100PBP, SNRNP70, STAC3, THOC5, TMCC1, TMEM167A, TMPO, TOP2B, TRIP4	40	32	[Cancer, Protein Synthesis, RNA Damage and Repair]

Supplementary Table 9. Continued

Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
AREL1, ATXN7, BTD, CKB, CPTP, DAP3, DUB, FBXO38, GPALPP1, HERC2, Histone h2a, HPDL, HSP90AB1, IKBKE, LRRK2, MARCHF7, MYLK4, NAA20, NME8, PDHB, PHYHIP, PRR23B, RASL11B, RIPK2, RNF11, SLC5A6, TMEM127, TRIM17, TRIM37, UBC, UBE2L3, UBE2QL1, Ubiquitin, USP39, USP48	40	32	[Post-Translational Modification, Small Molecule Biochemistry, Vitamin and Mineral Metabolism]
ATP2C1, CEP68, COQ9, DCAKD, DLK1, FAR1, GABA-A receptor, GABRR3, GSTK1, KCNC1, KIF5A, KRTCAP2, MTORC1, NKX2-1, NOTUM, NUP93, PDS5A, POU1F1, RAB5IF, RBM6, RCC1, RUBCNL, SCO2, SEH1L, SETD9, SLAIN2, STRIP2, Tap, TRAF3IP3, TRAK1, TSH, USO1, WNT4, WNT7A, WNT7B	38	31	[Embryonic Development, Organismal Development, Tissue Development]
ABCA9, ACAP2, ACKR1, ADRA2C, ADTRP, AK2, APCS, APEH, APEX1, CD3EAP, CTCF, CYP8B1, EBNA1BP2, F Actin, INKA1, LDB1, LMNA, MPV17, NOVA1, NPTX2, PICK1, PPARA, Proinsulin, RBPJ, Spectrin, T2r, TAS2R14, TAS2R42, TAS2R50, TLNRD1, TRIM46, TRIM54, ZNF649, ZNF691, ZSCAN12	38	31	[Hereditary Disorder, Infectious Diseases, Neurological Disease]
ABCC3, AK3, ASXL2, ATP13A2, ATPase, C4orf17, CHD1L, CHD3, CLSPN, COQ8B, DDX25, DDX4, ESCO2, FAM111A, FAM111B, H2AF, Histone H2b, HP1BP3, HSPD1, IGSF10, KLHL33, MBD6, MRPL19, NIPSNAP1, NT5DC3, P glycoprotein, pyruvate dehydrogenase, RAD54L2, SCAF1, SLFN11, SPRYD4, THOC7, TMEM201, WDR3, WRNIP1	36	30	[Cell Death and Survival, Connective Tissue Disorders, DNA Replication, Recombination, and Repair]
AKAIN1, ANAPC1, ANK3, ARL6IP5, ARRDC4, ATP1B3, Camk, CAMK2D, CAPN3, caspase, CDC27, CEP76, CEP97, CHMP2B, EMC1, FAM110A, Fgf, HSPA12A, LGALS1, LMO4, MAP4, MYO6, NMT1, ORC2, PLK1, PNMA1, PREB, proprotein convertase, PRR3, ROPN1, TMEM43, TRMT61A, UBIQUITIN LIGASE, WIPI1, ZNF564	36	30	[Cellular Assembly and Organization, Cellular Function and Maintenance, DNA Replication, Recombination, and Repair]
AARSD1, AGR2, AGTR1, Caspase 3/7, CFAP57, DBNDD2, FBXO7, FOSB, HNRNPF, HNRNPH3, HNRNPK, HNRNPM, KIAA2026, LAMB4, LIAS, LRRC70, LSMEM2, MAP3K1, Ngf, Nos, OR2K2, Pkg, PLN, QRICH2, RAB3IP, RAN, RNF123, Rsk, SAFB, SAFB2, TGIF2, TOE1, YAF2, ZNF462, ZNF575	36	30	[Cellular Development, Connective Tissue Development and Function, RNA Post-Transcriptional Modification]
Akt, ALG3, B3GALT6, CLIP3, CNTN5, cytochrome C, FNDC4, Hif1, HPS6, MAN2A2, MGAT4B, Mt, MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1M, MT1X, MT2A, MT3, MT4, NRF1, OSBPL9, PARM1, Pdi, Ppp2c, PROK2, RNF170, SEC16B, SLC35B2, SPINK7, TCL1B, TRPM4	34	29	[Organismal Injury and Abnormalities, Psychological Disorders, Reproductive System Disease]

Supplementary Table 9. Continued

Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
AHCYL2, AKR1C1/AKR1C2, CAMKV, CDNF, CHD5, CMYA5, CTIF, ENOSF1, FAM160B1, HEXIM2, HIBCH, HSD17B, HSD17B12, HSD17B4, HSD17B7, HSD17B8, KANSL3, KDELR1, MANF, METAP1D, MSL1, N-Cadherin, P38 MAPK, PARP2, PBK, PIP4K2C, RIT1, RSBN1L, SERCA, SIRT, SIRT3, SNX20, TTC7A, tubulin (family), ZNF414	34	29	[Endocrine System Development and Function, Energy Production, Small Molecule Biochemistry]
ABI2, ADAM19, ADAM23, Aldose Reductase, AMPK, Arf, ARL5C, CG, CLDN12, DPH1, E2F1, EXOC1, FSCN1, GINS1, GINS4, glutathione peroxidase, GPX4, HMGA2, HS6ST1, IDH3A, IGFBPL1, INSL5, KHK, MICALL2, MMRN2, OGA, PARP, PIAS3, PLCD3, PUS3, SENP1, SEPTIN14, TDP2, TP73, TRIM32	34	29	[Carbohydrate Metabolism, Cell Death and Survival, Embryonic Development]
AFTPH, Alpha tubulin, Ap1 gamma, AP1AR, BETA TUBULIN, C8orf33, CLINT1, CNBP, DDX31, DNA-PK, DNAH10, Dynein, GGA1, Importin beta, KCTD6, KIF22, LGALS2, LRRC47, MBOAT1, MOB4, NOL12, PIGR, PRDM15, PRR11, SLA2, SNX22, SPINK2, TCR, TMF1, TTLL7, TUBA8, UGP2, VPS33B, ZBTB11, ZNF22	32	28	[Developmental Disorder, Neurological Disease, Ophthalmic Disease]
ASTE1, cAMP-dependent protein kinase, CIAO1, CPLX3, CROCC, Cyclin B, DNAAF5, ELAC2, FASTKD2, GMPPB, GOLT1B, HEPHL1, IL-1R, IL31RA, Il8r, INTS11, Lpa receptor, MTMR12, N4BP2, NABP1, NSMAF, OAZ1, OLFM2, PRORP, PUSL1, RAB11B, Ras homolog, RHOF, RNF214, RTEL1, SMG8, THBS3, Tnf receptor, TRMT10C, UBA3	32	28	[Cancer, Metabolic Disease, Organismal Injury and Abnormalities]
ADA2, APOBEC3A, B-cell receptor, CAVIN2, CDIPT, CIITA, CMPK2, CSRNP1, CXADR, FLRT2, GJD3, IgG2a, IgG2b, Igg3, IgG4, Interferon alpha, ITPKB, NIPAL3, NIPAL4, NKX2-3, NPM3, POU2AF1, RNF19B, SHFL, SIDT2, SLC35A5, snRNP-IgG Immune complex, TMEM100, TMEM130, TMEM72, TRIM22, TRIM34, TRIM5, TRIOBP, UQCC1	32	28	[Cardiovascular Disease, Dermatological Diseases and Conditions, Developmental Disorder]
aldehyde dehydrogenase, aldehyde dehydrogenase (NAD), ALDH, ALDH1A3, ALDH1B1, ALDH3A1, ANKRD28, ARHGAP44, ASAP3, C/EBP, CEP120, CEP131, CEP192, CNKSR3, CRLF3, DHX37, DIP2B, EDC3, Eph Receptor, EPHB3, GRB10, GTPase, HOOK1, HOOK3, HPCAL1, LTV1, PDE4DIP, PPP2R2D, RCAN3, RCCD1, Sfk, SH3BP1, WASHC2A/WASHC2C, WHRN, ZBTB21	32	28	[Cell Cycle, Cellular Assembly and Organization, Cellular Function and Maintenance]

