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ORIGINAL ARTICLE

A genome-wide association study of European advanced cancer patients treated with opioids identifies regulatory variants on chromosome 20 associated with pain intensity

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Abstract

Background: Opioids in step III of the WHO analgesic ladder are the standard of care for treating cancer pain. However, a significant minority of patients do not benefit from therapy. Genetics might play a role in predisposing patients to a good or poor response to opioids. Here, we investigated this issue by conducting a genome-wide association study (GWAS).

Methods: We genotyped 2057 European advanced cancer patients treated with morphine, buprenorphine, fentanyl and oxycodone. We carried out a whole-genome regression model (using REGENIE software) between genotypes and the opioid response phenotype, defined as a numerical score measuring patient pain intensity.

Results: The GWAS identified five non-coding variants on chromosome 20 with a *p*-value $<5.0\times10^{-8}$. For all of them, the minor allele was associated with lower pain intensity. These variants were intronic to the *PCMTD2* gene and were 200 kbp downstream of *OPRL1*, the opioid related nociceptin receptor 1. Notably according to the eQTLGen database, these variants act as expression quantitative trait loci, modulating the expression mainly of *PCMTD2* but also of *OPRL1*. Variants in the same chromosomal region were recently reported to be significantly associated with pain intensity in a GWAS conducted in subjects with different chronic pain conditions.

Conclusions: Our results support the role of genetics in the opioid response in advanced cancer patients. Further functional analyses are needed to understand the biological mechanism underlying the observed association and lead to the development of individualized pain treatment plans, ultimately improving the quality of life for cancer patients.

Significance Statement: This genome-wide association study on European advanced cancer patients treated with opioids identifies novel regulatory variants on chromosome 20 (near *PCMTD2* and *OPRL1* genes) associated with pain intensity. These findings enhance our understanding of the genetic basis of opioid response, suggesting new potential markers for opioid efficacy. The study is a significant advancement in pharmacogenomics, providing a robust dataset and new insights into the genetic factors influencing pain intensity, which could lead to personalized cancer pain management.

1 | INTRODUCTION

Pain is one of the most distressing symptoms in patients with malignant diseases. Therefore, advanced cancer patients often require analgesic therapy for pain relief. Currently, opioids are the primary analgesics capable of effectively managing moderate to severe cancer-related pain (Caraceni et al., 2012). The World Health Organization (WHO) recommends the use of strong opioids from the third step of the WHO analgesic ladder (such as morphine, fentanyl, oxycodone and buprenorphine) to treat cancer pain. Unfortunately, there is significant variability among patients in terms of efficacy and side effects of these drugs (Klepstad et al., 2005; Teunissen et al., 2007).

Genetic factors potentially influence the response to opioids. Studies in monozygotic and dizygotic twins have shown that genetics may explain a percentage of variance, ranging from 12% to 60%, in alfentanil-induced analgesia (Angst et al., 2012). Many research efforts have explored the genetic underpinnings of individual diversity in opioid responsiveness, focusing on a limited number of candidate genes involved in opioid metabolism and mechanism of action. However, these studies often lack consistency due to the small sample size (as reviewed in (Subramaniam et al., 2019)).

For instance, the opioid receptor mu 1 (*OPRM1*) gene, which encodes the primary target of opioids, harbours a coding polymorphism (rs1799971) that has been extensively investigated for its impact on opioid response variability (as reviewed in (Yu et al., 2019)). It has been proposed that this variation might affect the affinity of opioids for the receptors and, consequently, their effectiveness in relieving pain (Mura et al., 2013). However, the clinical utility of this polymorphism, especially in European patients, remains uncertain (Yu et al., 2019).

The complexity of genetic factors underlying interindividual differences in opioid response is evident when considering the multifaceted nature of drug response and pain control. Opioid effects are influenced by factors such as drug absorption, distribution, and metabolism, receptor efficacy and downstream signalling pathways. Each of these aspects of pain control is influenced by multiple genes, each with its allelic variations in the population. To comprehensively identify all the genetic factors at play, genome-wide analyses (GWAS) might be helpful. However, although some GWAS have evaluated opioid response genetics, these studies have been performed

mainly for opioid use disorders. Additionally, the few available GWAS in opioid-treated cancer patients (Galvan et al., 2011; Nishizawa et al., 2022) are based on small sample sizes and lack of independent validation.

With the main aim of investigating, at genome-wide level, the genetic factors affecting the response to opioids of advanced cancer patients, in this study we individually genotyped more than 2000 European cancer patients treated with opioids for pain and carried out the largest genome-wide association study on opioid analgesic efficacy to date.

2 | METHODS

2.1 | Patient series, data collection and materials

This study used genotyping data and clinical information from 2057 European adult patients with locally advanced or metastatic tumours. These data were already available and analysed by our group for another phenotype, as reported in (Minnai et al., 2024). Patients received step III WHO opioids to treat cancer pain (i.e. buprenorphine, fentanyl, morphine and oxycodone). They were part of three studies: CERP (Corli et al., 2016), an Italian multicentre, randomized and longitudinal phase IV clinical trial; EPOS (Klepstad et al., 2011), a European multicentric and cross-sectional study; and MOLO (Shkodra et al., 2022), an Italian longitudinal study. The Committees for Ethics of each recruiting hospital contributing to the EPOS and CERP studies and the Ethics Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy), for the MOLO study (INT 153/13) and for the genetic study (INT 20/20) approved the protocol of this study, which was performed in agreement with the tenets of the Declaration of Helsinki. Written informed consent was provided by each enrolled patient before biological sample and data collection to allow researchers to use them for opioid pharmacogenomic research purposes. Data were treated according to the European General Data Protection Regulation.

The following personal and clinical information was available: age, sex, country of origin, cancer diagnosis, chemotherapy treatments at the time of recruitment, opioid taken and the efficacy of opioid treatment. In the three studies, clinical information about pain was collected using the short form of the Brief Pain Inventory questionnaire (Daut et al., 1983). The pain intensity phenotype was measured through a numerical rating scale from 0 to 10, with increasing values indicating growing pain. Specifically for this study, the response to opioids was defined for each patient as the average pain intensity reported at all the visits (at baseline and on days 3, 7, 14, 21 and 28), when available, for the CERP and MOLO cohorts. Since the EPOS series was a cross-sectional study, pain intensity was measured at a single time-point. Genomic DNA samples from the EPOS and CERP were obtained by Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy) from the HUNT biobank of the Norwegian University of Science and Technology and Istituto di Ricerche Farmacologiche 'Mario Negri'-IRCCS, respectively, for previous studies (Colombo et al., 2020; Galvan et al., 2011; Minnai et al., 2024). Blood samples from MOLO patients were available at Fondazione IRCCS Istituto Nazionale dei Tumori, where DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) and fluorometrically quantified using the Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific).

2.2 | Genotyping

Genotyping data were obtained using Axiom Precision Medicine Research Arrays on a GeneTitan multi-channel instrument (Thermo Fisher Scientific) and Axiom Analysis Suite v.5.01.38 (following the 'Best Practice Workflow' and default quality check (QC) settings, except for the average call rate for passing samples ≥97%). After removing samples for which Axiom QC failed, we extracted the genotypes of our patients and converted them into binary PLINK format. Per-sample and per-marker QC was carried out using PLINK software v1.921 (Purcell et al., 2007). In detail, we filtered out samples with sex inconsistencies, missing call rate >5%, and duplicated or related individuals (Figure S1A). Variants in high Hardy–Weinberg disequilibrium (HWD, p-value $<1.0\times10^{-6}$), with a missing genotype rate >1% and a minor allele frequency (MAF) <1% were removed. We also filtered out single nucleotide polymorphisms (SNPs) mapping in regions with extended linkage disequilibrium (LD) (Price et al., 2008); finally, we retained only biallelic and autosomal variants. Principal component analysis (PCA) was carried out using PLINK 2, to remove non-European patients and correct for population stratification. The first four PCs of our series were plotted together with those of 2504 individuals from five different populations selected from the 1000 Genomes Project (Delaneau et al., 2014) (Africans, Americans, East Asians, Europeans, and South-East Asians) and non-European patients were removed from the dataset (Figure S1B). The TopMED Imputation Server was used to impute genotypes to the whole-genome sequence, with setting GRCh38/hg38 as the build and TopMED as the reference panel, and the data were phased with Eagle v2.4 (Das et al., 2016; Fuchsberger et al., 2015; Loh et al., 2016; Taliun et al., 2021). Post-imputation filters (MAF <2%

and R^2 info score ≤0.3 (Verlouw et al., 2021)) were finally applied.

