Research Paper

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Human pain genetics database: a resource dedicated to human pain genetics research

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Abstract

The Human Pain Genetics Database (HPGDB) is a comprehensive variant-focused inventory of genetic contributors to human pain. After curation, the HPGDB currently includes 294 studies reporting associations between 434 distinct genetic variants and various pain phenotypes. Variants were then submitted to a comprehensive analysis. First, they were validated in an independent high-powered replication cohort by testing the association of each variant with 10 different pain phenotypes (n = 1320-26,973). One hundred fifty-five variants replicated successfully (false discovery rate 20%) in at least one pain phenotype, and the association *P* values of the HPGDB variants were significantly lower compared with those of random controls. Among the 155 replicated variants, 21 had been included in the HPGDB because of their association with analgesia-related and 13 with nociception-related phenotypes, confirming analgesia and nociception as pathways of vulnerability for pain phenotypes. Furthermore, many genetic variants were associated with multiple pain phenotypes, and the strength of their association correlated between many pairs of phenotypes. These genetic variants explained a considerable amount of the variance between different pairs of pain phenotypes, indicating a shared genetic basis among pain phenotypes. In addition, we found that HPGDB variants show many pleiotropic associations, indicating that genetic pathophysiological mechanisms are also shared among painful and nonpainful conditions. Finally, we demonstrated that the HPGDB data set is significantly enriched for functional variants that modify gene expression, are deleterious, and colocalize with open chromatin regions. As such, the HPGDB provides a validated data set that represents a valuable resource for researchers in the human pain field.

Keywords: Database, Human, Pain, Genetic variant, Single nucleotide polymorphism

1. Introduction

The estimated heritability of pain phenotypes varies between 25% and 50%.[55] Studies aiming to decipher the genetic components of this heritable trait have spiked during the last 2 decades, producing a large amount of data and stimulating unprecedented growth in the understanding of genetic factors contributing to the human pain experience.[25,52] These data come primarily from genetic association studies, in which one looks for variations in the genome (variants) of unrelated individuals exhibiting the phenotype of interest (cases) or not (controls). Several types of frequent and rare variants of different lengths exist— the most commonly investigated in human genetics being single nucleotide polymorphisms (SNPs), low-penetrance single substitutions in the genome that occur frequently enough in a population that the locations are termed polymorphic.

There are 2 major types of genetic association studies: candidate gene studies and genome-wide association studies (GWASs). In candidate gene studies, the selection of variants is usually performed based on previous knowledge or evidence-based assumption of their involvement in pain pathophysiology. Genome-wide association studies, however, are hypothesis free with respect to genomic location and involve millions of variants in large samples of cases and controls. This allows for the discovery of new variants associated with pain phenotypes.[77] Our current understanding of the genetic contribution to human pain phenotypes comes largely from candidate gene studies, although the field is moving fast toward data-driven approaches, such as GWAS and whole genome sequencing. As a notable exception, GWASs have already been conducted on many migraine cohorts.[3,4,17,26,34]

Databases hosting human genetic association data currently exist, none of which are sufficient for human pain genetics. Some are focused exclusively on GWAS,[33,44,45] and although the database of clinical variants (ClinVar)[42] aggregates information from both GWAS and candidate gene studies, almost no human pain association studies have been submitted to ClinVar. Another database, the Pain Genes Database, offers an exclusive and comprehensive catalog of pain-related genes and their associated traits.[41] However, it is limited to results from mouse studies and thus can be an incomplete proxy for the complex human pain experience.[50,71] The Pain Research Forum offers a catalog of human pain-related genes and their associated traits/conditions,

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but it relies on submission of association results by readers and gives limited information about genetic loci and source publications. As such, the human pain genetics field lacks a detailed, centralized, curated, and regularly updated repository of relevant data. Thus, we present here the Human Pain Genetics Database (HPGDB), freely accessible at https://humanpaingenetics.org/hpgdb.

The HPGDB offers an extensive and interactive web-based data browser that is easy to navigate and encapsulates the current relevant findings in human pain genetics. To validate the genetic variants previously reported to be associated with pain phenotypes and deposited into the HPGDB, we have tested them for replication in a large independent cohort. We further evaluated their functionality by assessing their enrichment for pleiotropy, effects on gene expression, deleteriousness, and colocalization with regions of open chromatin.

2. Methods

2.1. Search strategy

Quarterly searches were conducted on the MEDLINE/PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) using Medical Subject Headings (MeSH) terms of the U.S National Library of Medicine's indexing system. The first query includes search terms "pain measurements" AND "genetic polymorphism" AND "human" NOT "review." Until May 11, 2017, this query had returned 187 results. The second query consists of the terms "single nucleotide polymorphism" AND "pain/genetics" AND "human" NOT "review." This search returned 284 results. Next, we replace "pain measurements" in the first set and "single nucleotide polymorphism" in the second set with one of the following predefined phenotypes: "analgesia," "nociception," "musculoskeletal pain," "fibromyalgia," "postoperative pain," "cancer pain," "neuropathic pain," "temporomandibular disorder," "migraine," and "back pain."

Initial screening of all publications retrieved by the search queries was conducted by X.W. and N.T. Each publication passing the first screening was then reviewed by a second team of pain researchers (C.B.M., R.B., X.W., K.Z.-L., S.C., A.-J.C.-D., M.H.P., V.V., R.K., S.K., and M.P.). A publication was included in the HPGDB only if it reported at least one statistically significant ($P < 0.05$) association between a genetic variant and sensitivity to experimental nociceptive stimuli; analgesic response to opioid and nonopioid pharmacological treatment, as well as their side effects (such as nausea, vomiting, drowsiness, and confusion); clinical pain conditions described as chronic, such as musculoskeletal pain, neuropathic pain, and migraine (both risk of having an existing condition and risk of developing one during a prospective study were included); or pain conditions described as acute (such as postoperative pain).

Studies showing statistically insignificant results (absence of association) were not included in the database. Definition of terms used as part of the inclusion criteria are genotype, the genetic constitution with respect to the alleles at one or more genetic loci under observation; haplotype, a combination of alleles at closely linked gene loci that are inherited together; and diplotype, specific combination of 2 haplotypes.

The final decision on study inclusion was made by the pain researchers and clinicians C.B.M. and R.B., who reviewed all publications and their reported associations and assigned reported phenotypes to broad categories intended to group publications investigating similar phenotypes. Created categories were based on the phenotype solely as described by each publication and are the following: analgesia, which includes both opioid and nonopioid analgesia; cancer pain; fibromyalgia; migraine; neuraxial pain (pain that originates from the spinal cord or supporting structures, eg, lumbar radiculopathies); neuropathic pain; nociception (for publications that investigated aspects of experimental pain); postoperative pain; musculoskeletal pain (which includes conditions such as temporomandibular disorders, widespread pain, osteoarthritis, and pain after minor vehicle collision); and other clinical pain, including conditions such as chronic regional pain syndrome, pain in the major depressive disorder, pain in Parkinson disease, epigastric pain, endometriosis, burn injury pain, multiple sclerosis pain, vestibulodynia, labor pain, and sickle cell anemia pain. The HPGDB will be updated every 6 months after a similar procedure: initial screening by trained personnel and final decision on study inclusion by the pain researchers and clinicians C.B.M. and/or R.B.