Supplementary Table 9. Continued

Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
Activin, ANGPTL6, Ap2, Ap2 alpha, ARID4A, Cg Beta, CGB3 (includes others), CGB7, CIDEA, Cohesin, FST, HOXA2, HOXB1, HOXB2, HOXB3, HOXB4, HOXB5, HOXB6, HOXB7, HOXB8, IL20, INHBC, INHBE, Inhibin, IRX2, LY75, MPC1, NUCB2, NXPH4, POU4F1, PRC2, TCF23, TFAP2B, Vegf, ZP4	30	27	[Embryonic Development, Organismal Development, Skeletal and Muscular System Development and Function]
CLPSL1, COL4A2, CR2, Ctbp, CYP, CYP1A2, DCAF1, Focal adhesion kinase, HIC1, HOXA4, IFI35, IFN type 1, IFNA1/IFNA13, IFNA10, IFNA14, IFNA17, IFNA21, IFNA4, IFNA5, IFNA6, IFNA7, Ifnar, IRF, IRF2, ITLN2, JAK, PCYOX1L, Pias, RNF216, SAMHD1, SP100, TAGLN, UBA7, USP18, ZNF816	30	27	[Cellular Development, Hematological System Development and Function, Lymphoid Tissue Structure and Development]
AAK1, ACOX2, CARD16, CASP1, CD300C, CD300E, CD300LD, CGREF1, coactivator-Dtx-Notch-Rbpsuh, coactivator-Mam-Notch-Rbpsuh, DKK4, DTX3, DTX4, HCST, IKKA/B, Inflammasome, MAML1, MAML2, MEX3A, NALP, NFkB (complex), NLR, NLRP11, NLRP13, NLRP4, NLRP5, NLRP8, Notch, NUMBL, Pro-inflammatory Cytokine, SH2D5, SKAP2, TIFA, TMSB10/TMSB4X, TYROBP	28	26	[Cell Death and Survival, Cellular Function and Maintenance, Hematological System Development and Function]
BARX1, BMP, CER1, DEPP1, DHH, DKK, DKK1, DKK2, FGF18, FGF8, FOXC2, Gli, GLI1, HAND2, Hedgehog, HJV, LRP, Mapk, MAZ, MESP2, MSX1, NEK10, NEUROG3, Patched, PAX9, PPAN-P2RY11, RGS19, RTN4RL1, SMAD1/5/9, SOSTDC1, STRA8, TBX1, TEP1, Wnt, ZNF100	28	26	[Cardiovascular Disease, Cardiovascular System Development and Function, Digestive System Development and Function]
AChR, arginase, CAPZA3, CCDC102A, CHRNA6, CHRNB3, cytokine, DDN, DEXI, DSN1, FBXL7, GALP, HPCA, IL6ST, ITPRIPL1, JAK1/2, KCNF1, LRRCC1, Lymphotoxin, MLLT10, nicotinic acetylcholine receptor, NRG (family), PMF1/PMF1-BGLAP, PPIL3, Rab11, RALYL, RNASE10, RUFY2, SCAMP5, SEMA4A, SGTB, SKA2, SOCS, SOX18, SYT11	28	26	[Neurological Disease, Organismal Injury and Abnormalities, Psychological Disorders]
ALT, ARHGAP31, ARL6IP1, C19orf54, CEP85L, FANCL, FMO5, Glycogen synthase, GOT, Histone H1, IKK (complex), KHDC4, LDL-cholesterol, MFSD14B, MTTP, PPP1R3G, PRKAB2, PXR ligand-PXR-Retinoic acid-RXRÎ±, PYGB, RIMBP3 (includes others), RMI1, SCD, SERTAD2, SLC13A1, SLC2A8, STARD4, TARS1, THSD7B, TLX3, TMEM176A, TMEM258, TP53I11, VLDL, VLDL-cholesterol, ZNF44	28	26	[Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism]

Supplementary Table 10. Genetic correlations with pain treatment outcome. Rg, the estimated genetic correlation. SE, the bootstrap standard error of the genetic correlation estimate. Z, the z-statistic (rg/se) for testing whether the genetic correlation is significantly different from zero. P: p-value from the z-statistic. (Here lists top 20 records based on P-value. The complete table can be found online.)

Trait1	Trait2	Category	Ethnicity	Rg	SE	Z	P
switching from NSAIDs to opioids	Overall health rating	ukbb	European	0.5316	0.2025	2.6254	0.0087
switching from NSAIDs to opioids	Years of schooling 2016	education	European	-0.5431	0.2115	-2.5682	0.0102
switching from NSAIDs to opioids	Qualifications: None of the above	ukbb	European	0.5253	0.2047	2.5662	0.0103
switching from NSAIDs to opioids	Pain type(s) experienced in last month: Stomach or abdominal pain	ukbb	European	0.5639	0.2206	2.5568	0.0106
switching from NSAIDs to opioids	Qualifications: College or University degree	ukbb	European	-0.4668	0.1838	-2.5392	0.0111
switching from NSAIDs to opioids	Pain type(s) experienced in last month: Neck or shoulder pain	ukbb	European	0.6480	0.2562	2.5298	0.0114
switching from NSAIDs to opioids	Qualifications: CSEs or equivalent	ukbb	European	0.6807	0.2768	2.4592	0.0139
switching from NSAIDs to opioids	Age completed full time education	ukbb	European	-0.5905	0.2400	-2.4602	0.0139
switching from NSAIDs to opioids	Current employment status: Unable to work because of sickness or disability	ukbb	European	0.7586	0.3106	2.4422	0.0146
switching from NSAIDs to opioids	Job involves heavy manual or physical work	ukbb	European	0.5294	0.2170	2.4395	0.0147
switching from NSAIDs to opioids	Qualifications: A levels/AS levels or equivalent	ukbb	European	-0.4684	0.1937	-2.4180	0.0156
switching from NSAIDs to opioids	Pain type(s) experienced in last month: None of the above	ukbb	European	-0.4603	0.1912	-2.4077	0.0161
switching from NSAIDs to opioids	Number of operations_ self-reported	ukbb	European	0.5689	0.2427	2.3435	0.0191
switching from NSAIDs to opioids	Sleeplessness / insomnia	ukbb	European	0.4260	0.1818	2.3434	0.0191
switching from NSAIDs to opioids	Age at first live birth	ukbb	European	-0.4744	0.2029	-2.3375	0.0194
switching from NSAIDs to opioids	Types of physical activity in last 4 weeks: Other exercises (eg: swimming_ cycling_ keep fit_ bowling)	ukbb	European	-0.5149	0.2210	-2.3304	0.0198

Supplementary Table 10. Continued

Trait1	Trait2	Category	Ethnicity	Rg	SE	Z	P
switching from NSAIDs to opioids	Pain type(s) experienced in last month: Back pain	ukbb	European	0.4725	0.2056	2.2984	0.0215
switching from NSAIDs to opioids	Pain type(s) experienced in last month: Headache	ukbb	European	0.3535	0.1560	2.2661	0.0234
switching from NSAIDs to opioids	Age at last live birth	ukbb	European	-0.5150	0.2272	-2.2669	0.0234
switching from NSAIDs to opioids	Mouth/teeth dental problems: None of the above	ukbb	European	-0.5030	0.2223	-2.2634	0.0236

Supplementary Table 11. Nominal significant genetic correlations enrichment. Nr. of insignificant genetic correlations (P > 0.05), number of tested traits with insignificant genetic correlations with pain treatment response; Nr. of nominal significant genetic correlations (P < 0.05), number of tested traits with nominal significant genetic correlations with pain treatment response.

	Pain, education, employment traits (%)	**Other tested traits (%)**
Nr. of insignificant genetic correlations (P > 0.05)	26 (56.52%)	503 (91.45%)
Nr. of nominal significant genetic correlations (P < 0.05)	20 (43.48)	47 (8.55%)
Total	46	550

Chapter 8

General Discussion

Chronic pain significantly affects the quality of life and poses a substantial economic burden. Understanding the mechanisms underlying pain development could improve the management of pain. However, it is challenging to understand the mechanisms of pain as pain is a multifactorial trait with a complex interplay between genetic, neurobiological, psychosocial, and environmental factors. These factors lead to variations in individual pain development, pain experiences, and treatment outcomes. Among these factors, there is evidence that genetic factors are an important contributor to pain development and treatment as suggested by heritability studies [1]. Therefore, a deeper understanding of genetic factors contributing to pain development is essential. This will inform us about the mechanisms involved in pain development and might facilitate personalized pain management. In this thesis, we aimed to better understand the genetic architecture of chronic pain by identifying genetic variation associated with pain development and treatment.

This thesis addresses the knowledge gap regarding the role of genetics in pain development, encompassing both Mendelian and multifactorial pain disorders, the latter with a specific focus on chronic postsurgical pain. In **Chapter 2**, we explore the genetic contribution to a Mendelian pain disorder, primary erythermalgia. Using whole genome sequencing, we identified 97 potentially disease-causing candidate genes in both families and narrowed this number down to ten top candidate genes by focusing on genes related to neurology, nociception, and pain functions. However, none of these genes and variants could be causally linked to primary erythermalgia. The inability to pinpoint a causal gene suggests that EM is more heterogeneous than expected, with a complex underlying genetic mechanism. In **Chapter 3**, we reviewed 57 full-text articles and identified 30 loci reported to be associated with pain in more than one study. These genes, associated with different pain phenotypes, highlighted a role for neurological functions and inflammation in pain. In **Chapter 4**, we performed a genome-wide association study (GWAS) on 27,603 participants from the UK Biobank who underwent abdominal surgery. Two identified loci containing pain-related genes (*SRPK2*, *PDE4D*) were selected for further validation in clinical samples of adhesions from patients with and without pain. Although the results did not show statistical significance, the RT-PCR detection rate and expression level of PDE4D were modestly higher in patients with pain compared to the control group. In **Chapter 5**, we included all types of surgeries and conducted GWAS in a cohort of 95,931 individuals from the UK Biobank who had undergone various surgical procedures. One genetic locus within *GLRA3* displayed a genome-wide significant association ($P < 5 \times 10^{-8}$) with the development of chronic postsurgical pain (CPSP). Both **Chapter 4** and **5** provide new insights into the genetic factors contributing to chronic postsurgical pain

development. However, it is advisable to validate these findings in other cohorts. In **Chapter 6**, we presented a protocol outlining ongoing research at our center. The prospective, observational study described aims to recruit approximately 10,000 patients undergoing elective surgery. This study serves as an example of conducting pain research by considering the multidimensional and multifactorial nature of pain. It may also simplify risk profiling assays for future use, yielding a simpler, more accurate, and cost-efficient assay or potentially identifying new targets for treatment. In **Chapter 7,** we investigated genetic factors influencing pain treatment outcomes using a GWAS with ~23,000 UK Biobank participants, comparing NSAID and opioid users as a reflection of the treatment outcome of NSAIDs users. We found one genome-wide significant hit and annotated genes by suggestive loci ($P < 1\times10^{-6}$). These loci included four linked to neuropathic pain or musculoskeletal development. Pathway analyses highlighted immunity-related processes and suggested a central role for EGFR. This study represents an initial step in understanding the genetic basis of musculoskeletal pain treatment outcomes.