2.3 | Multivariable linear regression with clinical variables and the normalized phenotype

We first tested the normality of the distribution of pain intensity values (Shapiro–Wilk test). Then, we carried out a multivariable linear regression to test which clinical variables among those available (and listed above) were significantly associated with the pain intensity phenotype. To do this, we used the glm() function in the R environment. The model formula was as follows:

$$\text{Pain intensity} \sim \text{age} + \text{sex} + \text{opioid} + \text{study} + \text{country of origin} + \text{cancer diagnosis} + \text{chemotherapy} + \text{genotyping batch}$$

The normality of the distribution of the residuals of this model was then tested. The inverse normal transformation (INT) formula was as follows:

$$qnorm\left(\left(rank\left(x, na.last = \text{"}keep\text{"}\right) - 0.5\right) / sum(!is.na(x))\right)$$

was used to normalize the residuals (as in (Yang et al., 2012)) that, finally, were used in the GWAS.

2.4 | Genome-wide association study and heritability calculation

REGENIE software (Mbatchou et al., 2021) was used to perform the GWAS for pain intensity. The default settings of the pipeline (https://rgcgithub.github.io/regenie/options/) were used. In step 1, the non-imputed dataset was analysed using the ridge regression with the Leave One Chromosome Out method to define genetic predictors to be used in the second step, where 7,669,761 imputed germline variants were tested. The genome-wide significance threshold was set at a p-value $<5.0\times10^{-8}$. The qqman library (Turner, 2014) and the function manhattan() in R environment were used to draw the Manhattan plot. Additionally, a locus zoom for the top-significant variants was drawn using the function locus. Zoom in R environment (Pruim et al., 2010). The LD square matrix was calculated with PLINK v2 software.

SNP-based heritability was estimated from GWAS summary statistics using linkage disequilibrium score regression (LDSC) (Bulik-Sullivan et al., 2015). In detail, the summary statistics from our INT-pain intensity GWAS summary statistics were standardized and alleles were aligned and merged with a reference SNP list from HapMap3, using the munge_sumstats.py provided by LDSC. Then, through the ldsc.py script and specifying the flag –h2, we merged our summary statistics with precomputed LD scores, which were calculated on approximately 500 European samples from 1000 Genome project, and the heritability of our phenotypic trait was calculated.

Genetic association results were reported herein following the STREGA guidelines (Little et al., 2009).

2.5 | In silico functional and post-GWAS analyses

The identified germline polymorphisms (on chromosome 20, associated with pain intensity at $p<1.0\times10^{-5}$) were annotated using the SNPnexus tool (Oscanoa et al., 2020). In addition, we searched for candidate *cis*-regulatory regions (cCREs), according to ENCODE data (Abascal et al., 2020) (Registry of cCREs V3, accessed on 08/05/2024), that matched the positions of the identified variants. We also mapped the identified polymorphisms to transcription factor binding sites, using the SNP2TFBS tool (Kumar et al., 2017) (accessed on 09/05/2024), which relies on the JASPAR database of transcription factor binding sites (Mathelier et al., 2014). Functional annotation with RegulomeDB 2.2 (Boyle et al., 2012; Dong et al., 2022) were also performed (accessed on 21/05/2024). Then, we investigated the same polymorphisms for their possible regulatory role by searching for them in three public expression quantitative trait locus (eQTL) databases: GTEx (Analysis V8 release, GTEx_Analysis_v8_eQTL_EUR.tar), eQTLGen (Võsa et al., 2021) (accessed on 17 April 2024) and MetaBrain (de Klein et al., 2023) (accessed on 17 April 2024).

Colocalization analyses were performed using the coloc R package (Giambartolomei et al., 2014) with default priors, integrating our GWAS summary statistics and data from the eQTLGen dataset. This analysis tested four hypotheses in addition to the null hypothesis: there is no association with either trait (H0); there is association with pain intensity or expression, only (H1 and H2, respectively); there is association with both traits, but with independent causal variants (H3); and there is a shared causal variant associated with both traits (i.e. colocalization, H4). As commonly assumed (Chen et al., 2024), a posterior probability ≥0.8 for H4 was considered strong evidence of colocalization, while values between 0.5 and 0.8 were considered as suggestive of a moderate colocalization. The same coloc package was used to construct colocalization plots.

Finally, we searched for the top-significant variants, identified in our GWAS, in the Open Target Genetics portal (Ghoussaini et al., 2021; Mountjoy et al., 2021) to

retrieve phenome-wide association study (PheWAS) information across UK Biobank phenotypes. We reported only traits associated with the searched variants with *p*-values $<1.0\times10^{-5}$.

2.6 | Comparison of our GWAS results with previously reported associations with pain phenotypes

We searched for polymorphisms associated with several different pain phenotypes reported in the Human Pain Genetics Database (Meloto et al., 2018) (HPGDB, accessed on 31/05/2024) in our GWAS summary statistics. In addition, we searched for variants associated with pain-related phenotypes in previous studies, such as our previous GWAS on pain relief in cancer patients treated with opioids (Galvan et al., 2011), a smaller GWAS on opioid analgesic requirements in Asiatic cancer patients treated for pain (Nishizawa et al., 2022), a joint analysis of 17 pain-related traits in the UK Biobank (Mocci et al., 2023), a study on 24 chronic pain conditions (Zorina-Lichtenwalter et al., 2023) and a recent multi-ancestry GWAS of pain intensity in participants in the Million Veterans Program, with different types of chronic pain (Toikumo et al., 2024).

3 | RESULTS

After the quality control and imputation steps, the GWAS dataset included the genotypes of 7,588,110 polymorphisms and full phenotypic data from 2057 opioid-treated cancer patients (Figure S1), whose personal and clinical information is shown in Table 1. Patients were equally distributed between the two sexes. Among the four administered drugs, buprenorphine was the least common (5%) whereas approximately the same proportion of patients (~33%) received morphine or fentanyl. However, oxycodone was prescribed to 26% of patients. Patients had several different types of cancer, with gastro-enteric (~18%), lung (~18%) and breast (~14%) cancers the most frequent. Two-thirds of patients belonged to the EPOS study, 12% were recruited in the CERP study and the remaining 21% subjects were enrolled in the MOLO study. Approximately half of the patient series had Italian origins (46%) and the second most frequent nationality was Norwegian (18%). The median pain intensity was 3.67 with an interquartile range of 3.

With a multivariable linear regression model, we identified clinical variables (among sex, age, administered opioid, chemotherapy, study, genotyping batch, country of enrolment and tumour type) that were significantly associated with the pain intensity phenotype. We observed that females had higher pain intensity values than male patients. In addition, considering patients in the CERP

TABLE 1 Clinical characteristics of patients treated with opioids included in the GWAS for the pain intensity phenotype.

Patient characteristic	(*n* = 2057)
Age, years, median (range)	64 (18–91)
Sex, *n* (%)	
Female	1044 (50.8)
Male	1013 (49.2)
Opioid, *n* (%)	
Buprenorphine	109 (5.3)
Fentanyl	694 (33.7)
Morphine	707 (34.4)
Oxycodone	547 (26.6)
Tumour diagnosis, *n* (%)	
Lung	362 (17.6)
Breast	299 (14.5)
Gastro-enteric	366 (17.8)
Pancreas	90 (4.4)
Prostate	207 (10.1)
Urinary traits	142 (6.9)
Head and neck	98 (4.8)
Gynaecological	169 (8.2)
Other or unknown	324 (15.8)
Chemotherapy, n (%)	
Yes	656 (31.9)
No	1401 (68.1)
Study	
EPOS	1372 (66.7)
CERP	247 (12.0)
MOLO	438 (21.9)
Country of enrolment	
Switzerland	83 (4.0)
Germany	127 (6.2)
Denmark	12 (0.6)
Finland	28 (1.4)
Great Britain	204 (10.0)
Iceland	122 (5.9)
Italy	963 (46.8)
Lithuania	46 (2.2)
Norway	377 (18.3)
Sweden	95 (4.6)
Average pain intensity, median (IQR)	3.67 (2–5)

Abbreviation: IQR, interquartile range.

group as reference, MOLO patients registered the highest pain intensity values. Finally, the four administered opioids were not equally effective since patients receiving morphine had lower pain intensity levels than patients treated with fentanyl and oxycodone (Table 2). No significant differences due to age, chemotherapy, tumour type, country, study of enrolment or genotyping batch were observed.

TABLE 2 Multivariable linear regression between clinical variables and pain intensity phenotype (statistically significant results are in bold).