2.2. Navigation of the Human Pain Genetics Database

The HPGDB is a variant-focused database intended to present as much information as possible about each entry without requiring additional navigation. When browsing the website, the user views a table that contains 7 columns (Supplementary Fig. 1, available online at http://links.lww.com/PAIN/A520). In the first column, "loci," users will find the genetic locus attributed to the entry-generating variant, according to the latest release of NCBI's Single Nucleotide Polymorphism Database (dbSNP Human Build 147, https://www.ncbi.nlm.nih.gov/SNP/).[65] Single Nucleotide Polymorphism Database assigns a variant to a gene if it is within 500 base pairs (bp) upstream or 2000 bp downstream of a gene, and it considers both plus and minus DNA strands. In addition, transcripts may overlap in dbSNP, and thus, a variant may be mapped to more than one genetic locus. Mousing over a specific locus will display its official name, chromosome, number of bp, and an image of its genetic architecture showing its exons in gray, the entry-generating variant in yellow, and other variants that were also included in the HPGDB within that gene in purple. The number of bp and all information shown on the genetic architecture image are unavailable for intergenic variants (those located beyond the limits for gene assignment by dbSNP), and for uncharacterized genetic loci, cases in which the "loci" column was left empty. Clicking on the locus will take the user to the NCBI's gene database (www.ncbi.nlm.nih.gov/gene/),[24] containing comprehensive information regarding that locus. By default, the HPGDB displays data sorted alphabetically by "loci," but users can choose to sort data by any column by clicking on its header. The second column, termed "suggested loci," will display the genetic locus attributed to that variant in the article.

The third column, "variants," indicates the reference ID number (rs number) of the variant underlying the entry. Mousing over the variant will display its chromosome, position, minor allele, and global minor allele frequency. In case of haplotypic associations, the entry at this column will read "haplotype," and clicking on the zoom icon to its left will display all the variants composing that haplotype. Clicking on the zoom icon to the left of the variants will open a new tab on the browser with additional relevant information from external resources, namely from the Combined Annotation Dependent Deletion (CADD, www.cadd.gs.washington.edu) database,[40] the Genome-Wide Repository of Associations between SNPs and phenotypes (GRASP, https://grasp.nhlbi.nih.gov/Overview.aspx),[33] the GWAS catalog of the NHGRI-EBI (http://www.ebi.ac.uk/gwas/),[44] as well as the Genotype-Tissue Expression (GTEx) project (www.gtexportal.org,[23] patched version 6) and a dorsal root ganglia (DRGs)

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expression quantitative trait loci (eQTLs) data set (https://humanpaingenetics.org/DRG-eQTLs/).[58] A direct link to each of these repositories is also provided. Details on the information available at each of these repositories are provided in the Methods section under the subheading "functional analyses of Human Pain Genetics Database variants."

The next column is "alleles," and it displays the allele associated with the investigated phenotype. In case of haplotypic associations, mousing over the allele will display the rs number and allele of every variant composing that haplotype.

The fifth column displays the "direction" of association and consists of either an upward or downward arrow to indicate the direction of association with the phenotype.

Next, the column "phenotype" displays the phenotype category for which the association was described, namely analgesia, cancer pain, fibromyalgia, migraine, neuraxial pain, neuropathic pain, nociception, postoperative pain, musculoskeletal pain, or other clinical pain. Mousing over the phenotype category will display, where relevant, more detailed information on the phenotype for which the association was described.

The last column ("publication") displays the first author's name and the year of the publication that generated each entry. Mousing over the publication will show its details (title, authors, journal, volume, issue, year, and abstract), and clicking on, it will take users to the dedicated publication page on PubMed.

At any point while navigating the HPGDB site, users may search the database by typing specific search terms into the search box in the top right.

An additional resource that allows for the visualization of multiple summary charts of the data included in the HPGDB is also available under the user interface (UI) element "charts." Users may select their gene or variant of interest under "results by gene" or "results by variant" to find how many studies from each phenotype category have found an association for that particular gene or variant, respectively. Under "results by phenotype," a graph displays the number of studies that have reported a genetic association for each phenotype category, and under "results by year," the number of studies per year is displayed. Additional options include "variants by functional effect," "phenotypes by gene," "phenotypes by variant," "genes by phenotype," and "variants by phenotype."

Furthermore, a "downloads" dialog box provides access to back-end data for direct, denormalized download in a variety of textual formats, including tab-delimited text (tsv), eXtensible Markup Language (XML), and JavaScript Object Notation (JSON). Next to the "downloads" is the "contact us" UI element. This function allows users to submit new association findings and to contact the website administrators about any issues or questions regarding the database. Finally, the "about the HPGDB" UI element allows users to see the date of the most recent update of the website's underlying data and information and version data for contributing external databases.

2.3. Replication of Human Pain Genetics Database variants

To validate the data included in our database, we tested the HPGDB variants for replication in the UK Biobank (UKBB), a large prospective multicenter study of people living in the United Kingdom that has recruited 503,325 individuals between 2006 and 2010 (UKBB application number 20802). Participants were 40 to 69 years old and lived less than 25 miles from a study center. Informed consent was obtained from all study participants, and their participation involved completing questionnaires, undergoing an interview with a trained nurse during which a range of physical measures were collected, and donating a sample of blood, urine, and saliva. Details of the study can be found elsewhere.[1,69] Our analyses were performed on the interim release of genotype data for 152,000 individuals. As distinct pathophysiological pathways are implicated in cancer vs noncancer pain (eg, factors related to cancer surgery, treatments, and/or tumor growth), only HPGDB variants associated with noncancer pain phenotypes were tested for replication in the UKBB. The replication analyses were performed using a case–control design, and 9 "case" groups were composed of participants self-reporting headache, facial pain, neck or shoulder pain, back pain, stomach or abdominal pain, hip pain, knee pain, pain all over the body, and/or neuropathic pain. As part of the UKBB data collection framework, participants were asked the following question on a touchscreen questionnaire: "In the last month, have you experienced any of the following that interfered with your usual activities?" (UKBB data-field 6159). Participants could choose all that apply from the following options: headache, facial pain, neck or shoulder pain, back pain, stomach or abdominal pain, hip pain, knee pain, pain all over the body, none of the above, and prefer not to answer. The choice of "pain all over the body" was exclusive, meaning that participants were not able to select additional specific body sites. For each pain site that participants indicated interfered with their usual activities in the last month, they were asked whether they had experienced that pain for more than 3 months. Those who chose "pain all over the body" were solely asked whether they had experienced pain all over their bodies for more than 3 months. Thus, aside from the neuropathic pain group, case groups were composed of individuals self-reporting pain that interfered with their usual activities in the last month and that had been present for more than 3 months. Sample sizes were headache (UKBB data-field 3799, n = 13,456), facial pain (4067, n = 1320), neck or shoulder pain (3404, n = 24,388), back pain (3571, n = 26,973), stomach or abdominal pain (3741, n = 7330), hip pain (3414, n = 13,461), knee pain (3773, n = 25,862), and pain all over the body (2956, n = 2173). We also generated an additional quantitative trait ranging from 1 to 8 to track the number of sites reported as painful for more than 3 months. Those reporting to have "pain all over the body" were assigned the maximum score of 8.