The main findings across all chapters in this thesis are summarized and discussed below, followed by the implications for clinical care and research into chronic pain. In the discussion, I cover the different genetic methods and various phenotype definition in (our) pain research, the identified genetic associations with pain development and treatment, the pleiotropic effects of identified loci, challenges of applying genetic findings in the clinical care, and future recommendations for research and pain management.

Different genetic methods applied in this thesis

This thesis adopted two key methods in genetic research: Genome-Wide Association Studies (GWAS) and Whole Genome Sequencing (WGS). GWAS involves scanning the genome for common genetic variants in different individuals to identify associations with specific traits or diseases. It is particularly suitable for studying multifactorial disorders, where multiple genetic variants with small effects contribute to the condition, making it efficient and cost-effective for large-scale studies. Large-scale cohorts with homogeneous phenotype definitions are needed for GWAS to detect variants with smaller effect sizes and to ensure sufficient power, which remains an unmet need in pain research. However, GWAS typically captures only common variants and may miss rare or structural variants. In contrast, WGS provides a comprehensive analysis of the entire genome, identifying both common and rare variants and structural changes, offering a more complete genetic picture. WGS is suitable for studying Mendelian disorders, where genetic variants have strong effects, though its primary disadvantage is the relatively high cost. However,

WGS is not limited to the study of Mendelian disorders. For instance, the UK Biobank is implementing WGS on its participants to identify associations for rare variants with large(r) effects linked to multifactorial traits or disorders [2].

Variability in chronic postsurgical pain definition

In pain research, various cut-offs and settings have been applied for chronic postsurgical pain definition. Some studies used a 3-month cut-off [3-5], while others used a 6-month cut-off [6-8]. Additionally, some studies investigated CPSP using questionnaires distributed over a range of 2 months to 10 years [9, 10]. In addition, different types of surgeries were considered for genetic studies, including hysterectomy [3, 4], abdominal and knee surgeries [4, 5], mastectomy [9, 10], knee replacement [6], hernia [7], and knee arthroplasty [8]. Moreover, variations in demographic differences, cohort settings, and genotyping platforms add to the complexity. This is also reflected in this thesis. Although there is a questionnaire of self-reported CPSP (Data-Field 120005) in the UK Biobank, we did not utilize it to run a GWAS because, as per UK Biobank, there were significant issues with case definition due to the difficulties with assessing chronicity and determining sub-types of neuropathic pain. Therefore, we used analgesic prescription duration as a proxy phenotype for CPSP. The advantage of this phenotype definition is that it captures more severe pain symptoms necessitating medication, as indicated by the relatively low prevalence of CPSP in the UK Biobank. However, as evidence indicates, patients are experiencing significant pain but opting not to pursue treatment [11]. A drawback of this phenotype definition is that it excludes patients who experienced CPSP but did not require analgesics. Additionally, CPSP development could be a combination of genuine CPSP and a suboptimal response to analgesics.

Discovery of genetic associations with pain development and treatment

Looking across all chapters of this thesis, the analysis indicates two primary biological functions underlying pain development: neurological processes and (chronic) inflammation, both of which play crucial roles in pain development. This aligns with the systematic review conducted in **Chapter 3**, where an overview was presented of all identified (potential) genetic risk factors for pain from GWAS studies conducted so far. Among the 30 overlapping loci found between studies, more than half of the identified genes are implicated in neurological functions and inflammation.

The involvement of genes related to neurological function meets our expectations because pain is mediated by processes in the nervous system, regardless of the nature of pain, and by neurological effects that may alter the perception of pain

at the peripheral level and neuroplasticity at the central level. These findings underscore the importance of neuronal signaling in pain development. In **Chapter 3**, the identified risk loci for pain indicate that both the central and peripheral nervous systems are involved, encompassing various functions such as neurotransmission (*AMIGO3*), neurodevelopment (*ADAMTSL4*, *NGF*, *EPHA3*, *EPHA4*), brain development (*CA10*, *FOXP2*), peripheral nerve pathophysiology (*FGD4*), neurogenesis (*SPOCK2*), and nociceptive pathways (*DCC*). In **Chapter 4**, we identified *SRPK2* associated with CPSP after abdominal surgeries. *SRPK2* is involved in neuronal apoptosis both *in vitro* and *in vivo [12]*, underscoring the role of neurological processes. In **Chapter 7**, investigating the response to pain treatment, two genes implicated in neuropathic pain conditions were identified (*NPTX2* and *IPCEF1*). NPTX2 is downregulated in the brain in induced chronic neuropathic pain and induced endometriosis mouse models. The other gene, *IPCEF1*, has previously been related to neuropathic pain conditions [13]. These results underscore the diverse central and peripheral nervous functions of identified genetic loci affecting pain development and treatment.

The other significant group of contributors to pain are inflammatory and immunological mechanisms. Inflammation plays a crucial role in acute pain and the pathogenesis of chronic pain. The activation of immune cells in damaged tissues (i.e., inflammation) can cause hypersensitivity in peripheral pain-sensing neurons [14]. This inflammatory pain normally resolves as tissues heal. However, if pain does not resolve during tissue healing, it can contribute to the onset and maintenance of chronic pain by impacting neuronal plasticity in both peripheral (peripheral sensitization) and central pain pathways (central sensitization), leading to numerous changes within the somatosensory system [15-18]. The collection of identified genes implicated in inflammatory and immunological functions underscores this point. In **Chapter 3**, the function of identified genes includes inflammation onset (*SLC39A8*), inhibition of proinflammatory cytokine transcription (*C6orf106*), and prostaglandin transportation (*ABCC4*). In **Chapter 4**, the identified gene (*PDE4D*) belongs to the phosphodiesterases (PDE) protein family, which is responsible for cAMP hydrolysis in nerve and immune cells [19]. PDE4 inhibition can produce potent antinociceptive activity [19] and reduce neuroinflammation [20] in animal models. In **Chapter 5**, the genome-wide significant locus was mapped to *GLRA3*, which encodes a protein (GlyRα3) of the glycine receptor subfamily. The glycine receptors are widely distributed throughout the central nervous system, particularly within the hippocampus, spinal cord, and brain stem. Although being investigated in both inflammatory and neuropathic pain models, GlyRα3 seems to play an important role specifically in inflammatory pain [21, 22]. In **Chapter 7**, the identified gene, *TXK*, plays a role in regulating the adaptive immune response, and

this gene has previously been linked to Immune-Inflammatory Diseases [23]. The identified genes underscore the critical roles of inflammation and immunological responses in the development and modulation of pain. Besides, the interplay between inflammation and the neurological system is crucial for influencing pain perception by modulating neuronal excitability and leading to neuroinflammation in chronic pain conditions. Our results reaffirm the neurological and immunological axis of pain development [24].

In addition to their role in physiological and pathophysiological biological processes, certain identified genes are associated with underlying diseases where pain is a symptom. For instance, in **Chapter 3**, the identified genes associated with chronic back pain are implicated in chondrogenesis (*SOX5* and *SOX6*) or lumbar disc degeneration (*CCDC26*/*GSDMC*). Likewise, in **Chapter 7**, two genes linked to muscular or skeletal dystrophy were identified: *SGCB* and *FN1*. These genes are interesting candidate genes because musculoskeletal dystrophy often involves pain. Therefore, genes contributing to the underlying disease pathology may also play a role in pain development. Although it is difficult to determine if these genes contribute to pain development directly or through the progression of the underlying disease, understanding how these genetic factors contribute to disease progression can provide insights into mechanisms driving pain and potentially guide strategies for managing both the disease and associated pain more effectively.

Not all identified loci had a clear (pain-related) biological function. The function of some loci remains unclear as they were mapped to an intergenic region or non-protein coding genes with unknown functions (such as *LINC01065* and *C8orf34* identified in **Chapter 3**). Rather than influencing protein coding, these variants might regulate gene expression levels. However, this warrants future research using gene expression mapping methods, such as eQTL mapping or chromatin interaction mapping [25].

Discovery of genetic correlations of pain and the pleiotropic effects of identified loci

Genetic correlation is the proportion of variance that two traits share due to genetic causes. Genetic correlations can estimate the degree of pleiotropy or causal overlap between complex traits and diseases. The genetic correlation results in this thesis suggest that pain subtypes may not be entirely genetically independent. Although the results are statistically insignificant, genetic correlation among GWASes on CPSP development after different surgery subtypes (visceral surgeries, musculoskeletal surgeries, nervous surgeries, otorhinolaryngology and eye surgeries, and vascular

surgeries) show high correlation coefficients in **Chapter 5.** We identified an overrepresentation of genetic correlation between pain treatment outcomes with pain traits compared to other traits in **Chapter 7**. This indicates that these traits are not genetically independent. A shared genetic architecture and/or biological pathways might contribute to pain traits and other traits. Further research, such as Mendelian randomization, is warranted to investigate the causal relation between these genetically correlated traits. Mendelian randomization is a method that uses genetic variants as instrumental variables to infer causality between an exposure and an outcome, mimicking randomization in controlled trials to reduce confounding and reverse causation.

SNP pleiotropic effects denote the potential of genetic variants to be associated with multiple phenotypes. The pleiotropic effects of identified SNPs align with the functions of the identified genes, including neurological, psychiatric, immunological, and metabolic traits. **Chapter 4** showed that four identified lead SNPs were associated with regional brain volumes (e.g., cerebellum, pallidum) in the pleiotropic effects analysis. This might link to CPSP as previous studies showed various anatomic sites of altered brain morphology involved in pain perception and modulation [26, 27]. In addition, depression and lipid profiles were identified as pleiotropic effects for lead SNPs. In **Chapter 5**, the pleiotropic effects analysis of the lead SNPs revealed the importance of other traits in CPSP development, such as psychiatric traits (depression, posttraumatic stress disorder, and anxiety) and BMI, which are associated with pain development [28, 29]. Additionally, the GWAS catalog indicates SNPs with pleiotropic effects of red blood cell count. This connection between red blood cell traits and pain is intriguing, as red blood cell distribution width has been linked to chronicity in nonspecific low back pain [30]. The identified pleiotropic effects indicate that not only phenotypic correlations exist between pain and these traits [31-33] but that there are also shared genetic mechanisms.