Characteristic	Beta Reference	*p*-value
Sex		
Male		
Female	0.24	**0.021**
Age	0.0026	0.47
Study		
CERP	Reference	
MOLO	0.79	$\mathbf{3.5\times10^{-6}}$
EPOS	0.10	0.66
Country of enrolment		
Italy	Reference	
Other	0.065	0.63
Opioid		
Morphine	Reference	
Buprenorphine	0.20	0.35
Fentanyl	0.38	**0.0031**
Oxycodone	0.29	**0.031**
Tumour site		
Lung	Reference	
Gastro-enteric	−0.25	0.071
Breast	−0.18	0.28
Prostate	−0.34	0.050
Pancreas	−0.42	0.065
Urinary tracts	0.037	0.85
Head and neck	0.26	0.24
Gynaecologic	−0.0073	0.97
Other	−0.021	0.89
Genotyping batch		
I	reference	
II	0.14	0.36
III	0.26	0.067
IV	0.15	0.24

3.1 | Five variants associate with pain intensity, at genome-wide significance level

We then performed a genome-wide association analysis between the imputed genotypes and the inverse-normal transformed residues of the model described above. This analysis identified five germline variants associated with the pain intensity phenotype, at the genome-wide significance level (p-value $<5.0\times10^{-8}$). These variants (i.e. rs6062363, rs6062365, rs13043326, rs6089804 and rs1806952) mapped to a non-coding region of chromosome 20, downstream *PCMTD2* gene coding isoforms. Considering a suggestive threshold of p-value $<1.0\times10^{-5}$, a total of 66 variants were identified (Table S1), 31 of which mapped to the chromosome 20 locus (spanning less than 30 GWAS catalog, from position 64,257,449 to 64,285,790). The Manhattan plot in Figure 1 shows the results of this analysis, for all the tested variants; the complete summary statistics are available in the GWAS catalog (GCST90435150).

Specifically, we observed that the minor alleles of the identified variants were inversely correlated with pain intensity values (beta <0). Indeed, subjects who were homozygous for the minor alleles or were heterozygous experienced less severe pain than patients who were homozygous for the major alleles. The boxplot in Figure 2 shows the median pain intensity in the three patient genotyping groups according to the top-significant variant, rs6062363 (G/A), as an example. Although the differences were quite small, the median pain intensity of patients carrying at least one minor allele of this variant (A) was significantly lower than that of patients homozygous for the major allele (G; median pain intensity = 3.17, 3.33 and 4.00, in AA, GA and GG patients; Kruskal–Wallis p-value $=3.11\times10^{-5}$). Similar results were observed for the other four top-significant variants (Figure S2).

The 31 variants on chromosome 20 (p-value $<1.0\times10^{-5}$) were in strong linkage disequilibrium (LD) with the leading variant. In detail, the other four polymorphisms above the genome-wide significance threshold had r^2 >0.97 while the others, except for rs58586141, rs41279358 and rs41279358 had r^2 >0.60. In the zoom plot reported in Figure 3, we show the LD between rs6062363 and the variants in a ~200 kb region.

The heritability calculation, performed between the GWAS summary statistics and pre-computed LD scores for 1,146,582 SNPs, revealed that the heritability of pain intensity was quite low, that is, h^2 was 0.0746 (SE: 0.2062; lambda: 1.0046; mean χ^2: 1.006), indicating that only the 7% of the phenotypic variance can be explained by common genetic variants. Nonetheless, this result is in agreement with the h^2 calculated from a larger GWAS on pain intensity in individuals with different chronic pain conditions (Toikumo et al., 2024).

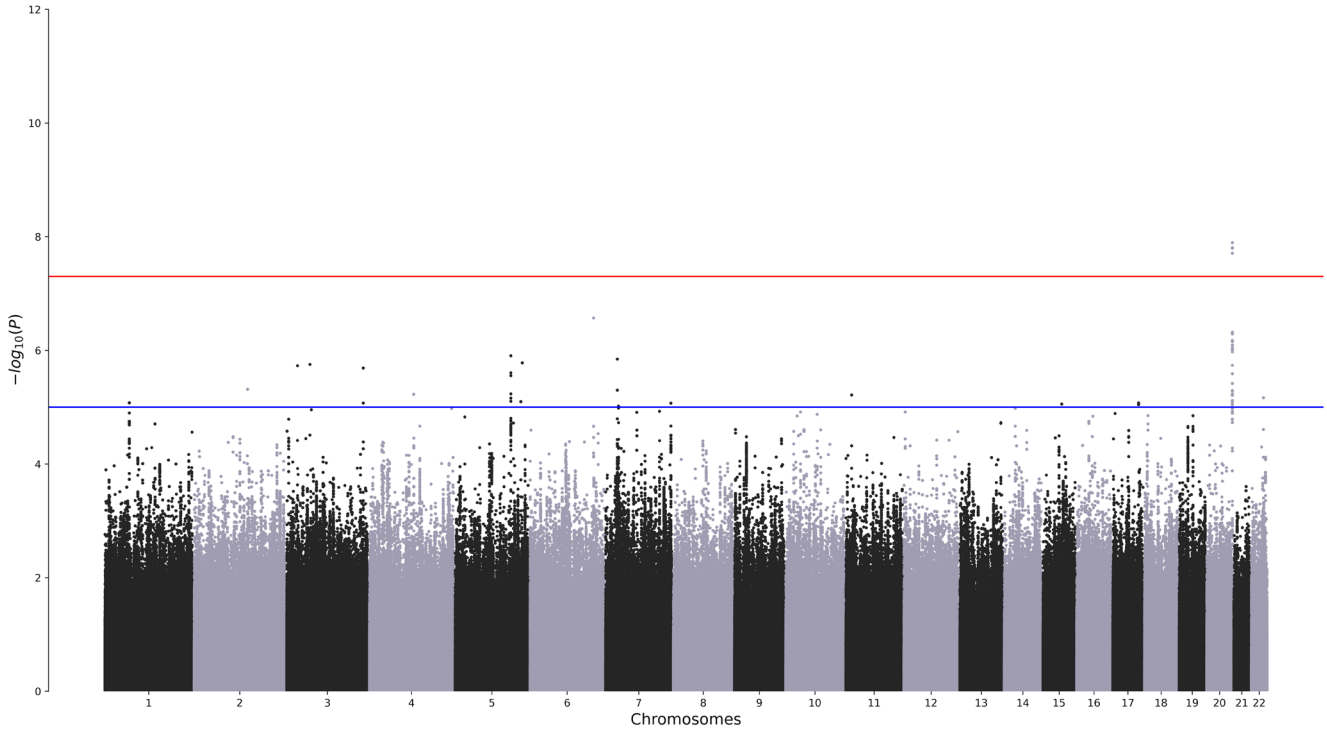
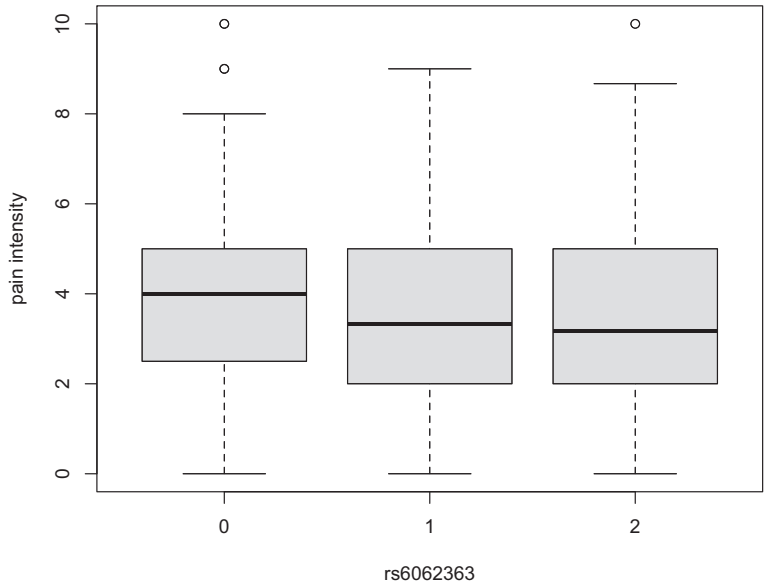


FIGURE 1 Polymorphisms on chromosome 20 (20q13.33) are significantly associated with pain intensity. Manhattan plot of the results from the GWAS with the inverse-normal transformed residuals of the linear regression model for pain intensity phenotype, with sex, age, opioid type, cancer diagnosis, chemotherapy treatment, country of origin, study of enrolment and genotyping batch, as covariates. Each dot represents a polymorphism whose coordinates are determined according to the genomic position (GChr38, hg38 release) on the x-axis and *p*-values (−log10(*P*)) of the association with the phenotype on the y-axis. The horizontal red line represents the threshold of significance (*p*-value $<5.0\times10^{-8}$), while the blue line is a suggestive threshold at *p*-value $<1.0\times10^{-5}$.

