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

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Article

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 was compared as a reflection of the treatment outcome of NSAIDs. We identified one genome-wide significant hit in chromosome 4 (rs549224715, P = 3.88×10^{-8}). Suggestive significant (P < 1×10^{-6}) loci were mapped to 28 target genes, including eight 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 lacked power and should be viewed as a first step to elucidate the genetic background of musculoskeletal pain treatment.

1. 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 panel and sample size and report contradictory results [11]. The most investigated genetic variant is the 118A to G basepair change in the *OPRM1* gene. 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 GWASs 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 long-term pain treatment outcomes 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 outcomes.

2. 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 ($P < 1 \times 10^{-6}$) signals.

2.1 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. UK Biobank obtained informed consent from all participants.

2.2 Phenotype definition

To define the pain treatment outcome, we first extracted all musculoskeletal pain patients with pain prescriptions (NSAIDs and opioids) from the GP data (see Supplementary data 1 and 2 for diagnosis and prescriptions codes included in this study). Only participants with a musculoskeletal pain diagnosis record and a pain prescription record occurring on the same date were included for analysis to ensure that the prescriptions are indeed for musculoskeletal pain treatment.

Pain treatment outcomes were 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 who go to the next level of the analgesic ladder. The following text will refer to this phenotype as pain treatment outcomes.

2.3 Genome-wide association study

A GWAS was conducted using binary phenotypes, i.e., NSAID users (controls) versus opioid users (cases). For markers on the autosomal chromosomes and PAR region of the X chromosome, GWAS on pain treatment outcomes was conducted using a linear function in GCTA [23], adjusting for age, sex, BMI, depression history, smoking status, drinking frequency, assessment center, genotyping array, and the first ten principal components (PCs). Markers on the non-PAR region of the X chromosome were analyzed by a sex-stratified analysis in the XWAS [24]. 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. Details on genotyping and quality control methods, covariates definition, and heritability/power calculation can be found in supplementary materials.

To examine the nature of pain between groups, all NMP 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.

Besides the binary case/control analysis, three additional analyses were performed: a GWAS using an ordinal outcome (NSAID, weak opioid, and strong opioid users), a subtype GWAS focusing on inflammatory pain, and a GWAS with a less stringent phenotype definition for validation purposes. Details on these analyses can be found in the supplementary materials.

2.4 Functional annotation

2.4.1 Bayesian fine-mapping of lead loci

Lead SNPs were analyzed using Bayesian fine-mapping in PAINTOR [25] 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.

2.4.2 Functional annotation of SNPs present in the 95% credible sets

SNPs in the 95% credible sets were annotated for regulatory functions in HaploReg v4.1 [26]. 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 S1). 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.

2.5 Gene mapping

To map suggestively significant (P-value $< 1 \times 10^{-6}$) GWAS SNPs and SNPs in LD (LD > 0.6) with them to genes, three strategies were adopted in FUMA: positional mapping, expression quantitative trait loci (eQTL) mapping, and chromatin interaction mapping. For the positional mapping, SNPs were mapped to known protein-coding genes based on physical distance (within a 10 kb window). For eQTL mapping, SNPs were mapped 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 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.

2.6 Post-GWAS analysis

We conducted the following post-GWAS analysis: pathway enrichment analysis and genetic correlation analysis (for details see supplementary materials).

3. Results

3.1 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).

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 chr4 reached genome-wide significance, in which the most significant SNP was rs549224715 (P $= 3.92 \times 10^{-8}$) (Fig. 1, **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. The SNP heritability was 0.16 (P-value $= 0.16$). A GWAS was conducted using ordinal phenotypes (NSAID, weak opioid, and strong opioid users), and the results were consistent with the GWAS using binary outcomes (Figure S1).

We conducted a subtype GWAS in patients with inflammatory pain to investigate if a more homogeneous phenotype would yield additional signals and a secondary GWAS with less strict criteria for diagnosis definition to validate our results (see supplementary materials). However, we did not find any genome-wide significant loci or overlapped suggestively significant loci with the GWAS using binary outcomes as described in detail above (Figure S2, Figure S3, Table S7, Table S8).

3.2 Functional annotation

3.2.1 Statistical fine-mapping of loci and functional annotation of SNPs

As GWAS signals can caused by SNPs in linkage disequilibrium with the likely causal SNPs, we calculated the posterior probability for variants in each genomic locus and created 95% credible sets (see methods). In five out of eight loci, the lead SNPs in the locus had the maximum PP (PP_{max}) (Figure S4).

Since all variants in the 95% credible sets were in non-coding regions, the regulatory effects of these variants were investigated by examining overlap with epigenomic markers of active enhancers or promoters in Haploreg. The results suggested that most genetic variants were potentially involved in transcriptional regulatory modulation (Figure S4).