Recommendations for future genetic research on pain

As indicated in **Chapter 3,** genetic studies in the field of pain research are still limited, and conducting these studies is challenging due to the complex genetic background of pain, the scarcity of large subject cohorts, inconsistent phenotype definitions, and the small effect sizes of identified genetic variants. Adding to these challenges are the occasional contradictory results and inadequate statistical power observed in previous studies. Still, studies clearly suggest a complex genetic architecture of pain, reflected by the heritability of pain [34]. Interestingly, the gene functions of the genes identified in this thesis nicely align with the hypotheses about the origins and mechanisms of pain. Especially the genes implicated in

neurological and immunological functions warrant prioritization for validation and further investigation.

As reflected in the GWAS on CPSP described in **Chapter 5**, individual studies typically include only a few hundred samples [35]. Even the meta-analyses reached only a sample size of maximally 1,350 subjects [36]. Therefore, collaboration and consortium efforts are needed for both Mendelian and multifactorial pain diseases to achieve larger sample sizes and adequate statistical power. These initiatives facilitate data sharing and resource pooling, thus enabling a more comprehensive exploration of genetic causal variants for both Mendelian and multifactorial diseases, which can help identify undetectable loci in individual studies and yield more robust results. This has been proven to work in diseases other than pain. [37, 38].

Looking at pain as a complex phenotype, it is unsurprising that the definitions of pain vary across studies. As reviewed in **Chapter 3** and the discussion above, genetic studies employ diverse definitions of pain, complicating comparisons and conclusions. Specifically, different diseases leading to pain (e.g., osteoarthritis, diabetes) are investigated, along with various types of pain (e.g., nociceptive, neuropathic, and nociplastic). Additionally, pain measurements also vary, ranging from pain questionnaires and numeric scales to ICD codes. On top of that, studies differentiate between acute and chronic pain and use diverse criteria to define chronic pain. To empower accurate replication studies, meta-analyses, and international collaborations, it is highly recommended that future studies use clear, consistent phenotype definitions aligned with the current diagnosis definition/ system, such as the ICD-11 classification for chronic pain [39]. For instance, given the current lack of definitive evidence on whether different pain subtypes share the same genetic background, it is essential to specify the subtype of pain under investigation. The research protocol in **Chapter 6** could serve as a valuable reference for future studies investigating chronic postsurgical pain. In this protocol, we outline the methods to assess postoperative pain from multiple perspectives that impact various aspects of a person's life. Taking such a comprehensive approach provides a better understanding of the effects of postoperative pain.

Findings from genetic studies in Mendelian disorders and multifactorial pain disorders can be interconnected. Genes linked to Mendelian disorders can be dysregulated by (non-coding) variants in complex traits displaying similar phenotypes. Therefore, findings from related Mendelian disorders and functional genomic datasets can be used to prioritize genes that are putatively dysregulated by GWAS variants [40]. Still, confirmation of findings in independent cohorts is essential for genes identified

with GWAS (in **Chapters 4, 5, and 7**) or sequencing (in **Chapter 2**). On top of that, functional studies are needed to validate the function of genetic markers, and this can be approached in several ways. In vitro assays can be used to explore cellular effects (such as CRISPR gene editing and reporter assays), and animal models might be helpful to reveal physiological implications. Combining several approaches ensures a thorough understanding of genetic markers in disease mechanisms and their potential as therapeutic targets.

For the identified genetic correlations, it is unclear whether there is causality between the associated traits. Further research can focus on Mendelian randomization analysis to determine causal relationships between one trait as modifiable exposures influence the other trait as outcomes, thus enhancing our understanding of disease mechanisms and informing clinical interventions. To assess the directionality of these relationships (e.g., does trait A influence trait B or vice versa), bidirectional Mendelian randomization can be conducted in future research. The SNPs with pleiotropic effects are promising candidates for further investigation. They influence multiple traits or diseases, suggesting they play a central role in key biological pathways, potentially providing insights into the fundamental processes and the shared etiology between different traits or diseases. Sometimes, pleiotropic SNPs are involved in two not closely phenotypically related traits; this may indicate that general biological processes are involved in these traits, or it suggests that the studied traits are more biologically related than previously thought [41]. Moreover, pleiotropic SNPs can be utilized to improve the accuracy of risk prediction [42] and provide valuable information in Mendelian randomization analysis [43].

Other than genetic markers, we also advocate for broadening the scope of biomarker research in the field of pain. In this thesis, we only investigate DNA variants as biomarkers for pain. However, the biological process from genetic markers to final protein expression is complex and involves multiple regulatory layers. Preliminary research has explored different biomarkers involved in (postoperative) pain development, including epigenetic modifications (such as DNA methylation regulating gene expression) [44], transcriptomic profiles (mRNA expression level changes) [45], and post-translational profiles (protein expression and modifications) [46]. Future research should validate these results, screen markers using non-hypothesis methods, and integrate these biomarkers to provide a comprehensive understanding of pain mechanisms.

In summary, for future pain genetic studies, we recommend collaborations to perform analysis in larger sample sizes, validate the function of genetic markers,

investigate the causality between identified genetic correlations, expand the scope of biomarkers beyond genetic markers. Conducting future research with careful experimental designs is essential, as well as ensuring sufficient statistical power and employing robust statistical methods to minimize incidental findings.

Recommendations for clinical care

For clinical care, a clear definition of pain will also optimize pain treatment and management. Now, studies have used pain as a broad umbrella concept encompassing various subtypes. However, the etiology and clinical manifestations may vary in each subtype. This inherent heterogeneity poses challenges in accurately diagnosing and treating pain-related conditions. Recently, a systematic classification of chronic pain in ICD-11 was developed by IASP [39], integrating existing pain diagnoses to provide precise definitions and characteristic features. We believe this will significantly enhance clinical care through clear definitions. Besides, it will aid genetic studies, allowing the selection of actual cases and analysis with homogeneous patient groups, which will ultimately inform clinical care.

Regarding the application of genetic findings in pain management and treatment in clinical care, insights from Mendelian disorders may lead to identifying therapeutic targets for pain. While we did not identify novel causal genes for erythermalgia, previous studies have identified SCN9A (encoding Nav 1.7) as the causal gene for most cases of erythermalgia. Multiple (selective) Nav 1.7 inhibitors have been tested in clinical trials for various pain indications, including pain in Nav1.7-related small fiber neuropathy [47], patients with postherpetic neuralgia with moderate or greater pain [48], or pain models in healthy subjects [49].

Regarding genetic findings for multifactorial pain disorders, while multiple genetic variants have been identified, most of these variants exhibit small effect sizes. Therefore, they may not serve as potential treatment targets on their own. However, applying a polygenic risk score (PRS) based on GWAS results holds promise for predicting (chronic postsurgical) pain and facilitating personalized pain management in clinical settings. There is a growing trend to incorporate PRS in risk prediction for clinical care [50, 51]. PRS has demonstrated translational potential as predictive and prognostic biomarkers for various common diseases, such as breast cancer [52], cardiovascular disease [53], and Alzheimer's disease [54]. For prediction models to be clinically useful, they must demonstrate adequate discrimination between sensitivity and specificity, be externally validated, and should have a significant clinical effect on patient care. In the context of CPSP, risk prediction is still in its early stages. Although many risk prediction models have

been proposed, incorporating multiple identified risk factors (as mentioned in the introduction section of this thesis), these models are at high risk of bias and are challenging to apply in clinical settings [55]. One of the reasons is that most current models lack generalizability, as they are limited to specific populations and surgical procedures. Secondly, there is significant heterogeneity in the tools used to assess CPSP, pain intensity cut-off values to distinguish between individuals with and without CPSP and follow-up times. Thirdly, there is still room for improvement of prediction to improve the models for chronic postsurgical pain, e.g., one study has explored incorporating genetic risk factors like polygenic risk scores in the model [56]. Integrating PRS into risk assessment shows higher predictive accuracy for CPSP compared to non-genetic models, with the area under the ROC Curve (the overall ability of the model to discriminate cases) increased from 0.70 to 0.96) [56]. However, these findings must be replicated and validated before being integrated into clinical practice. To improve clinical care for pain, robust prediction models including polygenic risk scores (PRS), are needed. This highlights the importance of large-scale GWAS efforts in extensive cohorts to construct reliable PRS models.

Additionally, pain is a multidimensional experience. Low pain scores do not guarantee that patients find their pain acceptable, nor do high pain scores invariably mean patients are dissatisfied with their pain levels [11]. In fact, one in ten patients experiences unacceptable pain even if they report low pain scores [11]. Utilizing multiple-item pain questionnaires to understand better acceptable pain levels for individual patients may help clinicians facilitate more effective CPSP treatment [55]. An example of such an initiative is the PPG cohort at our research center, as mentioned in **Chapter 6**.