FIGURE 2 The minor allele of the lead variant is associated with low pain intensity. Pain intensity values in the three genotyping groups of patients, according to the top-significant variant, rs6062363 (0, GG; 1, GA; 2, AA). The line within each box represents the median pain intensity values; the upper and lower edges of each box are the 75th and 25th percentiles, respectively; the upper and lower bars indicate the highest and lowest values, respectively; outliers are indicated as circles.



3.2 | Pain intensity associated variants regulate *PCMTD2* gene expression

Since all variants on chromosome 20, with *p*-value $<1.0\times10^{-5}$, mapped to non-coding regions of the genome (i.e. introns, 3′-UTRs or downstream *PCMTD2* gene), we hypothesized that they may play a regulatory role in gene expression. Indeed, three of these polymorphisms mapped to candidate cis-regulatory elements (cCREs). In detail, we found that rs1806952 mapped

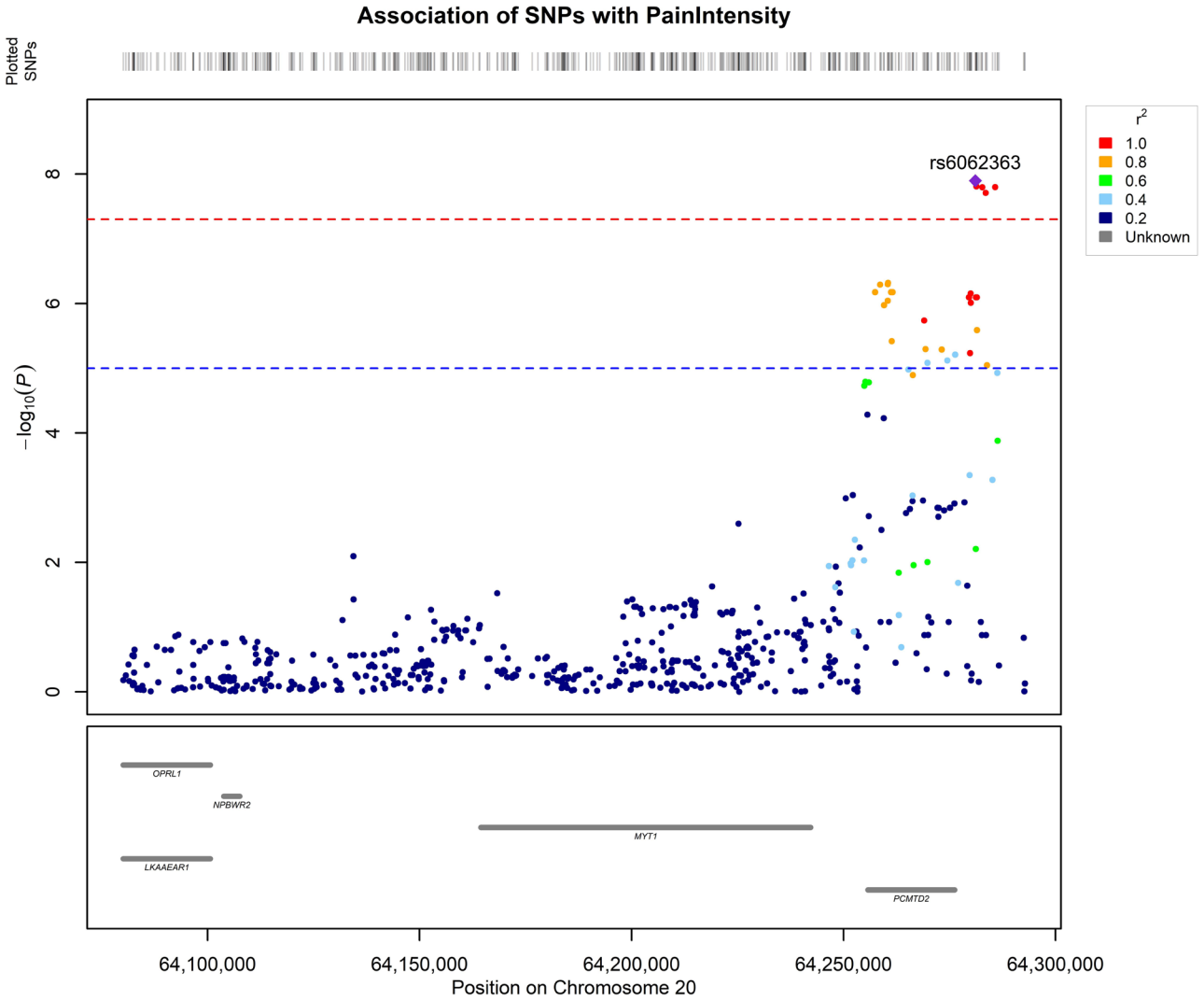


FIGURE 3 Zoom plot of the locus on chromosome 20 identified in the GWAS. The plots span the region from 64,292,800 to 64,080,100 bp. Polymorphisms are plotted according to their position on chromosome 20, along the *x*-axis, and to *p*-values ($-\log_{10}P$) for their association with pain intensity, on the y-axis. Genome-wide (*p*-value $<5.0\times10^{-8}$) and suggestive (*p*-value $<1.0\times10^{-5}$) thresholds of significance are represented as red and blue dashed lines, respectively. Dot colour indicates linkage disequilibrium (r^2) between each polymorphism and the lead variant (rs6062363, purple diamond).

to a CTCF-binding region (EH38E3450000), whereas rs6512309, rs6062357 and rs7270745 mapped to a distal enhancer-like region (EH38E2130198). Additionally, according to the SNP2TFBS tool, nine variants (rs6062681, rs6512309, rs11474881, rs6062359, rs58586141, rs1570520, rs6089801 and rs6089804) are predicted to affect transcription factor binding sites, by disrupting or creating a new binding site in the presence of the minor allele (Table S2). RegulomeDB functional annotation indicated that three polymorphisms (rs6062357, rs6089801 and rs76240558) had a high probability (>0.9) of being functional. Additionally, rs6062357, rs1570520 and rs6089801 had five supporting data points (including transcription factor (TF) binding sites, motifs and footprints, chromatin states and accessibility peaks and expression quantitative trait loci (eQTLs); ranking = 1b) that support their possible functional role. Similarly, rs6512309 and rs11474881 had four supporting data points (ranking = 1d and 2b, respectively), while the other 24 polymorphisms had at least two (ranking = 1f and 4, Table S3).

Then, we searched for the 31 polymorphisms in the GTEx database, and we found that 29 of them acted as expression quantitative trait loci (eQTLs) for *PCMTD2* in 18 tissues, including several brain tissue types (*p*-value $<1.0\times10^{-5}$; Table S4). We also searched for the same variants in the eQTLGen database: 29 of them were found to be eQTLs for three genes (*PCMTD2*, *OPRL1* and *PPDPF*) in blood (*p*-value $<1.0\times10^{-5}$; Table S5). Finally, looking at brain-specific eQTLs in the MetaBrain dataset, we

found 24 polymorphisms reported as eQTLs for *PCMTD2* in two brain regions, the cerebellum and cortex (p-value $<1.0\times10^{-5}$; Table S6). All datasets reported an inverse correlation between the number of minor alleles in individuals' genotype and the expression levels of *PCMTD2*. A lower expression of the *OPRL1* and *PPDPF* genes was also associated with the minor alleles of the identified polymorphisms, in blood (data from eQTLGen).

To determine whether the genetic factors modulating pain intensity in our opioid-treated cancer patients were the same as those regulating *PCMTD2*, *OPRL1* and *PPDPF* gene expression, we performed colocalization analyses. To do this, we used eQTLGen data since our variants were reported as eQTLs of more than one gene within this dataset. Figure 4 shows the results obtained from these analyses. In detail, we did not find evidence for colocalization between pain intensity and the expression of the three genes (Table S7). Therefore, the results suggested that the expression phenotypes and pain intensity did not share the same causal variants.