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.

3.2.2 Gene mapping

After mapping GWAS candidate SNPs (SNPs that are in LD (r2 > 0.6) of any independent significant SNPs) to genes, a total of 28 unique mapped genes were identified (**Table 3**). Five genes were mapped by genomic location, nine genes were identified by cis-eQTL mapping, 18 genes were annotated as SNPs in regions where 3D chromatin interactions occurred, and four genes were identified by at least two mapping strategies.

3.3 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 S3).

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 S4, Figure S5).

3.4 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 [27] (rg = -0.5431, P = 0.0102) (Table S5). 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 S6).

4. Discussion

To our knowledge, this is the first GWAS reporting on long-term pain treatment outcomes. We identified one genome-wide significant hit and seven loci with suggestive significance. 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 chr4p11 and pain treatment outcome remains unclear. The nearest gene, *CWH43*, is associated with Seckel Syndrome, characterized by growth delays before birth. Another gene, *TXK*, was mapped by eQTL to this SNP and played a role in regulating the adaptive immune response [28]. Therefore, this association is worth further validation and investigation.

This study is the first to report the narrow-sense heritability of the NSAID treatment outcome. Our study indicates that NSAID treatment outcome has a moderate heritability, although the P-value is insignificant. The insignificant P-value could be due to the lack of power of GWAS results. Since there are no comparable previous results, we examined whether the heritability is in line with the heritability of response to opioid analgesics or chronic pain. The heritability in our study is in agreement with the heritability of opioid response (60% in cold pressor induced pain and 12% in heat pressor) in a twin study [29] and chronic pain (0.08 to 0.31) [7]. One possible reason for the low heritability is that the narrow-sense heritability only captures the additive genetic components of common variants without the contributions of non-additive effects, rare variants, and structural variants. Another reason could be that pain treatment outcome is a highly complex phenotype with other contributing factors such as employment status and psychological factors [30].

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 [31] and pain treatment [32]. Some preliminary published results indicate that epigenetic restructuring can happen in response to opioid analgesic use. For instance, hypermethylation in both the promoter region of a candidate gene (*OPRM1)* and global DNA methylation was observed after opioid use [33, 34].

In total, we pinpointed 28 genes that linked to the identified SNPs based on physical, eQTL, and chromatin interaction mapping. Four identified genes are prioritized as these 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). This gene encodes a member of the neuronal pentraxins family, which are involved in excitatory synapse formation. NPTX2 is thought to play a role in anxiety [35] and is downregulated in the brain in induced chronic neuropathic pain [36] and induced endometriosis [37] mouse models. The other three genes are involved in: spinal sensitization and neuropathic pain states after peripheral nerve injury [38, 39] (*THBS4*); synaptic plasticity associated with chronic inflammatory pain [40] and neuropathic pain processing after nerve injury [41] (*HOMER1*); nerve injury-induced membrane receptor trafficking in dorsal root ganglions in neuropathic pain conditions [42] (*IPCEF1*). In addition, we identified four genes linked to muscular or skeletal dystrophy: *CMYA5*, *SGCB*, *TMEM130*, and *FN1*. These genes are of interest as musculoskeletal dystrophy is characterized by pain.

In addition, no candidate genes were implicated in the metabolism and working mechanisms of NSAIDs, which could indicate that participants are more likely to use opioids because of pain or disease

progression. However, our results do not exclude the role of those genes in pain treatment outcomes. One reason is that the subcategories in NSAIDs, such as non-selective NSAIDs and selective COX-2 inhibitors, were analyzed as a whole, which may dilute their effect on the pain treatment outcomes difference. However, stratified analyses per drug were impossible as the groups would become too small to obtain sufficient power. No variants involved in opioid processing were identified in line with our expectation because our phenotype is a proxy for NSAIDs treatment outcome. The other possible reason is that rare variants in drug-metabolizing genes can contribute significantly to treatment response differences [43]. Our study had 80% power to identify SNPs with a risk allele frequency of 5% and genotypic relative risk of 1.135, but we lack the power to detect variants with lower frequencies or smaller effect sizes. Therefore, it would be interesting to investigate the effect of rare variants in a larger sample with a Next-Generation Sequencing-based method.

Although no correlations with pain treatment outcomes remained significant after Bonferroni correction, the enrichment of top correlations with nominal significance was perhaps expected. The overrepresentation of pain phenotypes indicates that opioid users tend to have more chronic and severe pain conditions [44]. The correlations between pain treatment outcomes and education/occupation also matched reports that people carrying out strenuous occupations (jobs involving heavy manual or physical work) are more likely to report pain [18, 45]. Our study indicates that people with strenuous occupations are more likely to require analgesics at a higher step on the analgesic ladder.