For the construction of the neuropathic pain phenotype, a different item of the UKBB touchscreen questionnaire was used: data-field 20,002. In this field, participants were asked: "Has a doctor ever told you that you have had any of the following conditions?," and they could select all that apply from a list of options that included heart attack, angina, stroke, high blood pressure, blood clot in the leg, blood clot in the lung, emphysema/chronic bronchitis, asthma, and hay fever or allergic rhinitis or eczema. They were instructed to choose "none of the above" if they did not know or were not sure whether they had had any of the listed conditions. Participants who chose "none of the above" were asked to describe their condition(s) to a trained nurse, who then assigned a UKBB disease code. We thus composed the neuropathic pain case group (n = 3924) with individuals describing conditions to which a trained nurse assigned one of the following UKBB disease codes: peripheral neuropathy (code 1255), diabetic neuropathy/ulcers (1468), shingles (1573), trigeminal neuralgia (1523), sciatica (1476), spinal stenosis (1536), peripheral nerve injury (1394), trapped/compressed nerve (1257), prolapsed/slipped disc (1312), varicella zoster virus (1674), or peripheral nerve disorder (1254).

All pain phenotypes, except for the number of pain sites, were considered as binary traits (presence or absence). For the association studies, all groups were contrasted against the same

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control group, composed of individuals answering "none of the above" to the question: "In the last month, have you experienced any of the following that interfered with your usual activities?" (UKBB data-field 6159, n = 59,504).

Association tests were run for 408 unique HPGDB variants available in the UKBB on the sample defined by the UKBB as whites, and included age, sex, and genotyping platform (AxiomUK or UKBileve) as covariates. Association with imputed genotyping (dosage) data was performed with the "expected" method of SNPTEST, under the additive assumption for mode of inheritance. UK Biobank data were prepared with qctool v1.4 (www.well.ox.ac.uk/~gav/qctool/), and association tests performed using SNPTEST, v.2.5.2.[48] Because research suggests that pain phenotypes are complex heritable traits of polygenic origin,[84] association test was not considered independent. Thus, correction for multiple testing was performed using false discovery rate (FDR) to limit the inclusion of false positive findings in our results.[12] Increasing levels of stringency that are normally used in large scale genetic studies (ie, 20%, 10%, and 5%[15,81,83]) were systematically applied to all results.

We also plotted the cumulative distribution of GWAS *P* values for the set of noncancer pain variants found in the HPGDB for each of the pain conditions in the UKBB. A one-tailed Kolmogorov–Smirnov test was used to assess enrichment for lower *P* values. Null distributions of GWAS *P* values were obtained with 10 sets of randomly matched variants generated by the SNPsnap web server (www.data.broadinstitute.org/mpg/snpsnap).[60] Random variants were matched for minor allele frequency (MAF), number of variants in linkage disequilibrium (LD), distance to the nearest gene, and gene density. We extracted 10 randomly matched sets and defined the mean set as their union.

All QQ plots and cumulative distribution function plots were made with the R statistical package.[73]

2.4. Pleiotropy of Human Pain Genetics Database variants

We first investigated pleiotropy within the UKBB pain phenotypes. For that, we calculated the correlation of the association strength of each HPGDB variant between all possible pairs of binary pain phenotypes that we obtained from the UKBB data set (headache, facial pain, neck or shoulder pain, back pain, stomach or abdominal pain, hip pain, knee pain, pain all over the body, and neuropathic pain). For each pair, we built an exclusion list with individuals presenting both phenotypes simultaneously. We then ran association tests between the HPGDB variants and each phenotype separately, while removing from the case group individuals on the exclusion list. For each variant, we combined the effect size (beta) and *P* value of the phenotype association into a pi-value, defined as $\text{pi} = -\text{beta} \times \log_{10}(P\ \text{value})$.[82] The pi-value can be interpreted as a *P* value–weighted effect size. Hence, for each pair of phenotypes, a variant has 2 pi-values: one pi for the association with the first phenotype and one for the association with the second phenotype. We then performed linear regression to assess the correlation between pi-values of a given pair of phenotypes. Regression slope, *P* value, and the percent of variance explained (r^2) were reported. Outlier pi-values (±2 SDs) were then removed and regressions were run a second time. Given the non–Gaussian distribution of pi-values, Spearman (rank) correlations between the pi-values of each pair of phenotypes were run, and correlation coefficients and *P* values were reported.

We also assessed whether the HPGDB data set is enriched for pleiotropic variants by investigating whether they were associated with other nonpain phenotypes. We used GRASP[33] and the GWAS Catalog of the NHGRI-EBI,[44] 2 extensively annotated databases of significant GWAS association results between alleles and phenotypes ($P \leq 0.05$). These catalogs provide for a wealth of information on human conditions: GRASP contains more than 8.8 million entries of genetic associations with more than 186,000 unique phenotypes, whereas NHGRI-EBI has 19,000 entries with 1249 different phenotypes. We extracted the *P* values of all associations between noncancer pain HPGDB variants and phenotypes reported in these databases. Then, we plotted each of these association *P* values (observed *P* values) against the expected *P* values, given the number of HPGDB variant–phenotype pairs present in each of the databases in a QQ plot displaying increasingly stringent levels of FDR correction (ie, 20%, 10%, and 5%).

2.5. Functional analyses of Human Pain Genetics Database variants

To determine the extent of regulatory effects of the HPGDB data set, we investigated its enrichment for *cis*-eQTL, variants that affect local gene expression. We considered an eQTL to be *cis*-acting when the distance separating it from the transcription start site of the associated gene was ≤200,000 nucleotides.[58] Five different tissues were used as sources for gene-level *cis*-eQTL analysis: anterior cingulate cortex (n = 72), frontal cortex (n = 92), tibial nerve (n = 256), and whole blood (n = 338) available through the GTEx Project,[23] and DRG (n = 214) available through the DRG-eQTL data set.[58] In QQ plots displaying increasingly stringent levels of FDR correction (ie, 20%, 10%, and 5%), we plotted the log-transformed observed association *P* values for each noncancer pain HPGDB variant–gene pair available in the abovementioned tissues against the log-transformed expected *P* value, given the number of variant–gene pairs available in each tissue. To identify genes whose expressions are consistently regulated by HPGDB *cis*-eQTL, we combined the absolute pi-values of a given eQTL in each tissue. *P* values for the combined pi-values were estimated from fitted negative exponential distribution on all summed values and then corrected for FDR at increasing levels of stringency (ie, 20%, 10%, and 5%).

As an additional indication of functional effects among HPGDB variants, we assessed their enrichment for high C-scores (a measure that strongly and positively correlates with deleteriousness) and location in open chromatin regions. For that, we used functional genomic annotation data available through CADD[40] version 1.3, specifically deleteriousness (Column 116—variable "Phred-scaled C-score") and evidence for open chromatin (Column 61—variable "EncOCCombPVal"). Combined Annotation Dependent Deletion estimates deleteriousness based on functional genomic annotation data available through 63 different sources that are combined to produce a C-score. We used Phred-scaled C-scores, which range from 1 to 99 based on the rank of each variant relative to all possible substitutions in the human genome. Variants in the top 10% of C-scores were assigned to a C-score of 10, the top 1% to a C-score of 20, the top 0.1% to a C-score of 30, and so on. Phred-scaled C-scores were plotted in a cumulative distribution plot. Evidence for open chromatin derives from a variety of selected experimental procedures that probe for DNA-encoded regulatory regions in the ENCODE project,[22] FAIRE- and DNase-Seq,[67] as well as ChIP-seq data from CTCF,[11] MYC, and polymerase II.[22] Phred-scaled *P* values were also plotted in a cumulative distribution plot. Enrichment of variants for these functionalities (higher C-scores and location in regions of open chromatin) was assessed with

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a one-tailed Kolmogorov–Smirnov test, explicitly testing for enrichment of greater Phred. Null distributions of evidence were obtained using randomly matched variants generated by the SNPsnap web server.[60] Matching parameters were the same as described previously.