Applying genetic findings in clinical pain care also involves tailored pain management (optimizing medication selection and dosing for each patient) by leveraging genetic information that influences drug metabolism and response. Some pharmacogenetic findings have been incorporated into clinical practice, such as therapeutic recommendations for using *CYP2D6* genotype results in prescribing codeine and tramadol [57, 58]. However, other pharmacogenetic findings in pain treatment still lack strong evidence, e.g., findings for *OPRM1* and *COMT* [59, 60], and validation studies are essential to firmly prove whether these genes might be of clinical value. In addition to the traditional approach of applying pharmacogenetics for medication guidelines based on variants in a single or maybe two genes per drug, recent studies have investigated the potential of applying polygenic risk scores (PRS) in pharmacogenetic studies [61, 62]. By integrating effects from multiple genes and pathways, PRS may advance pharmacogenomics to

the next level. Such an approach aligns nicely with the metabolization and working mechanisms of a drug involving many genes.

Recommendations on education and social impact

Medical training should include foundational knowledge of the role genetics in pain development and management. Understanding these factors helps physicians better grasp the underlying reasons for variations in pain experiences, as well as how genetic predispositions and environmental factors influence pain development and drug responses. While personalized pain management based on genetic data is still evolving, integrating these topics into medical education is essential to train physicians in an early stage and prepare them for possible use of genetics in delivering precise, patient-centered care and tailoring treatments to individual patients in the future.

We recommend promoting the societal impact of genetics and epigenetics in pain management while prioritizing funding for these programs from the EU and national health institutions. This approach will foster a comprehensive understanding of the role of (epi)genetic factors in pain management. Investing in such initiatives can lead to more accurate diagnoses and personalized treatments, ultimately making healthcare more efficient and cost-effective in the long run.

A summary of recommendations for future research, clinical care, medical education, and social impact can be found in Table 1.

Concluding remarks

Pain is a complex phenotype where combinations of genetic variants interact with environmental factors influencing pain development and treatment. Our study identified genetic variants associated with pain development and treatment, and the identified genes were linked to neurological and immunological functions. These findings contribute to a better understanding of the genetic architecture of pain, shedding light on the molecular mechanisms of pain etiology. While there is still a significant journey ahead to conduct large-scale genetic studies, validate these results in independent cohorts, and develop risk prediction models with validation and clinical impact analyses, harnessing genetic findings holds promise for improving pain management by enabling the prediction of pain development, customizing drug prescriptions, and potentially uncovering new drug targets for pain treatment.

Table 1. Summary of recommendations for future genetic research, clinical care, education, and social impact.

Recommendations	Benefits/Influence
Recommendations for future genetic research on pain	
Collaboration and consortium efforts	Larger sample sizes and adequate statistical power
	Data sharing and resource pooling, comprehensive exploration
Use clear, consistent phenotype definitions	Avoid heterogeneity in pain phenotype definition, facilitate the subtype of pain under investigation
Interconnect findings from genetic studies in Mendelian and multifactorial pain disorders	Gene prioritization for validation/further research
Validate the biological function of genetic markers	Understanding of genetic markers in disease mechanisms and their potential as therapeutic targets
Mendelian randomization analysis	Determine causal relationships between one trait and the other
Broaden the scope of biomarker research	Provide a comprehensive understanding of pain mechanisms
Recommendations for clinical care	
A clear definition of pain	Optimize pain treatment and management
Translate identified genes as targets for pain treatment	Discover novel pain treatment medications
Applying a polygenic risk score on pain prediction	Facilitate personalized pain management in clinical settings
Use multiple-item pain questionnaires	Help clinicians understand better acceptable pain levels and facilitate more effective pain treatment
Incorporate pharmacogenetic findings into clinical practice	Tailored pain management
Recommendations on education and social impact	
Include foundational knowledge of the role genetics and epigenetics in pain development and management	Help physicians better grasp the underlying reasons for variations in pain experience
Promote the societal impact of genetics in pain management	Prioritize funding for pain (genetic) research

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Chapter 9

Summary

Pain is characterized as an unpleasant sensory and emotional experience linked to actual or potential tissue damage. Conditions associated with pain in humans can manifest as Mendelian disorders (caused by single genes) or as multifactorial diseases (caused by numerous genes along with environmental and clinical factors). While Mendelian pain syndromes are generally rare, a common form of multifactorial pain is chronic pain, which affects approximately 20% of the adult population. Chronic pain is the leading primary cause of years lived with disability. Several risk factors for chronic pain have been identified, including sociodemographic factors (such as age, female gender, and occupation), psychological factors (such as depression), clinical factors (such as chronic diseases), and lifestyle. In addition to these factors, genetic susceptibility also plays a role in pain development. Genetic studies on pain aim to pinpoint genes causing Mendelian pain disorders and clarify the genes and genetic mechanisms linked to multifactorial disorders, thus explaining the variance in pain development within populations. Such genetic findings contribute to a better understanding of the functional mechanisms underlying pain development, hold promise for developing potential treatments for the disorders, and pave the way for improved pain management by incorporating genetic factors into clinical practice. With this thesis, we have contributed to understanding the genetic backgrounds of Mendelian and multifactorial pain disorders. Below is a summary of the content covered in the chapters of this thesis.

To explore the genes involved in Mendelian pain disorders, in **Chapter 2,** we investigated the genetic causes of the Mendelian pain disorder erythermalgia (EM). EM is a rare condition characterized by recurrent episodes of red, warm, and painful swollen extremities. It can be primary, caused by gain-of-function missense mutations in the *SCN9A* gene, or secondary, stemming from underlying diseases or medication use. We examined two families with primary EM without pathogenic variants in a known gene for this disorder, *SCN9A*. Whole-genome sequencing was conducted in six patients with EM and two unaffected family members to identify the disease-causing gene. Sixteen single nucleotide variants overlapped in both families (variants shared by all affected individuals in both families, while absent in unaffected controls), but none were considered pathogenic. After excluding intergenic and non-coding RNA variants, 97 overlapping genes were identified (genes with variants shared by all affected individuals in both families, while absent in unaffected controls). Further filtering based on neurology, nociception, and pain-related gene functions yielded ten top candidate genes. However, none of the genes and variants could be linked with the disease with certainty. For future research, it is essential to provide a clear definition of EM to discern primary and secondary EM. Collaboration through a consortium is recommended to increase

sample size, thereby enhancing statistical power. In addition to investigating autosomal dominant genes, it is important to explore other possible inheritance patterns, such as incomplete penetrance, as well as the combined effects of multiple genetic variants and non-genetic risk factors.

In addition to exploring the genetic background of the Mendelian pain disorder EM, we also explored genetic factors involved in multifactorial pain (disorders). In **Chapter 3**, a systematic review was conducted to provide an overview of the potential genetic risk factors for pain in genome-wide association studies (GWASes), investigating pain, nociception, neuropathy, and pain treatment responses in humans. A systematic literature search was performed, and 57 full-text articles met our selection criteria. We identified 30 genetic loci reported in more than one study, and the gene function of identified loci is mainly involved in neurological functions and inflammation. These findings highlight the critical role of inflammation and nerve injury at the peripheral level and neuroplasticity at the central level in the development of (chronic) pain.

In the further part of this thesis, we focused on chronic postsurgical pain (CPSP) as a multifactorial condition, aiming to elucidate its genetic background. CPSP refers to pain that develops or increases following a surgical procedure and persists beyond the expected healing period (usually three months). In **Chapter 4,** our objective was to identify single-nucleotide polymorphisms (SNPs) associated with CPSP development after abdominal surgery (one of the most prevalent types of surgery). A GWAS was conducted on 27,603 participants from the UK Biobank who underwent abdominal surgery. One locus (rs185545327) reached genome-wide significance, while ten loci surpassed the suggestively significant threshold ($P < 1 \times 10^{-6}$) for association with CPSP development. Among these, two loci containing pain-related genes (*SRPK2* and *PDE4D*) were chosen for further validation in clinical samples of adhesions obtained from patients with and without pain. Although the results did not exhibit statistical significance, the detection rate (the number of samples with expression) and if present the expression level of PDE4D detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR) were slightly higher in patients with pain compared to the control group. This study provides preliminary evidence for genetic risk factors implicated in CPSP following abdominal surgery, particularly in the *PDE4D* gene.

In **Chapter 5,** we expanded our investigation beyond specific surgery types to encompass CPSP after a broad group of major and minor surgeries. In addition, to identify genetic variants associated with CPSP development following various

surgical procedures, we explored the genetic correlations of CPSP development across different surgical types. A GWAS was conducted on 95,931 individuals from the UK Biobank who had undergone surgical procedures. Notably, one genetic locus within *GLRA3* exhibited a genome-wide significant association ($P < 5 \times 10^{-8}$) with CPSP development. Furthermore, we identified nine additional loci that surpassed the suggestive significance threshold ($P < 1 \times 10^{-6}$). Genetic correlations between CPSP development after different surgical procedures were explored. Though none of the results achieved statistical significance, we observed high correlation coefficients ($|rg| > 0.4$). This study provides new insights into the genetic factors associated with CPSP (particularly highlighting *GLRA3*) and suggests that the genetic background of CPSP development after different surgeries might not be independent. In addition, this study provides a foundation for future investigations into the function of these risk variants and the mechanisms underlying CPSP by offering summary statistics for CPSP development.

Identifying the genetic background of postoperative pain development provides valuable insights into postoperative pain management, but the genetic studies and risk prediction for CPSP are still in the early stages. In **Chapter 6**, we present a protocol outlining ongoing research conducted at our center. This prospective, observational study aims to recruit approximately 10,000 patients undergoing elective surgery. Postoperative acute and chronic pain outcomes will be assessed via questionnaires at various time points over a six-month follow-up period. Genetic, demographic, and clinical risk factors will be collected through blood samples, questionnaires, and electronic health records, respectively. The primary objective is to identify specific genetic risk factors for acute and chronic postoperative pain and construct a prediction model for personalized pain management. Secondary objectives include building a databank to identify other risk factors, exploring factors predicting pharmacological pain relief, and investigating the relationship between acute and chronic postoperative pain. This study may open ways to identify new targets for treatment and potentially simplify the risk profiling assay for future use, yielding a simpler, more accurate, and cost-efficient assay or product.