The pathway enrichment and network analysis should be interpreted carefully as the input consisted of nominally significant genes from the GWAS analysis. Top prioritized pathways were mainly implicated in immunity-related processes. One of the identified pathways was retinoic acid-mediated apoptosis signaling. Studies on the link between this pathway and pain are inconsistent. Retinoic acid (RA) administration can reduce chemotherapy-induced neuropathy [46] or inhibit prostaglandin synthesis in astrocytes [47], an important mediator of inflammation and pain signaling. In contrast, topical application of RA can induce retinoid-elicited irritation [48]. The network analysis emphasizes the role of EGFR (a member of the ErbB family of receptors) in pain treatment outcomes. Some links between EGFR and pain can be found in the literature. For instance, EGFR inhibition can block inflammatory chronic pain progression in preclinical studies [49] and relieve neuropathic pain in clinical settings [50], suggesting that the role of EGFR in pain treatment outcomes is worth further investigating.

Our subtype GWAS focusing on inflammatory pain did not identify genes linked to inflammation, nor did it strengthen the associations found in our primary GWAS. This could be explained by the loss of power due to the decreased sample size, and it could also indicate that inflammatory factors may not be a predictor of the severity of NMP [51]. The secondary analysis aimed to validate the GWAS findings in a more heterogeneous group. However, we could not replicate our findings. 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 long-term pain treatment outcomes. However, it is still worth exploring the genetic background of pain treatment outcomes in a large cohort

with a specific pain treatment outcomes definition, such as the ongoing Pain Predict Genetics cohort in our center (NCT02383342).

By utilizing a derived phenotype in the UKB, a large sample size with long-term pain treatment outcomes to NSAIDs was available for analysis. However, it is difficult to validate this derived phenotype and assess pain chronicity because of a lack of appropriate diagnosis codes for pain in the current International Classification of Diseases (ICD). 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 [52], and the reported risk factors for using opioids are also in line with previous literature [44, 52].

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. However, this study should be viewed as an initial stepping stone for future research.

Declarations

Acknowledge

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 manusrcript. 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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Tables

Table 1 to 3 are available in the Supplementary Files section.
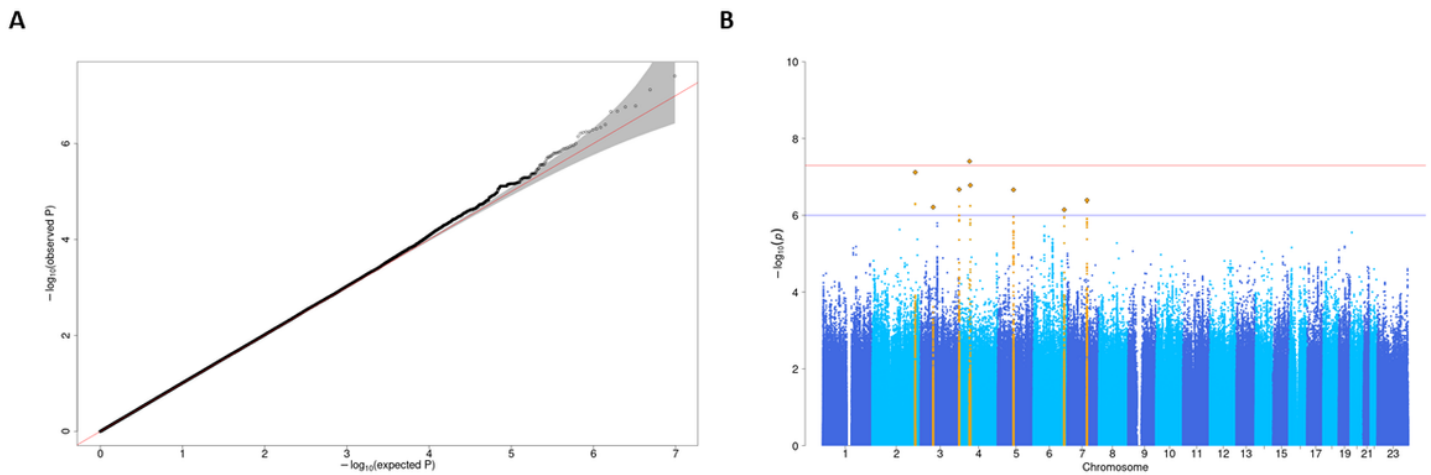
Figures

Figure 1

Figure 1

Q-Q plot and Manhattan plot of primary analysis for pain treatment outcome. (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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- supplementarymaterials.pdf