Lastly, we conducted gene pathway analysis to identify whether HPGDB variants admitting successful replication in at least one UKBB pain phenotype (FDR 20%) would suggest the implication of particular biological pathway(s). Genes were assigned to each HPGDB variant that admitted replication according to the following rules: variants within a genetic locus were assigned to the gene containing them; variants within more than one genetic locus were attributed to each gene containing them; and variants not intersecting with any genetic locus were assigned to the closest gene in nucleotide distance to them. The set of unique genes attributed to each variant that admitted replication was then used for gene pathway analyses. The online version of the PANTHER classification system[51] was used, and we tested for statistical overrepresentation of genes within pathways. To reduce redundancy between closely related pathways, a trimmed version of Gene Ontology's (GO) biological processes[5] was used including only pathways with at most 100 genes and with a maximum of 50% gene redundancy among them. As reduced redundancy among pathways can be considered as independent tests, *P* values were corrected using the Bonferroni procedure.

3. Results

3.1. Human Pain Genetics Database summary statistics

The HPGDB currently includes 925 entries from 294 studies published in the last 16 years, with results on 434 unique variants across 155 unique genetic loci and 22 chromosomes. A flow chart describing the process of identification of all publications included in the HPGDB is shown in Supplementary Figure 2 (available online at http://links.lww.com/PAIN/A520). The field of human pain genetics started expanding rapidly shortly after the completion of the Human Genome Project in 2003[19,20] and generally showed a steep growth (Supplementary Fig. 3, available online at http://links.lww.com/PAIN/A520). A decline in this growth since 2014 may be attributed to the rise of pressure on the field of human genetics for GWAS rather than candidate gene studies. Despite the fact that GWASs are time consuming and expensive because they require large sample sizes for sufficient statistical power, we expect an imminent increase in the number of such publications in the field of human pain genetics.

Underscoring the need for a centralized database hosting up-to-date pain genetics data, the HPGDB shows minimal overlap with existing databases. The overlap with GRASP and NHGRI-EBI is 0.5% and 1%, respectively, primarily because these databases are focused on GWAS rather than candidate gene association studies, which make up most studies reporting variants linked to pain states.

Because the current knowledge on the genetic contributions to the experience of human pain derives mainly from candidate gene studies, it was not surprising that gene-encoding molecules known to be involved in pain processing, namely the μ-opioid receptor 1 (*OPRM1*) and the catechol-O-methyltransferase (*COMT*) genes, were by far the ones most frequently reported to be associated with a pain phenotype (31.3% and 30% of the studies included in the HPGDB, respectively) (**Fig. 1A**). Reports of associations between pain phenotypes and the methylenetetrahydrofolate reductase (*MTHR*), tumor necrosis factor (*TNF*), GTP cyclohydrolase 1 (*GCH1*), estrogen receptor alpha (*ESR1*), ATP-binding cassette subfamily B member 1 (*ABCB1*), and sodium voltage-gated channel alpha subunit 9 (*SCN9A*) genes were less numerous, accounting for 5% to 9% of the studies included in the HPGDB only. Genes for which a genetic association was reported in less than 1% of the studies are listed in Supplementary Table 1 (available online at http://links.lww.com/PAIN/A520).

In addition, we found that the most investigated variants were the nonsynonymous SNPs rs4680 (22.6% of the studies included in the HPGDB) and rs1799971 (22.2%) (**Fig. 1B**) situated within the loci of *COMT* and *OPRM1*, respectively. Nonsynonymous SNPs result in an amino acid change in the protein sequence and are thus expected to have consequences on protein function, likely explaining why research interest in these variants is high. *COMT* SNP rs4680, commonly known as val158met, encodes a valine-to-methionine amino acid change that results in an enzyme with lower stability.[46] The next 3 most investigated variants, rs4818 (11%), rs4633 (11%), and rs6269 (9%), are also in *COMT*. Together with SNP rs4680, these 3 variants form

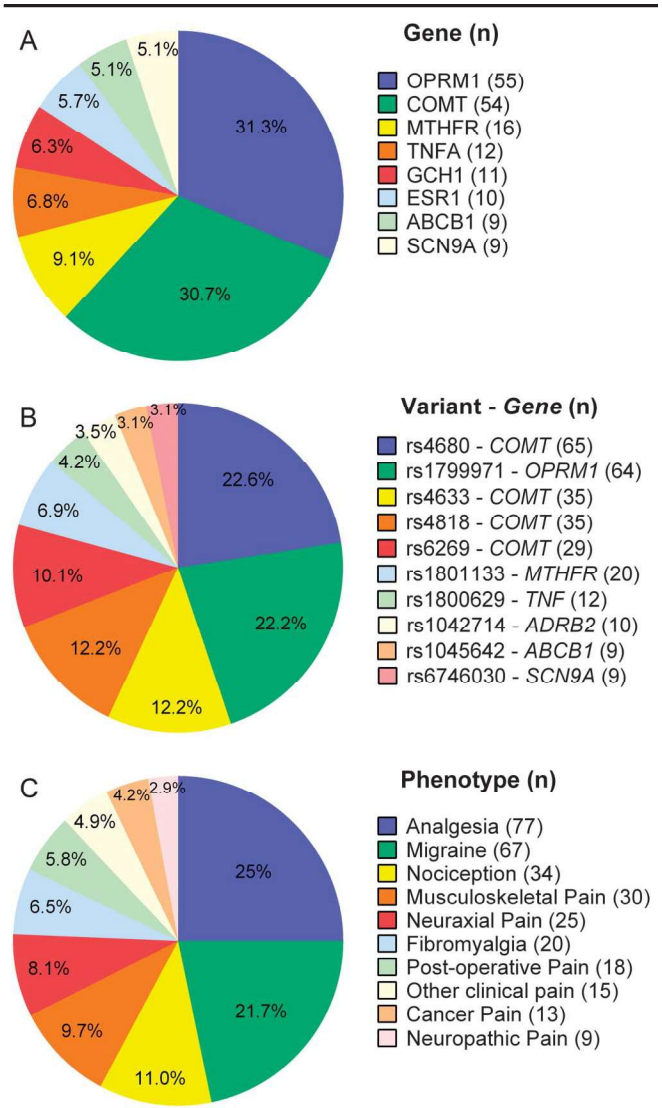

Figure 1. Pie charts of the genes, variants, and phenotypes included in the Human Pain Genetics Database (HPGDB). (A) Genes with highest frequency of report of associations with pain phenotypes (at least 1%); (B) variants with highest frequency of report of associations with pain phenotypes (at least 1%); and (C) phenotype categories by frequency of reporting.

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haplotypes that strongly affect protein translation and enzymatic activity[53] and are associated with different levels of pain.[30] SNP rs1799971, commonly referred to by its transcript nucleotide change, A118G, causes an aspartic acid-to-asparagine change that results in multiple functional effects,[14,56,57] of which a 3-fold increase in the affinity of the receptor for β-endorphin, associated with the minor G allele, is of notable importance.[14] Reports of associations between pain phenotypes and other variants ranged from 3% to 12% of HPGDB studies (**Fig. 1B**). Variants for which a genetic association has been reported in less than 1% of the studies included in the HPGDB are listed in Supplementary Table 2 (available online at http://links.lww.com/PAIN/A520).