Finally, looking at the PheWAS data in the Open Targets Genetics portal, we observed that two UK Biobank traits, tea and coffee intake, were associated (at p-value $<1.0\times10^{-5}$) with most of the polymorphisms associated with pain intensity in our study (Table S8). In particular, the tea intake phenotype is reportedly linked to all the investigated variants.

3.3 | The locus on chromosome 20 was previously reported to be associated with pain intensity

HPGDB reports several genetic variants that were found, in several different studies, to be associated with various pain phenotypes. Thus, we searched for all these variants ($n=1264$) in our GWAS summary statistics to test whether our study independently verified any of the previously reported associations with pain phenotypes. We found 1170 variants among our results, and 69 of which were associated with pain intensity in our opioid-treated cancer patients, at p-value <0.05 (Table S9). Six of these 69 variants (including an upstream variant of the *OPRM1* gene, encoding the mu-opioid receptor) were associated with the analgesia phenotype and four other variants were previously reported to be associated with cancer pain. However, none of these 69 variants survived multiple test correction (false discovery rate, FDR >0.05).

Then, we compared our results with those of four previously published papers, including our first GWAS in cancer patients treated with opioids for pain (Galvan et al., 2011). None of the eight variants identified in our previous study on pain relief were associated with pain intensity in this new, larger GWAS, in a partially overlapping patient series (p-value >0.05; Table S10). Neither the three variants that were statistically significant in the GWAS by Nishizawa, et al. (2022) were associated with pain intensity in our GWAS (p-value >0.05; Table S10).

Among the 99 independent loci associated with 17 different pain-related traits from the UK Biobank, in the joint analysis by Mocci et al. (2023), 97 were tested in our GWAS, but only five variants were associated with pain intensity, with p-values <0.05 (Table S11). Of the 33 independent SNPs identified by Zorina-Lichtenwalter, et al. (2023) in their general factor GWAS for 24 chronic pain conditions, only two had a p-value <0.05 in our GWAS (Table S12). Finally, we searched for the 126 lead variants identified by Toikumo., et al. (2024), in a recently published multi-ancestry GWAS: 109 of them

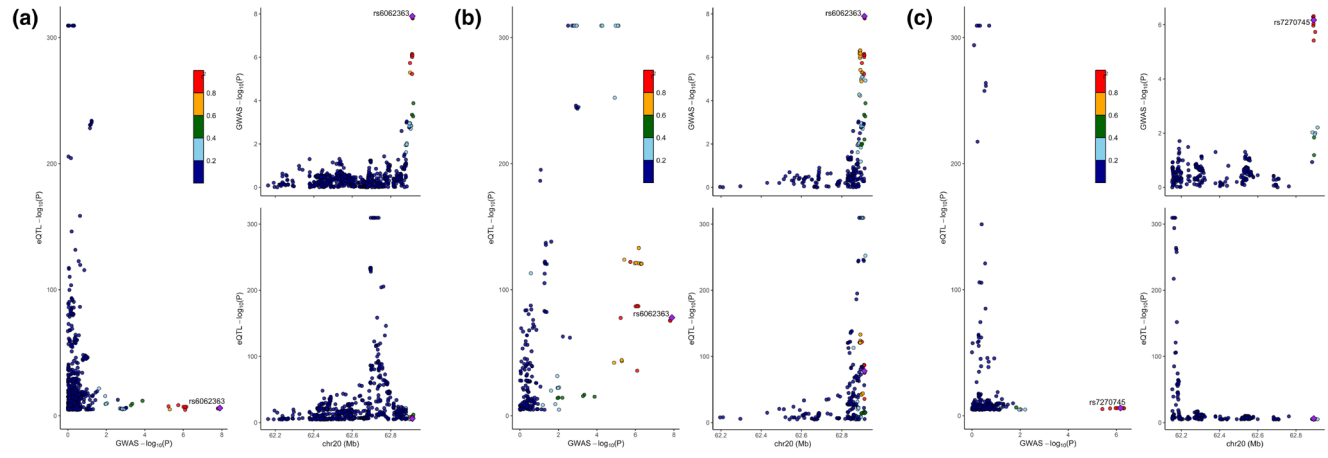


FIGURE 4 The genetic factors modulating pain intensity and gene expression in blood are not the same. Colocalization plots for the *OPRL1* (a), *PCMTD2* (b) and *PPDPF* (c) genes using the whole-blood tissue expression data from the eQTLGen database (numbers of SNPs included in the analyses were 755, 224 and 293, respectively). Dots are coloured based on linkage disequilibrium (r^2) with the lead variants, rs6062363 (a, b) and rs7270745 (c; *PPDPF* expression was not associated with the 5 top-significant variants associated with pain intensity).

TABLE 3 Polymorphisms associated with pain intensity in our opioid-treated cancer patients (at *p*-value $<1.0\times10^{-5}$) mapping less than 1 Mb from leading variants identified in previously reported pain GWASs.

rsID	Chr	Position (bp)[a]	Location	Distance[b] (bp)[a]	BETA	*p*-value	Study
rs6062363	20	64,281,110	20q13.33	441,591	−0.17	1.27×10^{-8}	Our GWAS
rs4809370	20	63,839,519			−0.011	1.79×10^{-9}	Toikumo et al.
rs9479734	6	150,123,675	6q25.1	411,779	0.19	2.69×10^{-7}	Our GWAS
rs1934534	6	149,711,896			0.002	2.38×10^{-8}	Mocci et al.
rs35961649	5	167,004,270	5q34	−265,686	−0.48	1.65×10^{-6}	Our GWAS
rs10053440	5	167,269,956			−0.01	3.03×10^{-9}	Toikumo et al.
rs79108599	1	66,447,783	1p31.3	−247,136	−0.31	8.37×10^{-6}	Our GWAS
rs1325266	1	66,694,919			0.016	8.94×10^{-11}	Mocci et al.
rs6484374	11	10,570,705	11p15.4	−81,487	−0.27	6.10×10^{-6}	Our GWAS
rs4909945	11	10,652,192			0.019	3.36×10^{-13}	Mocci et al.
rs7811696	7	22,482,241	7p15.3	990,028	−0.14	9.54×10^{-6}	Our GWAS
rs12672629	7	21,492,213			−0.019	1.67×10^{-10}	Toikumo et al.

Abbreviations: Chr, chromosome; rsID, variant ID in dbSNP.

[a]According to GRCh38 genomic release.

[b]Distance between the two variants.

were tested in our study and only nine were associated with pain intensity in our opioid-treated cancer patients, with a *p*-value <0.05 (Table S13). However, when we considered our variants with *p*-value $<1.0\times10^{-5}$, we observed that our lead variant on chromosome 20, rs6062363, was less than 500 kb from the lead variant (rs4809370) of one of the 126 loci identified in (Toikumo et al., 2024). Similarly, other variants above our suggestive threshold on chromosomes 1, 5, 6, 7 and 11 mapped near (i.e. less than 500 kb, except for the one on chromosome 7 that was less than 1 Mb apart) five lead variants reported in the previous studies (Table 3) (Mocci et al., 2023; Toikumo et al., 2024), suggesting possible shared genetic factors modulating chronic pain and opioid-treated cancer pain.

4 | DISCUSSION AND CONCLUSIONS

In this GWAS, which was carried out in more than 2000 cancer patients treated with opioids, we identified a locus on chromosome 20 significantly associated with pain intensity. Specifically, the minor alleles of five variants, in a non-coding region of the genome, were markers of good response to opioid analgesic therapy. Indeed, patients carrying these alleles, in heterozygosity or homozygosity, had lower pain intensity than patients homozygous for the major alleles. These five variants were in strong linkage disequilibrium with each other and were also linked to 26 other less significantly associated polymorphisms in a 30 kbp region where the protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2 (*PCMTD2*) gene is located. Additionally, nearby (less than 200 kbp apart) protein-coding genes included the *MYT1* (myelin transcription factor 1), *NPBWR2* (neuropeptides B and W receptor 2) and *OPRL1* (opioid-related nociceptin receptor 1) genes. All these genes are widely expressed in the brain, and the proteins encoded by the latter two genes are involved in pain modulation (as reviewed in (Chottova Dvorakova, 2018; El Daibani & Che, 2022)). In particular, *OPRL1* encodes a G protein-coupled receptor of the opioid receptor family, although it does not have high affinity for standard opiate ligands. Instead, it binds the endogenous neuropeptide nociceptin and has opioid modulatory activity, thus affecting analgesia (Toll et al., 2016). The function of *PCMTD2* is not well understood, but recently it has been shown to interact with the CUL5 protein, leading to the ubiquitination of IL2RB and negatively regulating IL2 signalling in CD^{8+} T cells (Liao et al., 2024). However, its role in pain modulation is unknown, although ILR2B plasma levels have been associated with neuropathic pain (but not after correction for multiple testing) in a study on subjects with neuropathy after traumatic nerve injuries (Miclescu et al., 2023).