In **Chapter 7**, we investigated genetic factors influencing pain treatment outcomes for another type of pain, i.e. musculoskeletal pain. The pharmacological management of musculoskeletal pain starts with non-steroidal anti-inflammatory drugs (NSAIDs), when the pain cannot be controlled by NSAIDs this is followed by weak or strong opioids until pain is under control. However, treatment outcomes vary among individuals. We conducted a GWAS with ~23,000 participants from the UK Biobank to explore genetic variants that showed association with treatment

outcomes by comparing NSAID and opioid users. One significant hit was identified on chromosome 4 (rs549224715, $P = 3.88\times10^{-8}$). Additionally, suggestive significant loci ($P < 1\times10^{-6}$) were functionally annotated to 18 target genes, including four genes linked to neuropathic pain processes or musculoskeletal development. Pathway and network analyses highlighted immunity-related processes and a potential central role of EGFR. Therefore, it should be kept in mind that this study represents an initial step in understanding the genetic basis of musculoskeletal pain treatment outcomes.

In summary, while the majority of genetic risk factors for pain remains to be identified, this thesis and emerging evidence implicate neuronal and immunological genes that might mediate pain development. The findings contribute to understanding the genetic background of pain, facilitating investigation of the function of these risk variants to explain the biological mechanisms underlying pain, and providing summary statistics for future research in this field. In future studies, we advocate for conducting genetic studies on pain with substantially larger sample sizes and consistent phenotype definitions. We are optimistic that these results will advance risk identification and will enable tailoring of personalized treatment for pain in the future.

Samenvatting

Pijn wordt gekenmerkt door een onaangename sensorische en emotionele ervaring die gepaard kan gaan met werkelijke of mogelijke weefselschade. Aandoeningen die bij mensen pijn veroorzaken kunnen zich genetisch gezien manifesteren als Mendeliaanse aandoeningen (veroorzaakt door een enkel gen) of als een multifactoriële ziekte (veroorzaakt door meerdere genen in combinatie met omgevings- en/of klinische factoren). Mendeliaanse pijnaandoeningen zijn over het algemeen zeldzaam, daarentegen is chronische pijn een veelvoorkomende multifactoriële vorm van pijn, die ongeveer 20% van de volwassen bevolking treft. Chronische pijn is de belangrijkste oorzaak van verloren levensjaren door een handicap. Verschillende risicofactoren voor chronische pijn zijn geïdentificeerd, waaronder sociaal demografische factoren (zoals leeftijd, vrouwelijk geslacht en beroep), psychologische factoren (zoals depressie), klinische factoren (zoals chronische ziekten) en levensstijl. Naast deze factoren speelt ook genetische vatbaarheid een rol bij de ontwikkeling van pijn. Genetisch onderzoek naar pijn is gericht op enerzijds de identificatie van genen die Mendeliaanse pijnaandoeningen veroorzaken en aan de andere kant het krijgen van inzicht in de genen en genetische mechanismen die de onderliggende oorzaak zijn van multifactoriële pijnaandoeningen. De inzichten helpen om de variatie in pijnontwikkeling binnen populaties te verklaren en deze genetische bevindingen dragen bij aan een beter begrip van de functionele mechanismen die ten grondslag liggen aan de ontwikkeling van pijn. Tevens levert dit onderzoek inzichten voor de ontwikkeling van potentiële behandelingen voor deze pijn aandoeningen en effenen ze de weg voor verbeterd pijnmanagement door genetische factoren in de klinische praktijk te integreren. Met dit proefschrift hebben wij een bijdrage geleverd aan het begrip van de genetische achtergronden van Mendeliaanse en multifactoriële pijnaandoeningen. Hieronder volgt een samenvatting van de inhoud van dit proefschrift.

Om de genen te identificeren die betrokken zijn bij Mendeliaanse pijnaandoeningen, onderzochten we in Hoofdstuk 2 de genetische oorzaken van erythermalgie (EM), een de Mendeliaanse pijnaandoening. EM is een zeldzame aandoening die wordt gekenmerkt door terugkerende episodes van rode, warme en pijnlijke gezwollen ledematen. Het kan primair zijn, veroorzaakt door gain-of-function of missense-mutaties in het *SCN9A* gen, of secundair, dan is de oorzaak te vinden in onderliggende ziekten of medicatiegebruik. In dit proefschrift onderzochten we twee families met primaire EM zonder pathogene varianten in het *SCN9A* gen, het gen wat normaal gesproken de oorzaak is van deze aandoening. Whole-genome sequencing werd uitgevoerd bij zes patiënten met EM en twee niet-aangedane familieleden om het

ziekte veroorzakende gen te identificeren. Zestien kleine genetische variatie in het DNA overlapten in beide families (variatie gedeeld door alle aangetaste individuen in beide families, terwijl deze afwezig waren bij niet-aangedane controles), maar geen enkele werd als pathogeen beschouwd. Na uitsluiting van intergene en niet-coderende RNA-varianten werden 97 overlappende genen geïdentificeerd (genen met variatie gedeeld door alle aangetaste individuen in beide families, terwijl deze afwezig waren bij niet-aangedane controles). Verdere filtering op basis van gen functie, zoals, neurologische, nociceptieve en pijn-gerelateerde genfuncties leverde tien kandidaat genen op. Echter, geen van de genen en variaties in de genen kon met zekerheid aan de ziekte worden gekoppeld. Voor toekomstig onderzoek is het essentieel om een duidelijke definitie van EM te hanteren om primaire en secundaire EM te kunnen onderscheiden. Samenwerking via een consortium wordt aanbevolen om een grotere groep patiënten te kunnen analyseren om zo de statistische power te verbeteren. Naast onderzoek naar autosomaal dominante genen is het belangrijk om andere mogelijke overervingspatronen, zoals incomplete penetrantie, en de gecombineerde effecten van meerdere genetische varianten en niet-genetische risicofactoren te onderzoeken.

Naast het onderzoeken van de genetische achtergrond van de Mendeliaanse pijnaandoening EM, onderzochten we ook genetische factoren die betrokken zijn bij multifactoriële pijn(aandoeningen). In Hoofdstuk 3 werd een systematische literatuuronderzoek uitgevoerd om een overzicht te geven van de potentiële genetische risicofactoren voor pijn die gevonden zijn door middel van genoom-wijde associatie studies (GWAS), waarbij pijn, nociceptie, neuropathie en pijnbehandelingsresponsen bij mensen werden onderzocht. Het systematische literatuuronderzoek leverde 57 artikelen op die voldeden aan onze selectiecriteria. In totaal werden 30 genetische loci in meer dan één studie gerapporteerd. De genen aanwezig in de geïdentificeerde loci bleken voornamelijk betrokken bij neurologische functies en ontsteking. Deze bevindingen benadrukken de cruciale rol van ontsteking en zenuwbeschadiging op perifeer niveau en neuroplasticiteit op centraal niveau bij de ontwikkeling van (chronische) pijn.

In de andere hoofdstukken van dit proefschrift richtten we ons op de multifactoriële aandoening chronische postoperatieve pijn (CPSP) , met als doel de genetische achtergrond van deze aandoening verder te ontrafelen. CPSP is pijn die ontstaat of toeneemt na een chirurgische ingreep en tevens blijft CPSP langer dan de verwachte genezingsperiode (meestal drie maanden) aanwezig. In Hoofdstuk 4 was ons doel om kleine veranderingen in het DNA te identificeren die geassocieerd zijn met CPSP-ontwikkeling na buikchirurgie (een van de meest voorkomende soorten

chirurgie). Een GWAS werd uitgevoerd op 27.603 deelnemers uit de UK Biobank die een buikoperatie hadden ondergaan. Eén locus (rs185545327) bereikte genoom-wijde significantie, terwijl tien loci de suggestief significante drempel ($P < 1 \times 10^{-6}$) voor associatie met CPSP-ontwikkeling overschreden. Van de geïdentificeerde loci, werden twee loci met pijn-gerelateerde genen (*SRPK2* en *PDE4D*) gekozen voor verdere validatie in klinische monsters van adhesies verkregen van patiënten met en zonder pijn. Hoewel de resultaten geen statistische significantie vertoonden, was het aantal monsters waarbij het gen gedetecteerd kon worden en indien aanwezig, het expressieniveau van *PDE4D* iets hoger bij patiënten met pijn in vergelijking met de controlegroep. Deze studie biedt een eerste bewijs voor genetische risicofactoren die betrokken zijn bij CPSP na buikchirurgie, met name het *PDE4D* gen lijkt betrokken te zijn.

In Hoofdstuk 5 hebben we het onderzoek uitgebreid naar meerdere chirurgietypes om CPSP te onderzoeken in een grote groep van patiënten met zowel grote en kleine chirurgische ingrepen. Om genetische varianten te identificeren die geassocieerd zijn met CPSP-ontwikkeling na verschillende chirurgische procedures, onderzochten we genetische correlaties van CPSP-ontwikkeling bij verschillende soorten chirurgie. Een GWAS werd uitgevoerd op 95.931 individuen uit de UK Biobank die chirurgische procedures hadden ondergaan. Opvallend was dat één genetische locus in het *GLRA3* gen een genoom-wijde significante associatie vertoonde ($P < 5 \times 10^{-8}$) met CPSP-ontwikkeling. Verder identificeerden we negen additionele loci die de suggestief significante drempel ($P < 1 \times 10^{-6}$) overschreden. Genetische correlaties tussen CPSP-ontwikkeling na verschillende chirurgische procedures werden onderzocht. Hoewel geen van de resultaten statistische significantie bereikte, observeerden we hoge correlatiecoëfficiënten ($|rg| > 0,4$). Deze studie biedt nieuwe inzichten in de genetische factoren die verband houden met CPSP (met name *GLRA3*) en suggereert dat de genetische achtergrond van CPSP-ontwikkeling na verschillende operaties deels overlappend is. Bovendien biedt deze studie een basis voor toekomstig onderzoek naar de functie van deze genetische varianten en de mechanismen die ten grondslag liggen aan CPSP. Data van deze studie kan bijvoorbeeld gebruikt worden in grote analyses waarin vele studies samengevoegd worden in zogenaamde meta-analyses.