As for the phenotype categories applied to the HPGDB (**Fig. 1C**), analgesia is the one for which genetic associations were most frequently reported (25% of studies included in the HPGDB), which was clearly driven by investigations concerning opioid analgesia (72 of 77 investigations). There is generally a great interest in the ability to explain the wide interindividual variability in both responses to opioids and their dose requirements.[6] The hope is that genetic studies will contribute considerably to the development of a tool that will allow for the prediction of the most effective drug and dose choices at the individual level.

Importantly, when combined, clinical pain conditions (migraine, musculoskeletal pain, neuraxial pain, fibromyalgia, postoperative pain, cancer pain, neuropathic pain, and other clinical pain) have clearly been the focus of most investigations, roughly 64% of studies included in the HPGDB. Of those, 21.7% refers to genetic contributions to migraine, with half the number of such reports for nociception (11%) and musculoskeletal pain (9.7%). Thus, the migraine field is noticeably leading human pain genetics in producing not only the largest number of relevant reports, but also almost all available GWAS. Genetic associations contributing to other phenotype categories (neuraxial pain, fibromyalgia, postoperative pain, cancer pain, neuropathic pain, and other clinical pain) were reported in 3% to 8% of studies included in the HPGDB (**Fig. 1C**).

3.2. Replication of Human Pain Genetics Database variants

Replication is the process by which genetic association results are validated.[18] Here, we used a highly powered study that has collected pain phenotypes to validate the data set included in the HPGDB. Specifically, we investigated the association between HPGDB variants and 10 pain phenotypes (headache, facial pain, neck or shoulder pain, back pain, stomach or abdominal pain, hip pain, knee pain, pain all over the body, number of pain sites, and neuropathic pain), using as control individuals who reported not to have any pain. In total, we tested 408 unique variants included in the HPGDB for their associations with noncancer pain phenotypes for replication in each UKBB pain phenotype. Variants included in the HPGDB for being associated with cancer pain are listed in Supplementary Table 3 (available online at http://links.lww.com/PAIN/A520). Noticeably, 38% of noncancer pain HPGDB variants contributed to at least one of the UKBB pain phenotypes, with 155 unique variants passing an FDR threshold of 20% at least once, more than half of those (94) passing a threshold twice as low (FDR 10%) at least once, and 70 surviving a much more stringent cutoff at 5% at least once (Supplementary Table 4, available online at http://links.lww.com/PAIN/A520). Of the 155 unique variants that admitted at least one successful replication at FDR 20%, 109 were deposited into the HPGDB for their exclusive association with clinical pain phenotypes, 21 with analgesia-related phenotypes, 13 with nociception-related phenotypes, and 12 for their associations with 2 or more of the aforementioned subgroups of phenotypes (Supplementary Table 4, available online at http://links.lww.com/PAIN/A520).

Proportionally, most variants replicated at FDR 20% were originally identified by candidate gene studies investigating nociception-related phenotypes (22 of 49 or 44.9%), followed by studies investigating clinical pain phenotypes (120 of 306 or 39.2%) and then by candidate gene studies investigating analgesia-related phenotypes (29 of 89 or 32.6%). Of note, after dividing the variants included in the HPGDB for their associations with clinical pain phenotypes into those identified in candidate gene studies and GWAS, only the latter group exhibited replication of most variants (19 of 36 or 52.8%), with those identified in candidate gene studies being 101 of 270 or 37.4%. Supplementary Table 6 (available online at http://links.lww.com/PAIN/A520) lists all variants included in the HPGDB for their associations with clinical pain phenotypes with the number of times each variant admitted successful replication at FDR 20%, 10%, or 5% in the pain phenotypes collected in the UKBB. Supplementary Table 7 (available online at http://links.lww.com/PAIN/A520) lists all variants included in the HPGDB for their associations with analgesia-related or nociception-related phenotypes with the number of times each variant admitted successful replication at FDR 20%, 10%, or 5% in the pain phenotypes collected in the UKBB. It should be noted that lack of replication of any HPGDB variant in the UKBB pain phenotypes investigated here does not necessarily contradict the published associations, possibly because there are considerable differences between the phenotypes of the original studies included in the HPGDB and the phenotypes of the replication cohort.

With regards to specific UKBB pain phenotypes, the count of minor alleles of a sizable number of HPGDB variants contributed to the number of pain sites reported by participants of the UKBB (**Fig. 2**). Association QQ plots show the observed distribution of log-transformed association *P* values (y-axis) against the expected log-transformed *P* values, given the number of variants being tested (x-axis). Any deviation from the x = y line implies a consistent difference between cases and controls across the variants being tested. As shown in **Figure 2**, a total of 113 associations corresponding to 99 of 408 unique variants passed an FDR threshold of 20% for the association with the number of pain sites, more than half of those (54) passed a threshold twice as low (FDR 10%), and 33 survived a much more stringent cutoff at 5% (Supplementary Tables 8 and 9, available online at http://links.lww.com/PAIN/A520). These variants are distributed across 65 unique genetic loci, with the most commonly implicated ones being *GCH1* (number of variants replicated in the number of pain sites = 7) and opioid receptor delta 1 (*OPRD1*, n = 7), followed by *SCN9A* (n = 5), adenosine deaminase RNA-specific B2 (*ADARB2*, n = 4), and *COMT* (n = 3) (Supplementary Table 10, available online at http://links.lww.com/PAIN/A520).

Proportionally, most variants replicated were originally identified in migraine GWAS (14 of 36 or 39%, FDR 20%, Supplementary Table 7, available online at http://links.lww.com/PAIN/A520), followed by variants identified by candidate gene studies investigating experimental aspects of pain (15 of 49 or 31%, FDR 20%, Supplementary Table 8, available online at http://links.lww.com/PAIN/A520) and finally followed by variants identified by candidate gene studies investigating clinical pain (68 of 270 or 25%, FDR 20%, Supplementary Table 7, available online at http://links.lww.com/PAIN/A520).

Several HPGDB variants also admitted successful replication at an FDR rate of 20%, 10%, and 5% in back pain, knee pain,