The polymorphisms identified in our GWAS were previously reported to act as eQTLs, mainly for *PCMTD2* but also *OPRL1*. These findings indicate that these genetic variants are regulatory polymorphisms that can modulate the expression levels of these genes. Subjects carrying one or two copies of the minor alleles expressed lower levels of these genes in blood. However, colocalization analyses did not support the hypothesis that the same

variants affecting gene expression are also responsible for the modulation of pain intensity. Nevertheless, several tools predict these polymorphisms to be functional, potentially affecting transcription factor binding sites or other regulatory elements. However, further studies are needed to better understand the regulatory role of these variants and the mechanism underlying their association with pain intensity. Indeed, it would be interesting to investigate whether these variants also affect *OPRL1* levels in the brain or in other nervous system tissues involved in pain signalling. Although we searched for eQTL data in brain tissue the sample sizes of the specific datasets were smaller (n_{GTEx} <100 and $n_{MetaBrain}$ <500) than those of the blood dataset ($n_{eQTLGen}$ >25,000), where the identified polymorphisms were significantly associated with the expression of *OPRL1*. Thus, the brain datasets had lower statistical power to detect *OPRL1* eQTLs.

Although our results did not replicate those of previous GWAS (Galvan et al., 2011; Nishizawa et al., 2022), this lack of validation is not surprising for different reasons. Our previous GWAS was smaller, based on genotyping data from pooled DNA (i.e. patients were not individually genotyped) and focused on a slightly different phenotype (pain relief instead of pain intensity). The Asiatic study was even smaller and probably underpowered, analysed a different phenotype (opioid requirement) and, additionally, the different patient ancestry might have led to different results. Notably, a locus on chromosome 20 (whose lead variant mapped at approximately 400 kbp from our top variants) was recently reported to be significantly associated with pain intensity in a multi-ancestry GWAS for chronic pain (Toikumo et al., 2024). Although the lead variant of that locus was not significantly associated with pain intensity in our GWAS for cancer pain, we cannot exclude that the identified locus could be the same. Similarly, five additional loci that were less significantly associated with pain intensity in our opioid-treated cancer patients were mapped near the lead variants identified in previous GWASs (Mocci et al., 2023; Toikumo et al., 2024). Thus, our results independently validate previous findings under different pain conditions. These partially overlapping findings indicate that genetic factors may be shared between different types of pain.

Furthermore, a PheWAS with UK Biobank traits revealed significant associations of our variants on chromosome 20 with tea and coffee intake phenotypes. A recent study in a US cohort (23andMe, Inc. research participants) confirmed the association between coffee intake and variants near the *PCMTD2* gene (Thorpe et al., 2024). These findings suggest a possible relationship between pain intensity and caffeine (and other related natural xanthines) consumption. Caffeine is a well-known analgesic adjuvant and has been shown to induce pain relief (as reviewed in (Faudone et al., 2021)) due to its antagonistic effect on adenosine receptors (Jacobson et al., 2022). Moreover, caffeine and theophylline have been found to interact with morphine, enhancing its analgesic effect in animal models (Malec & Michalska, 1988). Investigating the association between these polymorphisms and caffeine consumption in our opioid-treated cancer patients as well as understanding the shared mechanisms underlying the association with coffee/tea intake, pain intensity and opioid response would be of interest.

Although this is the largest pain intensity GWAS conducted in opioid-treated cancer patients to date, our sample size was underpowered for detecting genome-wide associations with rare variants, which were not investigated in our study for this reason. Large studies focused on opioid-treated cancer are needed to confirm our findings and, possibly, discover additional genetic loci modulating this specific type of pain. Additional investigations would also help in overcoming other limitations of the present study. Indeed, our results might not be generalizable to patients of ancestry different from the European one, as we found them investigating only European individuals and genetic variants affecting opioid response might vary in other populations. Another limit of our study might reside in the analysed phenotype itself: pain intensity is a subjective phenotype and the use of standardized questionnaires to report this patient outcome is helpful to overcome bias. However, in our study we analysed patients from a cross-sectional study together with those from two longitudinal studies and this might have contributed to increase the heterogeneity of such a complex phenotype. Additionally, pain intensity might be influenced by other external factors (e.g. co-existent mood disorders or other comorbidities) that we could not take into account in our regression model, because we did not have this kind of information.

In conclusion, the locus identified on chromosome 20q13.33 has already been found in an independent, larger study in individuals with different types of chronic pain (Toikumo et al., 2024). Our results confirm that genetics plays a role in modulating pain phenotype and opioid efficacy in cancer patients, although this role is quite limited, as the calculated heritability was quite low. Functional studies are needed to elucidate the molecular mechanisms through which the identified variants modulate pain and to determine whether the *OPRL1* gene is the effector of the associated genetic locus.

AUTHOR CONTRIBUTIONS

F.C. and F.M. Conceptualization; F.M., M.E. and F.C. formal analysis; A.T.C., M.SH., A.P., E.Z., F.S., P.K., S.K., O.C., M.C.M. and M.C.P. resources; S.N. and F.C. investigation—performing experiments; M.SH., C.B., A.P., E.Z., F.S., P.K., S.K., O.C., M.C.M. and M.C.P. investigation—data

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author upon reasonable request. The GWAS summary statistics are publicly available upon publication in the GWAS Catalog (GCST90435150).

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REFERENCES

Abascal, F., Acosta, R., Addleman, N. J., Adrian, J., Afzal, V., Ai, R., Aken, B., Akiyama, J. A., al Jammal, O., Amrhein, H., Anderson, S. M., Andrews, G. R., Antoshechkin, I., Ardlie, K. G., Armstrong, J., Astley, M., Banerjee, B., Barkal, A. A., Barnes, I. H. A., … Ecker, J. R. (2020). Expanded encyclopaedias of DNA elements in the human and mouse genomes. *Nature*, *583*, 699–710.

Angst, M. S., Phillips, N. G., Drover, D. R., Tingle, M., Ray, A., Swan, G. E., Lazzeroni, L. C., & Clark, D. J. (2012). Pain sensitivity and opioid analgesia: A pharmacogenomic twin study. *Pain*, *153*, 1397–1409.

Boyle, A. P., Hong, E. L., Hariharan, M., Cheng, Y., Schaub, M. A., Kasowski, M., Karczewski, K. J., Park, J., Hitz, B. C., Weng, S., Cherry, J. M., & Snyder, M. (2012). Annotation of functional variation in personal genomes using RegulomeDB. *Genome Research*, *22*, 1790–1797.

Bulik-Sullivan, B. K., Loh, P.-R., Finucane, H. K., Ripke, S., Yang, J., Patterson, N., Daly, M. J., Price, A. L., & Neale, B. M. (2015). LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nature Genetics*, *47*, 291–295.

Caraceni, A., Hanks, G., Kaasa, S., Bennett, M. I., Brunelli, C., Cherny, N., Dale, O., De Conno, F., Fallon, M., Hanna, M., Haugen, D. F., Juhl, G., King, S., Klepstad, P., Laugsand, E. A., Maltoni, M., Mercadante, S., Nabal, M., Pigni, A., … European Palliative Care Research Collaborative (EPCRC), European Association for Palliative Care (EAPC). (2012). Use of opioid analgesics in the treatment of cancer pain: Evidence-based recommendations from the EAPC. *The Lancet Oncology*, *13*, e58–e68.

Chen, B., Wang, L., Pu, S., Guo, L., Chai, N., Sun, X., Tang, X., Ren, Y., He, J., & Hao, N. (2024). Unveiling potential drug targets for hyperparathyroidism through genetic insights via Mendelian randomization and colocalization analyses. *Scientific Reports*, *14*, 6435.

Chottova Dvorakova, M. (2018). Distribution and function of neuropeptides W/B signaling system. *Frontiers in Physiology*, *9*, 981.

Colombo, F., Pintarelli, G., Galvan, A., Noci, S., Corli, O., Skorpen, F., Klepstad, P., Kaasa, S., Pigni, A., Brunelli, C., Roberto, A., Piazza, R., Pirola, A., Gambacorti-Passerini, C., & Caraceni, A. T. (2020). Identification of genetic polymorphisms modulating nausea and vomiting in two series of opioid-treated cancer patients. *Scientific Reports*, *10*, 542.