Het identificeren van de genetische achtergrond van postoperatieve pijn-ontwikkeling biedt waardevolle inzichten in postoperatief pijnmanagement, maar genetische studies en risicovoorspelling voor CPSP bevinden zich nog in de beginfase. In Hoofdstuk 6 presenteren we een protocol dat lopend onderzoek binnen ons centrum beschrijft. Deze prospectieve, observationele studie heeft tot

doel ongeveer 10.000 patiënten te werven die een electieve operatie ondergaan. Postoperatieve acute en chronische pijnuitkomsten zullen worden verzameld via vragenlijsten op verschillende tijdstippen gedurende een periode van zes maanden na de operatie. Genetische, demografische en klinische risicofactoren zullen worden verzameld via bloedmonsters, vragenlijsten en elektronische medische dossiers. Het primaire doel is het identificeren van specifieke genetische risicofactoren voor acute en chronische postoperatieve pijn en het construeren van een voorspellingsmodel voor gepersonaliseerde pijnmanagement. Secundaire doelen omvatten het opbouwen van een databank om andere risicofactoren te identificeren, het onderzoeken van factoren die farmacologische pijnverlichting voorspellen en het bestuderen van de relatie tussen acute en chronische postoperatieve pijn. Deze studie kan wegen openen voor het opstellen van een persoonlijk behandelplan voor patiënten die een operatie zullen ondergaan.

Appendices

Data management plan

The data management of this thesis is described below.

Ethics and privacy

Chapter 2 is based on the results of research involving human participants. Information on the informed written consent procedure and ethics approval can be found in this paper: [1]. The privacy of the participants in these studies was warranted by the use of pseudonymization.

For Chapter 4, 5, 7, data was obtained from the UK Biobank, informed consent has been obtained by UK Biobank. UK Biobank has approval from the North West Multicentre Research Ethics Committee (MREC) as a Research Tissue Bank (RTB) approval. This approval means that researchers do not require separate ethical clearance and can operate under the RTB approval. The privacy of the participants in these studies was warranted by the use of pseudonymization. The summary de-identification protocol of UK Biobank can be found at here: https://www.ukbiobank.ac.uk/media/5bvp0vqw/de-identification-protocol.pdf.

For the study described in Chapter 6, this study will be conducted according to the principles of the Declaration of Helsinki version 2013, and in accordance with the Medical Research Involving Human Subjects Act and Good Clinical Practice. In the Netherlands to be called: WMO, WGBO, WBP and BIG- laws. The Institutional Review Board of the Radboud university medical center approved the study (authorization number: 2012/117). The ClinicalTrials.gov ID for this study is NCT02383342. The privacy of the participants is guaranteed by storing encrypted data. When storing clinical data and data on human tissue, no identifying data is recorded within the meaning of the law. Every participant will receive an pseudonymous study number. The key is only accessible to the study team and monitors.

Data collection and storage

For Chapter 2, DNA collected from subjects was stored at the Radboudumc Human Genetics department. The whole genome sequencing and the variants prioritization programming code is stored at Radboudumc Human Genetics server. More information can be found in Table 1.

For Chapter 4, 5, 7, the data was obtained from the UK Biobank and was stored on the Dutch national super computer Snellius with budget name EINF-9855. The data obtained from the UK Biobank, including both phenotype data and primary

care data, has been deleted following the completion of the project, as required by the UK Biobank's data usage policy (*If the project is complete please confirm that data has been deleted or rendered inaccessible*). The analysis programming code and corresponding results have been stored in separate repositories. Further details regarding their location and accessibility can be found in Table 1. The original UKB data can be accessed upon application, and the scripts are available, allowing the analysis to be reproducible.

Data sharing according to the FAIR principles

For Chapter 2, the analysis programming code and results are stored on the Radboudumc Human Genetics server. The analysis programming code and data can be shared if requested for subsequent studies.

For Chapter 4, 5, 7, the analysis code and findings can be found in the table below. The table details where the data and research documentation for each chapter can be found on the Radboud Data Repository (RDR) or other data repositories. All data archived as a Data Sharing Collection remain available for at least 15 years after termination of the studies.

Reference

1. Burns, T.M., et al., *Genetic heterogeneity and exclusion of a modifying locus at 2q in a family with autosomal dominant primary erythermalgia.* Br J Dermatol, 2005. **153**(1): p. 174-7.

Table 1. An overview of data management.

Chapter	Paper DOI	Data repository
Chapter 1: Introduction	N/A	N/A
Chapter 2: Investigating Genetic Variants in Primary Erythermalgia Patients without SCN9A Mutations: findings from Whole Genome Sequencing	N/A	The data and programming code are stored at Human Genetics department Server
Chapter 3: A systematic review of genome-wide associated studies for pain, nociception, neuropathy, and pain treatment responses	10.1097/j.pain.0000000000002910	N/A
Chapter 4: Genome-wide association study on chronic postsurgical pain after abdominal surgeries in the UK Biobank	N/A yet	Summary statistics of the primary analysis are available at DANS archive: https://doi.org/10.17026/LS/X9DUS1 Gene mapping results are available at FUMA: https://fuma.ctglab.nl/browse/689 Programming and statistical code used in the phenotype definition and data analysis can be found at GitHub: https://github.com/lisongmiller/UKB_GWAS_CPSP_abdominal
Chapter 5: Genome-wide association study on chronic postsurgical pain in the UK Biobank	N/A yet	Summary statistics of the primary analysis are available at DANS Archive: https://doi.org/10.17026/LS/486TJZ Gene mapping results are available at FUMA: https://fuma.ctglab.nl/snp2gene/266351 Programming and statistical code used in the phenotype definition and data analysis can be found at GitHub: https://github.com/lisongmiller/ UKB_GWAS_CPSP_all_operations
Chapter 6: Pain Predict Genetics: Protocol for a prospective observational study of clinical and genetic factors to predict the development of postoperative pain.	10.1136/bmjopen-2022-066134	ClinicalTrials.gov Registration number: NCT02383342

Chapter	Paper DOI	Data repository
Chapter 7: Genome-wide association study on pharmacological outcomes of musculoskeletal pain in UK Biobank	10.1038/s41397-023-00314-x	Summary statistics of the primary analysis are available at DANS archive: https://doi.org/10.17026/dans-xns-un6c Gene mapping results are available at FUMA: https://fuma.ctglab.nl/browse/378 Programming code used in phenotype definition can be found at GitHub: https://github.com/lisongmiller/UKB_GWAS_pain_treatment_outcome
Chapter 8: General Discussion	N/A	
Chapter 9: Summary	N/A	

About the author

Curriculum vitae

Song Li was born on the 23rd of November 1992 in Hebei, China. He started his bachelor's degree in pharmacy at Hainan Medical College. During his bachelor's studies, he completed an internship at the pharmacology department of Hainan Medical College under the supervision of Dr. Qibing Liu, where he examined the antihyperlipidemic and antioxidative effects of the aqueous extract of Carmona microphylla (Lam.) G. Don. (CAE) in Triton WR-1339-induced hyperlipidemic mice. He also involved in a project investigating the protective effect of compound astragalus pills on liver injury in rats induced by carbon tetrachloride.

He pursued a master's degree at the Department of Radiation Medicine of Peking Union Medical College. During this time, he was involved in two research projects. In one project, he studied the cancer transmission hypothesis and designed *in vitro* experiments to understand how breast cancer intratumor heterogeneity forms. In the other study, he designed *in vivo* and *in vitro* experiments to investigate the role of necroptosis in radiation-induced cell death. The internship was under the supervision Prof. Chenguang Wang and Dr. Qiang Liu.

In 2019, he began his PhD project in the Department of Human Genetics at Radboudumc under the supervision of Dr. Marieke Coenen, Dr. Regina L.M. van Boekel, Prof. Franke Barbara, and Prof. Kris C. P. Vissers. The PhD project focused on unraveling the genetic architecture of pain. He investigated the genetic factors contributing to Mendelian pain disorders, multifactorial pain disorders, and pain treatment outcomes. The results of this PhD project are detailed in his thesis.

Next to his PhD, he pursued his career in the pharmaceutical industry, where he was involved in clinical trial data analysis. He performed statistical analyses, generated statistical outputs to gather insights, and collaborated with statisticians to develop and implement analytical strategies.