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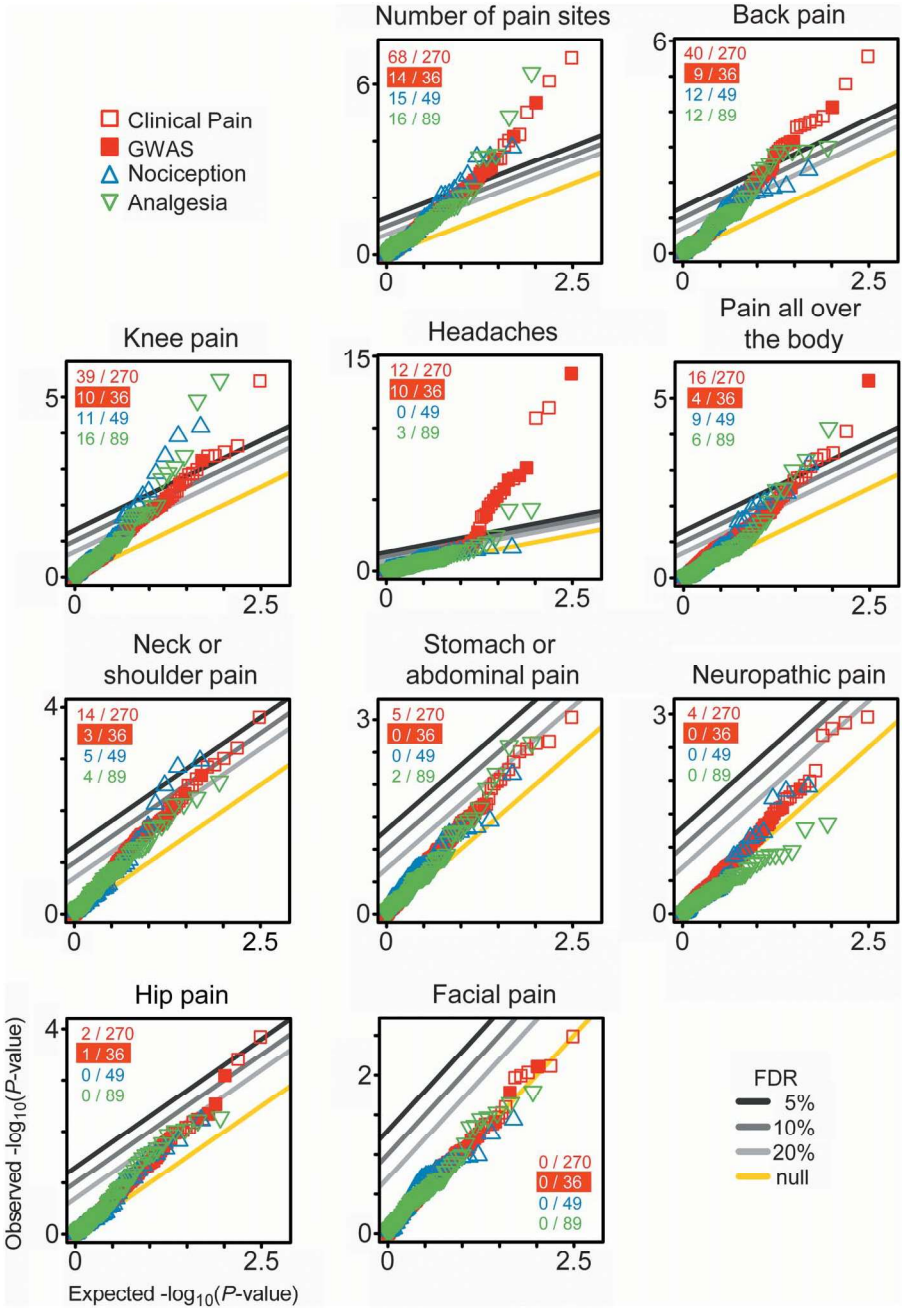

Figure 2. QQ plots for Human Pain Genetics Database (HPGDB) variants replicated in pain phenotypes of the UK Biobank (UKBB). Observed *P* values of association between HPGDB variants and pain phenotypes extracted from the UK Biobank were compared against expected *P* values. Variants are organized in 3 groups, according to the originally reported association in the HPGDB: clinical pain (red square), nociception (blue triangle pointing up), and analgesia (green triangle pointing down). Variants originally identified by genome-wide association study (GWAS) are shown as a red-filled square. Ratios are number of variants passing an false discovery rate (FDR) threshold of 20% relative to the total number of variants in the respective group.

headaches, pain all over the body, and neck or shoulder pain but very few in stomach or abdominal, neuropathic, and hip pain, and none in facial pain (Supplementary Tables 11–20, available online at http://links.lww.com/PAIN/A520). Genes most commonly implicated in each of the aforementioned UKBB pain phenotypes are shown in Supplementary Tables 21–28 (available online at http://links.lww.com/PAIN/A520).

As shown in **Figure 2**, the highest proportion of variants replicated in the headache group also derived from original associations reported in migraine GWAS (10 of 38 or 26%, FDR 20%). Interestingly, the same pattern is observed in the groups reporting knee (10 of 38 or 26%, FDR 20%) and back pain (9 of 38 or 23.7%, FDR 20%). In the latter group, the proportion of replicated variants originally identified in migraine GWAS was virtually the same as that of variants originally identified in nociception studies (12 of 49 or 24.5%, FDR 20%). Variants derived from candidate gene studies of nociception were also the largest proportioned majority of replications in the group reporting neck and shoulder pain (5 of 49 or 10%, FDR 20%).

When compared with a random set of variants matched for MAF, number of variants in LD, distance to the nearest gene, and gene density in a cumulative distribution plot, HPGDB variants

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associated with headaches, knee, stomach or abdominal, and neck or shoulder pain in the UKBB showed statistically significant enrichment for lower *P* values (**Table 1**). To assess whether this enrichment was driven by GWAS-originated variants, the cumulative distribution of association *P* values was plotted a second time for headaches, excluding all migraine GWAS variants. After this exclusion, the enrichment for lower *P* values remained significant ($P = 0.024$). The lower *P* value may be attributed to the smaller sample size. Enrichment for low *P* values among HPGDB variants associated with hip pain, number of pain sites, and back pain approached significance, whereas no enrichment was observed for neuropathic pain, facial pain, and pain all over the body (**Table 1**).

In total, 87 unique genes contributed to all UKBB pain phenotypes, with the most commonly implicated ones being the cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*), *ESR1*, and the potassium voltage-gated channel subfamily J member 6 (*KCNJ6*), each contributing to 6 UKBB pain phenotypes (Supplementary Table 29, available online at http://links.lww.com/PAIN/A520).

3.3. Pleiotropy of Human Pain Genetics Database variants

We also investigated a potentially shared genetic basis between different UKBB pain phenotypes that are associated with HPGDB variants, and learned that the variance explained by HPGDB variants pi-values ranged from 3% for the hip and neck or shoulder pain pair to 81% for the back and knee pain pair (**Table 2**). Such robust effects were largely driven by a group of 77 variants whose pi-values exceeded the others within the same phenotype by at least 2 SDs (Supplementary Tables 30–44, available online at http://links.lww.com/PAIN/A520). The top 3 variants exhibiting statistical significance and biological relevance across all 15 pairs of UKBB pain phenotypes tested were rs673, in the promoter region of *TNF*; rs28371759, a missense variant in *CYP3A4*; and rs28445017, downstream of the NFKB inhibitor–like 1 (*NFKBIL1*). Importantly, *TNF*'s rs673 and *NFKBIL1*'s rs28445017 are situated approximately 15 kb from each other and are in full LD, so that the minor allele of *TNF*'s rs673, whose frequency is roughly half of that of *NFKBIL1*'s rs28445017, is always inherited with *NFKBIL1*'s rs28445017 minor allele ($D' = 1$). All other variants exhibited strong effects in 1 to 10 of 30 of the pain phenotypes investigated and are listed in Supplementary Table 45 (available online at http://links.lww.com/PAIN/A520).

As these variants display strong individual effects, possibly inflating Pearson's correlation r^2 values or the percent of variance explained, we have refitted regression lines into the association pi-values while removing the variants indicated in Supplementary Tables 21–35 from the regression models (available online at http://links.lww.com/PAIN/A520). Effects remained significant in 10 out of 14 models, but the range of the percent of variance explained dropped substantially to 3 to 19% (**Table 2**). We then assessed rank correlations between the remaining variants of each phenotype pair because rank-based correlations are assumption-free with regards to the distribution of the data. Importantly, rank correlations were significant in all pairs of phenotypes tested, and correlation coefficients were moderate even after removing the variants with the strongest effects for back and knee pain, back pain and pain all over the body, pain all over the body and knee pain, pain all over the body and neck or shoulder pain, and knee and neck or shoulder pain (**Table 2**). Together, these results suggest that HPGDB SNPs contribute to a shared genetic basis across different pain conditions, and we have identified many variants with especially strong contributions.