Corli, O., Floriani, I., Roberto, A., Montanari, M., Galli, F., Greco, M. T., Caraceni, A., Kaasa, S., Dragani, T. A., Azzarello, G., Luzzani, M., Cavanna, L., Bandieri, E., Gamucci, T., Lipari, G., Di Gregorio, R., Valenti, D., Reale, C., Pavesi, L., … Molinari, E. (2016). Are strong opioids equally effective and safe in the treatment of chronic cancer pain? A multicenter randomized phase IV 'real life' trial on the variability of response to opioids. *Annals of Oncology*, *27*, 1107–1115.

Das, S., Forer, L., Schönherr, S., Sidore, C., Locke, A. E., Kwong, A., Vrieze, S. I., Chew, E. Y., Levy, S., McGue, M., Schlessinger, D., Stambolian, D., Loh, P. R., Iacono, W. G., Swaroop, A., Scott, L. J., Cucca, F., Kronenberg, F., Boehnke, M., … Fuchsberger, C. (2016). Next-generation genotype imputation service and methods. *Nature Genetics*, *48*, 1284–1287.

Daut, R. L., Cleeland, C. S., & Flanery, R. C. (1983). Development of the Wisconsin brief pain questionnaire to assess pain in cancer and other diseases. *Pain*, *17*, 197–210.

de Klein, N., Tsai, E. A., Vochteloo, M., Baird, D., Huang, Y., Chen, C.-Y., van Dam, S., Oelen, R., Deelen, P., Bakker, O. B., El Garwany, O., Ouyang, Z., Marshall, E. E., Zavodszky, M. I., van Rheenen, W., Bakker, M. K., Veldink, J., Gaunt, T. R., Runz, H., … Westra, H.-J. (2023). Brain expression quantitative trait locus and network analyses reveal downstream effects and putative drivers for brain-related diseases. *Nature Genetics*, *55*, 377–388.

Delaneau, O., Marchini, J., McVean, G. A., Donnelly, P., Lunter, G., Marchini, J. L., Myers, S., Gupta-Hinch, A., Iqbal, Z., Mathieson, I., Rimmer, A., Xifara, D. K., Kerasidou, A., Churchhouse, C., Delaneau, O., Altshuler, D. M., Gabriel, S. B., Lander, E. S., Gupta, N., … Leinonen, R. (2014). Integrating sequence and array data to create an improved 1000 genomes project haplotype reference panel. *Nature Communications*, *5*, 3934.

Dong, S., Zhao, N., Spragins, E., Kagda, M. S., Li, M., Assis, P., Jolanki, O., Luo, Y., Cherry, J. M., Boyle, A. P., & Hitz, B. C. (2022). Annotating and prioritizing human non-coding variants with RegulomeDB. *BioRxiv*, *55*, 724.

El Daibani, A., & Che, T. (2022). Spotlight on Nociceptin/Orphanin FQ receptor in the treatment of pain. *Molecules*, *27*, 595.

Faudone, G., Arifi, S., & Merk, D. (2021). The medicinal chemistry of caffeine. *Journal of Medicinal Chemistry*, *64*, 7156–7178.

Fuchsberger, C., Abecasis, G. R., & Hinds, D. A. (2015). minimac2: Faster genotype imputation. *Bioinformatics*, *31*, 782–784.

Galvan, A., Skorpen, F., Klepstad, P., Knudsen, A. K., Fladvad, T., Falvella, F. S., Pigni, A., Brunelli, C., Caraceni, A., Kaasa, S., & Dragani, T. A. (2011). Multiple loci modulate opioid therapy response for cancer pain. *Clinical Cancer Research*, *17*, 4581–4587.

Ghoussaini, M., Mountjoy, E., Carmona, M., Peat, G., Schmidt, E. M., Hercules, A., Fumis, L., Miranda, A., Carvalho-Silva, D., Buniello, A., Burdett, T., Hayhurst, J., Baker, J., Ferrer, J., Gonzalez-Uriarte, A., Jupp, S., Karim, M. A., Koscielny, G., Machlitt-Northen, S., … Dunham, I. (2021). Open targets genetics: Systematic identification of trait-associated genes using large-scale genetics and functional genomics. *Nucleic Acids Research*, *49*, D1311–D1320.

Giambartolomei, C., Vukcevic, D., Schadt, E. E., Franke, L., Hingorani, A. D., Wallace, C., & Plagnol, V. (2014). Bayesian test for Colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genetics*, *10*, e1004383.

Jacobson, K. A., Gao, Z., Matricon, P., Eddy, M. T., & Carlsson, J. (2022). Adenosine a 2A receptor antagonists: From caffeine to selective non-xanthines. *British Journal of Pharmacology*, *179*, 3496–3511.

Klepstad, P., Fladvad, T., Skorpen, F., Bjordal, K., Caraceni, A., Dale, O., Davies, A., Kloke, M., Lundström, S., Maltoni, M., Radbruch, L., Sabatowski, R., Sigurdardottir, V., Strasser, F., Fayers, P. M., & Kaasa, S. (2011). Influence from genetic variability on opioid use for cancer pain: A European genetic association study of 2294 cancer pain patients. *Pain*, *152*, 1139–1145.

Klepstad, P., Kaasa, S., Cherny, N., Hanks, G., De Conno, F., Torvik, K., Likar, R., Menten, J., Kakas, J., Pederson, L., Kalso, E., Poulain, P., Radbruch, L., Mystakidou, K., Sigurdardotii, V., O'Brien, T., Thill, B., Zylics, B., Ferraz Goncalves, J. A., … Nunez Olarte, J. M. (2005). Pain and pain treatments in European palliative care units. A cross sectional survey from the European Association for Palliative Care Research Network. *Palliative Medicine*, *19*, 477–484.

Kumar, S., Ambrosini, G., & Bucher, P. (2017). SNP2TFBS—A database of regulatory SNPs affecting predicted transcription factor binding site affinity. *Nucleic Acids Research*, *45*, D139–D144.

Liao, X., Li, W., Zhou, H., Rajendran, B. K., Li, A., Ren, J., Luan, Y., Calderwood, D. A., Turk, B., Tang, W., Liu, Y., & Wu, D. (2024). The CUL5 E3 ligase complex negatively regulates central signaling pathways in CD^{8+} T cells. *Nature Communications*, *15*, 603.

Little, J., Higgins, J. P. T., Ioannidis, J. P. A., Moher, D., Gagnon, F., von Elm, E., Khoury, M. J., Cohen, B., Davey-Smith, G., Grimshaw, J., Scheet, P., Gwinn, M., Williamson, R. E., Zou, G. Y., Hutchings, K., Johnson, C. Y., Tait, V., Wiens, M., Golding, J., … Birkett, N. (2009). STrengthening the REporting of genetic association studies (STREGA)—An extension of the STROBE statement. *Genetic Epidemiology*, *33*, 581–598.

Loh, P. R., Danecek, P., Palamara, P. F., Fuchsberger, C., Reshef, Y. A., Finucane, H. K., Schoenherr, S., Forer, L., McCarthy, S., Abecasis, G. R., Durbin, R., & Price, A. L. (2016). Reference-based phasing using the haplotype reference consortium panel. *Nature Genetics*, *48*, 1443–1448.

Malec, D., & Michalska, E. (1988). The effect of methylxanthines on morphine analgesia in mice and rats. *Polish Journal of Pharmacology and Pharmacy*, *40*, 223–232.

Mathelier, A., Zhao, X., Zhang, A. W., Parcy, F., Worsley-Hunt, R., Arenillas, D. J., Buchman, S., Chen, C., Chou, A., Ienasescu, H., Lim, J., Shyr, C., Tan, G., Zhou, M., Lenhard, B., Sandelin, A., & Wasserman, W. W. (2014). JASPAR 2014: An extensively expanded and updated open-access database of transcription factor binding profiles. *Nucleic Acids Research*, *42*, D142–D147.

Mbatchou, J., Barnard, L., Backman, J., Marcketta, A., Kosmicki, J. A., Ziyatdinov, A., Benner, C., O'Dushlaine, C., Barber, M., Boutkov, B., Habegger, L., Ferreira, M., Baras, A., Reid, J., Abecasis, G., Maxwell, E., & Marchini, J. (2021). Computationally efficient whole-genome regression for quantitative and binary traits. *Nature Genetics*, *53*, 1097–1103.