PhD portfolio of Song Li

Department: Human Genetics
PhD period: 1-Sep-2019 to 01-Jan-2025
PhD Supervisor(s): Prof. Franke Barbara
PhD Co-supervisor(s): Dr. M.J.H. Coenen, Prof. Kris C. P. Vissers, Dr. Regina L.M. van Boekel

Training activities	Hours
Courses	
• RIHS - Introduction course for PhD candidates (2019)	15.00
• RIHS PhD introduction course (2019)	21.00
• Hands-on: genome association analysis (2019)	84.00
• Cambridge English courses C1 Advanced (2020)	42.00
• Pain course on EFIC Education Platform (2020)	35.00
• Genetic Analysis of Population-based Association Studies (Virtual) (2020)	35.00
• Project management for PhD candidates (2021)	56.00
• Radboudumc - eBROK course (for Radboudumc researchers working with human subjects) (2021)	26.00
• RU - Achieving your Goals and performing more successfully in your PhD (2021)	28.00
• Radboudumc - Scientific integrity (2021)	16.00
• Scientific Integrity for PhD candidates (2021)	28.00
• Scientific Writing for PhD candidates (2021)	84.00
• RU - Language Development for Academic Writing (2021)	50.00
• RU - The Art of Presenting Science (2021)	36.00
• Statistics for clinicians (2021)	28.00
Seminars	
• StatGen Meeting by MPI (2019)	2.00
• Genetics beginner's R Course (2019)	11.20
• Genetics Advanced R Course (2019)	11.20
• Netherlands Network of Precision Medicine (NNPM) symposium (2019)	8.00
• UCSC genome browser workshop (2020)	22.40
• Netherlands Network of Precision Medicine (NNPM) symposium (2021) ^	16.00
• How to prepare your poster presentation (2021)	11.20
• Latest developments in Pharmacogenomics research and clinical implementation (2021)	3.00
• Logical reasoning in Human Genetics (2021)	15.00
• Statistics and bioinformatics workshop (2021)	24.00
• Radboudumc Science Café Anesthesiologie (2023) ^	2.00
• Clinical Genomics and NGS jointly organized by ESHG and CEUB (2023)	40.00
Conferences	
• 8th International Conference on Rare and Undiagnosed Diseases (2019)	16.00
• Workshop Literature review (2021)	5.60
• The European Human Genetics Conference (2021) ^	32.00
• ESPT Virtual Congress (2021)	8.00
• International Congress of the European Society for Pharmacogenomics and Personalised Therapy (2021)	8.00
• PhD retreat (2021)	16.00

Teaching activities	
Lecturing	
• Genetic Lab Practice (2019)	8.00
• MED-MMSTA Course: Omics data analysis and interpretation (2020)	8.00
• NWI-BM072: Translational Genomics course (2020)	16.00
• Genetic Lab Practice (2020)	25.20
• MED-MMSTA Course: Omics data analysis and interpretation (2021)	8.00
• NWI-BM072: Translational Genomics course (2021)	16.00
• MED-MMSTA Course: Omics data analysis and interpretation (2022)	8.00
Supervision of internships / other	
• Supervision Master student internships (2021)	60.00
• Supervision Master student internships (2022)	40.00
Total	**1,024.80**

^Indicate oral or poster presentation.

Acknowledgement

I would like to begin by expressing my heartfelt gratitude to the incredible colleagues and friends who have accompanied me on this journey. Their unwavering support, insightful conversations, and shared experiences have reminded me that even in the most challenging moments, I was never truly alone. This thesis is as much a reflection of their kindness and encouragement as it is of my own efforts, and for that, I am deeply thankful.

I would also like to extend my deepest gratitude to the patients from the pain family and the participants of the UK Biobank. Your generous contributions and willingness to participate in research have been the cornerstone of this work. Without your participation, this thesis would not have been possible.

I am deeply grateful to my promoter and supervisory team, without whom this thesis would not have been possible. You have been role models to me, exemplifying both scientific integrity and insightful research. To Franke Barbara, I deeply appreciate your critical comments and sharp observations, especially during presentations and in the process of refining my thesis. Your incisive feedback has challenged me to think more deeply and critically, and I have greatly benefited from your expertise. To Marieke Coenen, I am immensely thankful for the effort and guidance you have invested in me throughout this journey. Your patience at the beginning of my PhD was invaluable, and your accessibility and willingness to discuss every detail of my project have been a constant source of encouragement. Our conversations have not only provided clarity and direction but have also left me feeling motivated and energized. Thank you for being a source of unwavering support and inspiration.

To Rianne Boekel, thank you for your valuable input from a clinician's perspective. Your insights into the clinical aspects of pain and pain management have provided essential background knowledge that has greatly enriched this work. Your feedback has been critical in ensuring the clinical significance of this thesis. To Kris Visser, in addition to your input as a clinician, I am particularly grateful for the thoughtful questions you posed. These have encouraged me to consider how my work can ultimately contribute to clinical care and pain management. I have also learned a great deal from your guidance on presenting results in a way that resonates with clinicians and highlights the importance of pain research. Your perspectives have been instrumental in bridging the gap between research and practice.

To my mentor, Elke de Jong, I truly enjoyed all the conversations we had. Thank you for being warm, encouraging, and supportive!

To the members of my manuscript committee, prof. dr. B.J.F. van den Bemt, dr. H. Adams, and prof. dr. W.F.F.A. Buhre, thank you sincerely for taking the time to read and evaluate my thesis. Your thoughtful review and constructive feedback have been invaluable in shaping this work.

To my co-authors and collaborators, thank you for your invaluable contributions to the studies included in this thesis. I am especially grateful to Professor L. Diatchenko and Dr. Marc Parisien for generously sharing the summary statistics from the chronic postsurgical pain meta-analysis, which provided a critical foundation for this work. My sincere thanks also go to Drs. Masja Toneman, Judith Mangnus, and Dr. Richard ten Broek from the Department of Surgery for offering their insights and emphasizing the clinical significance of surgical procedures. Your expertise has greatly enriched the scope and relevance of this research. I also want to thank Professor Joost Drenth and Rene te Morsche for sharing the data and for your valuable input as clinicians, which greatly improved the chapter on the pain family analysis in my thesis.

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Thank you, Cris and Mire, for the great times we had playing table tennis and hanging out together. Those happy moments are ones I will never forget.

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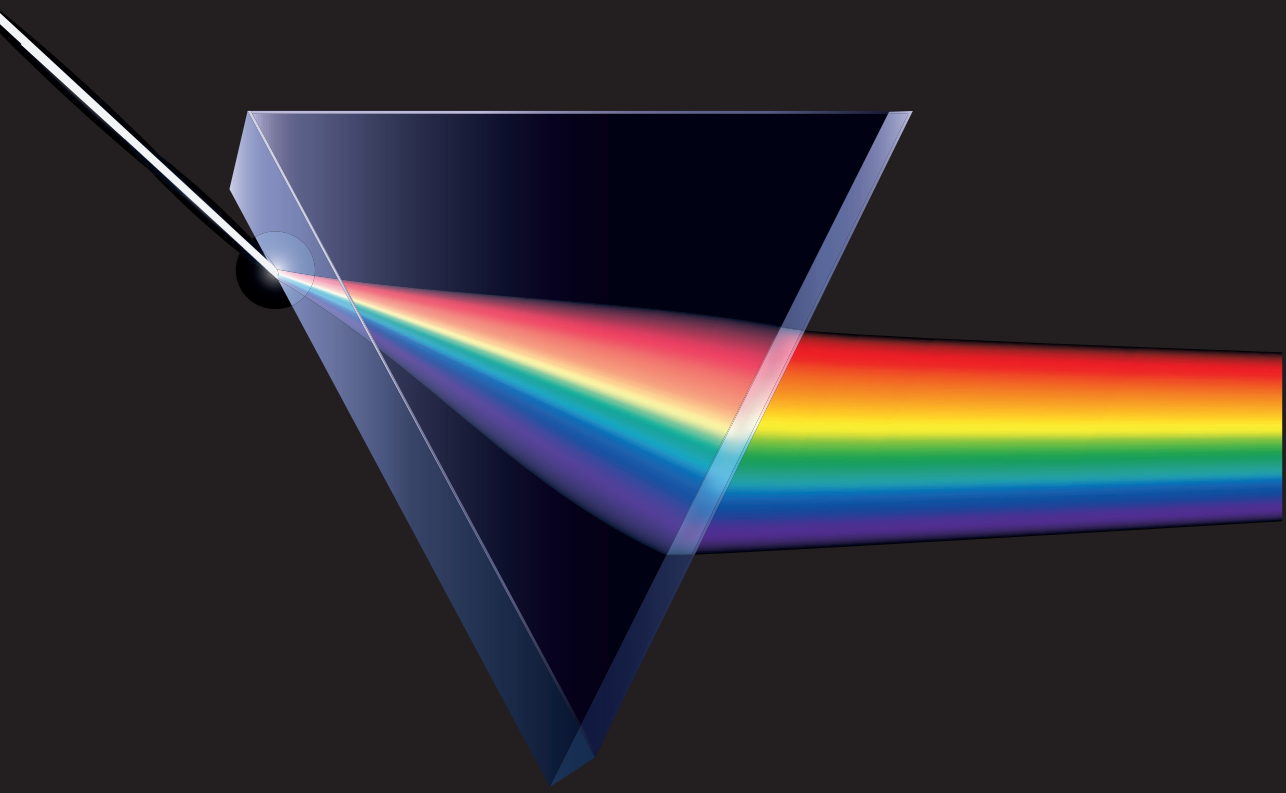
To Sander and Taoran, thank you for being such kind neighbors. Sander, I truly appreciated our language exchange sessions; they helped me improve my Dutch much faster and gave me a deeper understanding of Dutch culture.

To my friends in China, thank you for your unwavering support. 游伟 and 高嘉, your listening ears and encouragement lifted me when I felt down, making me feel better and less alone. 赵徵鑫, I've learned so much from our conversations and always felt comforted by your shared experiences. A special thanks to my 717 dorm mates, especially 赵明鹤—you've been like a big brother to me, always there to

listen and offer guidance. Each of you has contributed to my journey in unique and meaningful ways, and I am deeply grateful for your friendship and support.

I want to express my heartfelt gratitude to my family. Papa and Mama, thank you for supporting my decision to move to another country to pursue my dreams and career. Your understanding, encouragement, and unwavering support have been the foundation of my journey.

Finally, a special thank you to my girlfriend, Mao. Your constant companionship and support mean the world to me. Knowing that you are always there for me, no matter what happens, is a source of great strength and comfort. It has been a pleasure and a privilege to grow alongside you as we navigate the adult world together.



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