Single nucleotide polymorphism pleiotropy was also analyzed by investigating whether HPGDB variants are associated with nonprimarily painful phenotypes in NHGRI-EBI or GRASP. In total, 20 HPGDB variants were cataloged in NHGRI-EBI (Supplementary Table 46, available online at http://links.lww.com/PAIN/A520) with reported associations with 30 unique nonprimarily painful phenotypes. Genome-Wide Repository of Associations between SNPs and phenotypes included 273 HPGDB variants (Supplementary Table 47, available online at http://links.lww.com/PAIN/A520) with reported associations with 634 unique nonprimarily painful-related phenotypes. Pleiotropy QQ plots (**Fig. 3**) display the observed distribution of log-transformed association *P* values for each HPGDB variant–phenotype pair (x-axis) against the expected distribution of log-transformed association *P* values distribution (y-axis), given the number of pairs being tested. Because of the nature of both databases, which catalog only statistically significant associations ($P \leq 0.05$), it was expected that each report of association would survive an FDR threshold of 5%. Nonetheless, the strength of the association of many HPGDB variants with nonprimarily painful phenotypes in NHGRI-EBI and GRASP is remarkable, with very low observed association *P* values in NHGRI-EBI (as low as 10E-150) and in GRASP (as low as 10E-200), indicating that HPGDB variants are also implicated in other nonpainful diseases. It is noteworthy that the same variant (rs3024504, downstream of the interleukin 10 gene, *IL10*) exhibited the greatest number of pleiotropic associations both in NHGRI-EBI (n = 7, Supplementary Table 37, available online at http://links.lww.com/PAIN/A520) and in GRASP (n = 112, Supplementary Table 38, available online at http://links.lww.com/PAIN/A520). In NHGRI-EBI, the 3 nonprimarily painful phenotypes most commonly associated with HPGDB variants were coronary heart disease, asthma, and ulcerative colitis (Supplementary Table 48, available online at http://links.lww.com/PAIN/A520). In GRASP, the 5 nonprimarily painful phenotypes most commonly associated with HPGDB variants were LDL cholesterol, total cholesterol, height, age-related macular degeneration, and systolic blood pressure (Supplementary Table 49, available online at http://links.lww.com/PAIN/A520).

Table 1

Enrichment for lower association *P* values in pain phenotypes collected in the UK Biobank (UKBB).

Phenotype	*P*
Headache	0.00056
Knee pain	0.0081
Stomach or abdominal pain	0.0082
Neck or shoulder pain	0.029
Hip pain	0.052
No. of pain sites	0.059
Back pain	0.052
Facial pain	0.13
Pain all over the body	0.14
Neuropathic pain	0.25

Enrichment of single nucleotide polymorphism (SNPs) with lower *P* values assessed with a one-tailed Kolmogorov–Smirnov test.

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Table 2

Correlation of UK Biobank (UKBB) pain phenotypes explained by Human Pain Genetics Database (HPGDB) variants.

Pain condition 1	Pain condition 2	All HPGDB variants			All but statistical outliers			Rank correlations*	
		r^2	Slope	*P*	r^2	Slope	*P*	*r*	*P*
Back pain	Knee pain	0.81	0.57	1.80E-158	0.12	0.28	<0.0001	0.49	<0.0001
Back pain	Pain all over the body	0.75	1.39	2.56E-132	0.19	0.92	<0.0001	0.52	<0.0001
Pain all over the body	Knee pain	0.67	0.38	6.60E-105	0.16	0.14	<0.0001	0.48	<0.0001
Pain all over the body	Neck or shoulder pain	0.51	0.15	4.98E-70	0.11	0.09	<0.0001	0.45	<0.0001
Knee pain	Neck or shoulder pain	0.51	0.24	5.55E-70	0.09	0.19	<0.0001	0.40	<0.0001
Pain all over the body	Hip pain	0.46	0.16	4.43E-59	0.03	0.05	0.0005	0.21	<0.0001
Facial pain	Neuropathic pain	0.08	0.03	1.98E-09	0.04	0.08	<0.0001	0.26	<0.0001
Pain all over the body	Neuropathic pain	0.08	0.04	5.94E-09	<0.01	0.01	ns	—	—
Facial pain	Pain all over the body	0.06	0.13	4.19E-07	<0.01	−0.06	ns	—	—
Knee pain	Neuropathic pain	0.06	0.10	5.05E-07	0.03	0.12	0.0011	0.22	<0.0001
Back pain	Stomach or abdominal pain	0.06	−0.26	6.78E-07	0.07	0.24	<0.0001	0.18	0.0001
Hip pain	Knee pain	0.05	0.39	3.63E-06	0.04	0.28	<0.0001	0.36	<0.0001
Back pain	Neuropathic pain	0.03	0.45	1.82E-04	0.02	0.18	ns	—	—
Back pain	Facial pain	0.03	1.07	5.31E-04	<0.01	−0.01	ns	—	—
Hip pain	Neck or shoulder pain	0.03	0.21	9.21E-04	<0.01	0.08	ns	—	—

Percent variances explained (r^2), regression slopes, and *P* values were estimated using linear regression. Only significant regression models at Bonferroni threshold of 0.0014 are displayed; statistical outliers were determined by ±2 SDs of the mean effect values.

* Spearman rank correlations were run only for regression models that remained significant after removal of the statistical outliers and did not include them.

ns, not significant.

3.4. Functional analyses of Human Pain Genetics Database variants

Understanding the role of regulatory variants and knowing the tissues in which they are active is essential for the functional characterization of genetic variants and insights into disease pathophysiology. With this in mind, we assessed the HPGDB data set for enrichment of variants that modify local gene expression (ie, *cis*-eQTL) in tissues that are relevant to pain. expression quantitative trait locus QQ plots (**Fig. 4**) show the deviation of the observed log-transformed association *P* values for each HPGDB variant–gene pair (y axis) from the expected distribution, given the number of pairs being tested (x-axis). These plots reveal that a substantial number of HPGDB variants are significantly associated with modification in the expression of neighboring genes. In other words, many HPGDB variants are *cis*-eQTL in tissues that are relevant to pain, particularly in the nerve and in whole blood[70] (**Fig. 4**). Combined analysis revealed that the genes under strongest regulation of HPGDB *cis*-QTL are *GCH1*, interleukin 10 receptor subunit beta (*IL10RB*), elaC ribonuclease Z 2 (*ELAC2*), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), and zinc finger protein 555 (*ZNF555*). This analysis also revealed that a substantial number of HPGDB *cis*-QTL regulate the expression of genes that are immediate neighbors to those described in the original