Meloto, C. B., Benavides, R., Lichtenwalter, R. N., Wen, X., Tugarinov, N., Zorina-Lichtenwalter, K., Chabot-Doré, A.-J., Piltonen, M. H., Cattaneo, S., Verma, V., Klares, R., Khoury, S., Parisien, M., & Diatchenko, L. (2018). Human pain genetics database: A resource dedicated to human pain genetics research. *Pain*, *159*, 749–763.

Miclescu, A. A., Granlund, P., Butler, S., & Gordh, T. (2023). Association between systemic inflammation and experimental pain sensitivity in subjects with pain and painless neuropathy after traumatic nerve injuries. *Scandinavian Journal of Pain*, *23*, 184–199.

Minnai, F., Shkodra, M., Noci, S., Brunelli, C., Pigni, A., Zecca, E., Skorpen, F., Klepstad, P., Kaasa, S., Corli, O., Pallotti, M. C., Maltoni, M. C., Caraceni, A. T., & Colombo, F. (2024). Genomic study in opioid-treated cancer patients identifies variants associated with nausea-vomiting. *Journal of Pain and Symptom Management*, *S0885-3924*. https://doi.org/10.1016/j.jpainsymman.2024.10.033

Mocci, E., Ward, K., Perry, J. A., Starkweather, A., Stone, L. S., Schabrun, S. M., Renn, C., Dorsey, S. G., & Ament, S. A. (2023). Genome wide association joint analysis reveals 99 risk loci for pain susceptibility and pleiotropic relationships with psychiatric, metabolic, and immunological traits. *PLoS Genetics*, *19*, e1010977.

Mountjoy, E., Schmidt, E. M., Carmona, M., Schwartzentruber, J., Peat, G., Miranda, A., Fumis, L., Hayhurst, J., Buniello, A., Karim, M. A., Wright, D., Hercules, A., Papa, E., Fauman, E. B., Barrett, J. C., Todd, J. A., Ochoa, D., Dunham, I., & Ghoussaini, M. (2021). An open approach to systematically prioritize causal variants and genes at all published human GWAS trait-associated loci. *Nature Genetics*, *53*, 1527–1533.

Mura, E., Govoni, R., Carossa, R., Allegri, M., & van, S. (2013). Consequences of the 118A>G polymorphism in the OPRM1 gene: Translation from bench to bedside? *Journal of Pain Research*, 6, 331–353.

Nishizawa, D., Terui, T., Ishitani, K., Kasai, S., Hasegawa, J., Nakayama, K., Ebata, Y., & Ikeda, K. (2022). Genome-wide association study identifies candidate loci associated with opioid analgesic requirements in the treatment of cancer pain. *Cancers (Basel)*, *14*, 4692.

Oscanoa, J., Sivapalan, L., Gadaleta, E., Dayem Ullah, A. Z., Lemoine, N. R., & Chelala, C. (2020). SNPnexus: A web server for functional annotation of human genome sequence variation (2020 update). *Nucleic Acids Research*, *48*, W185–W192.

Price, A. L., Weale, M. E., Patterson, N., Myers, S. R., Need, A. C., Shianna, K. V., Ge, D., Rotter, J. I., Torres, E., Taylor, K. D., Goldstein, D. B., & Reich, D. (2008). Long-range LD can confound genome scans in admixed populations. *The American Journal of Human Genetics*, *83*, 132–135.

Pruim, R. J., Welch, R. P., Sanna, S., Teslovich, T. M., Chines, P. S., Gliedt, T. P., Boehnke, M., Abecasis, G. R., & Willer, C. J. (2010). LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics*, *26*, 2336–2337.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., De Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*, 559–575.

Shkodra, M., Brunelli, C., Zecca, E., Infante, G., Miceli, R., Caputo, M., Bracchi, P., Lo Dico, S., Kaasa, S., & Caraceni, A. (2022). Cancer pain: Results of a prospective study on prognostic indicators of pain intensity including pain syndromes assessment. *Palliative Medicine*, *36*, 1396–1407.

Taliun, D., Harris, D. N., Kessler, M. D., Carlson, J., Szpiech, Z. A., Torres, R., Taliun, S. A. G., Corvelo, A., Gogarten, S. M., Kang, H. M., Pitsillides, A. N., LeFaive, J., Lee, S. b., Tian, X., Browning, B. L., Das, S., Emde, A. K., Clarke, W. E., Loesch, D. P., … Lin, K. H. (2021). Sequencing of 53,831 diverse genomes from the NHLBI TOPMed program. *Nature*, *590*, 290–299.

Teunissen, S. C. C. M., Wesker, W., Kruitwagen, C., de Haes, H. C. J. M., Voest, E. E., & de Graeff, A. (2007). Symptom prevalence in patients with incurable cancer: A systematic review. *Journal of Pain and Symptom Management*, *34*, 94–104.

Thorpe, H. H. A., Fontanillas, P., Pham, B. K., Meredith, J. J., Jennings, M. V., Courchesne-Krak, N. S., Vilar-Ribó, L., Bianchi, S. B., Mutz, J., Aslibekyan, S., Auton, A., Babalola, E., Bell, R. K., Bielenberg, J., Bryc, K., Bullis, E., Coker, D., Partida, G. C., Dhamija, D., … Sanchez-Roige, S. (2024). Genome-wide association studies of coffee intake in UK/US participants of European ancestry uncover cohort-specific genetic associations. *Neuropsychopharmacology*, *49*, 1609–1618.

Toikumo, S., Vickers-Smith, R., Jinwala, Z., Xu, H., Saini, D., Hartwell, E. E., Pavicic, M., Sullivan, K. A., Xu, K., Jacobson, D. A., Gelernter, J., Rentsch, C. T., Pavicic, M., Stahl, E., Cheatle, M., Zhou, H., Waxman, S. G., Justice, A. C., Kember, R. L., & Kranzler, H. R. (2024). A multi-ancestry genetic study of pain intensity in 598,339 veterans. *Nature Medicine*, *30*, 1075–1084.

Toll, L., Bruchas, M. R., Calo, G., Cox, B. M., & Zaveri, N. T. (2016). Nociceptin/Orphanin FQ receptor structure, signaling, ligands, functions, and interactions with opioid systems. *Pharmacological Reviews*, *68*, 419–457.

Turner, S. D. (2014). Qqman: An R package for visualizing GWAS results using Q-Q and manhattan plots. *BioRxiv*, *005165*.

V Subramaniam, A., Salem Yehya, A. H., & Oon, C. E. (2019). Molecular basis of cancer pain management: An Updated Review. *Medicina (Kaunas, Lithuania)*, *55*, 584.

Verlouw, J. A. M., Clemens, E., de Vries, J. H., Zolk, O., Verkerk, A. J. M. H., am Zehnhoff-Dinnesen, A., Medina-Gomez, C., Lanvers-Kaminsky, C., Rivadeneira, F., Langer, T., van Meurs, J. B. J., van den Heuvel-Eibrink, M. M., Uitterlinden, A. G., & Broer, L. (2021). A comparison of genotyping arrays. *European Journal of Human Genetics*, *29*, 1611–1624.

Võsa, U., Claringbould, A., Westra, H.-J., Bonder, M. J., Deelen, P., Zeng, B., Kirsten, H., Saha, A., Kreuzhuber, R., Yazar, S., Brugge, H., Oelen, R., de Vries, D. H., van der Wijst, M. G. P., Kasela, S., Pervjakova, N., Alves, I., Favé, M.-J., Agbessi, M., … Lehtimäki, T. (2021). Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nature Genetics*, *53*, 1300–1310.

Yang, J., Loos, R. J. F., Powell, J. E., Medland, S. E., Speliotes, E. K., Chasman, D. I., Rose, L. M., Thorleifsson, G., Steinthorsdottir, V., Mägi, R., Waite, L., Vernon Smith, A., Yerges-Armstrong, L. M., Monda, K. L., Hadley, D., Mahajan, A., Li, G., Kapur, K., Vitart, V., … Hayward, C. (2012). FTO genotype is associated with phenotypic variability of body mass index. *Nature*, *490*, 267–272.

Yu, Z., Wen, L., Shen, X., & Zhang, H. (2019). Effects of the OPRM1 A118G polymorphism (rs1799971) on opioid analgesia in cancer pain. *The Clinical Journal of Pain*, *35*, 77–86.

Zorina-Lichtenwalter, K., Bango, C. I., Van Oudenhove, L., Čeko, M., Lindquist, M. A., Grotzinger, A. D., Keller, M. C., Friedman, N. P., & Wager, T. D. (2023). Genetic risk shared across 24 chronic pain conditions: Identification and characterization with genomic structural equation modeling. *Pain*, *164*, 2239–2252.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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