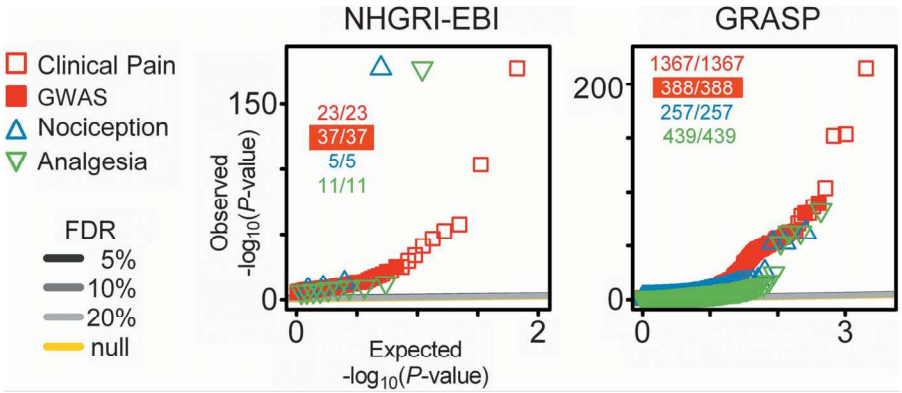

Figure 3. Human Pain Genetics Database (HPGDB) variants pleiotropy. QQ plots display variants replicated in the (A) NHGRI-EBI and (B) Genome-Wide Repository of Associations between SNPs and phenotypes (GRASP) databases. Observed *P*-values of association between HPGDB variants and various phenotypes in Genome-Wide Repository of Associations between SNPs and phenotypes or NHGRI were compared against expected *P*-values. Variants are organized in 3 groups, according to original reported association in the HPGDB: clinical pain (red square), nociception (blue triangle pointing up), and analgesia (green triangle pointing down). Variants originally identified by genome-wide association study (GWAS) are shown as a red-filled square. Ratios are number of variants passing an false discovery rate (FDR) threshold of 20% relative to the total number of variants in the respective group.

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publication that generated the entry of the variant in the HPGDB (Supplementary Table 50, available online at http://links.lww.com/PAIN/A520).

To gather additional evidence for enrichment of functional variants in HPGDB, we estimated their predicted deleteriousness and colocalization with open chromatin. The cumulative distribution track of HPGDB variant C-scores showed a robust shift to the right compared with that of a randomly selected set of matching variants (**Fig. 5A**). The CADD database suggests Phred-scaled C-scores between 10 and 20 as arbitrary cutoffs to identify potentially pathogenic variants, and our analysis indicated that there are roughly 20% of HPGDB variants with a C-score above or equal to 10 and only around 5% of such variants among the random set of matching variants. Likewise, there are roughly 5% of HPGDB variants with a C-score above 20, whereas no such variants exist among the random set of matching variants. Finally, we also obtained evidence that HPGDB variants are enriched in regions of open chromatin (**Fig. 5B**), the location of many types of active human regulatory DNA elements.[54]

Lastly, gene pathway analysis was performed on a set of 87 unique genes attributed to HPGDB variants that admitted replication (FDR 20%) in at least one UKBB pain phenotype (Supplementary Table 51, available online at http://links.lww.com/PAIN/A520). This analysis revealed that 6 pathways are significantly overrepresented after Bonferroni correction: sensory perception of pain (fold-enrichment = 29.25, Bonferroni-corrected P = 2.0E-13), regulation of neurological system process (23.93, P = 9.4E-11), regulation of amine transport (22.47, P = 3.4E-09), negative regulation of blood pressure (27.83, P = 9.8E-08), receptor metabolic process (14.24, P = 4.6E-06), and gland morphogenesis (10.07, P = 1.5E-04) (Supplementary Table 52, available online at http://links.lww.com/PAIN/A520).

4. Discussion

The HPGDB is introduced as a curated resource for researchers interested in genetic contributions to the human experience of pain. We showed here that HPGDB provides researchers not only with a convenient tool summarizing human pain genetics but also with insights into the pathophysiology of chronic pain conditions and, ultimately, new treatment alternatives.

Most human pain genetics data produced thus far derives from candidate gene studies. Debates on the validity of this approach have carried on during the past decades because they are susceptible to the confounding effects of population stratification, among others.[75] Since the publication of a landmark GWAS was 10 years ago,[79] the pain field has been slowly moving into this data-driven method of identifying disease-related variants. These findings should provide dependable information on the genetics of pain because these designs are not biased by any prevailing assumptions. Indeed, across all UKBB pain phenotypes investigated, the proportioned majority of replicated variants derived from migraine GWAS, reiterating the dependability of these findings. Nonetheless, many variants originating from candidate gene studies were also successfully replicated in the UKBB, associated with alternative phenotypes and demonstrated an increased probability of functionality. Thus, candidate gene studies still represent valuable resources to deepen our comprehension of the complex genetic regulatory networks that are connected to illness.

The variants for which a significant association with a pain-related phenotype have most been reported are *COMT*'s rs4680 and *OPRM1*'s rs1799971. The interest in these variants did not emerge from unbiased whole-genome studies but has certainly unveiled important aspects of their role in pain pathophysiology that have been previously reviewed.[72,78] In addition to *COMT* and *OPRM1*, *MTHFR* also figures among the most frequently studied

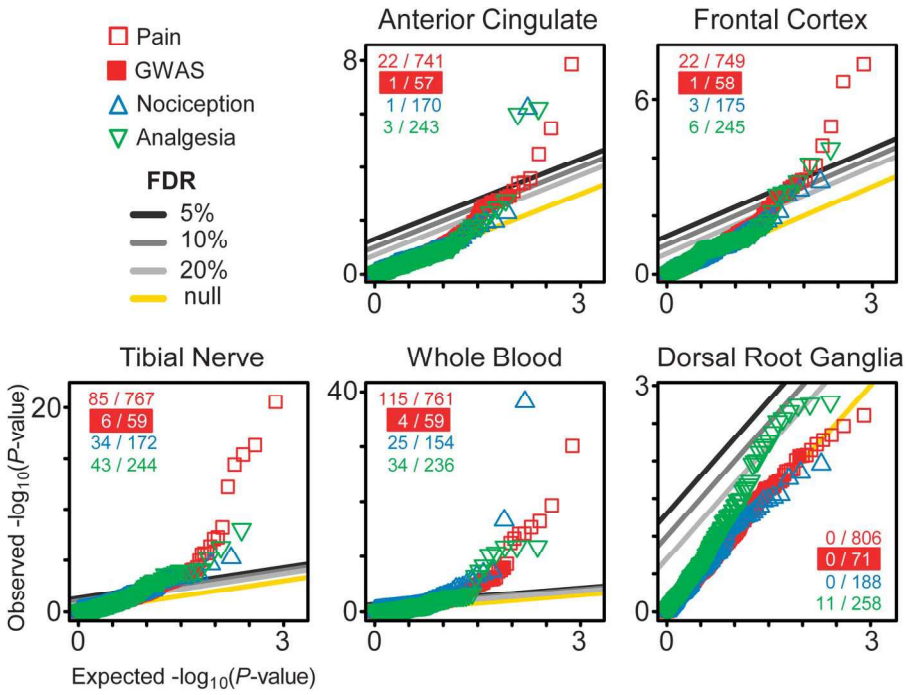

Figure 4. QQ plots of Human Pain Genetics Database (HPGDB) expression quantitative trait loci (eQTLs) in different tissues. Variants are organized in 3 groups, according to the originally reported association in the Human Pain Genetics Database: clinical pain (red square), nociception (blue triangle pointing up), and analgesia (green triangle pointing down). Variants originally identified by genome-wide association study (GWAS) are shown as a red-filled square. Ratios are number of variants passing an false discovery rate (FDR) threshold of 20% relative to the total number of variants in the respective group.

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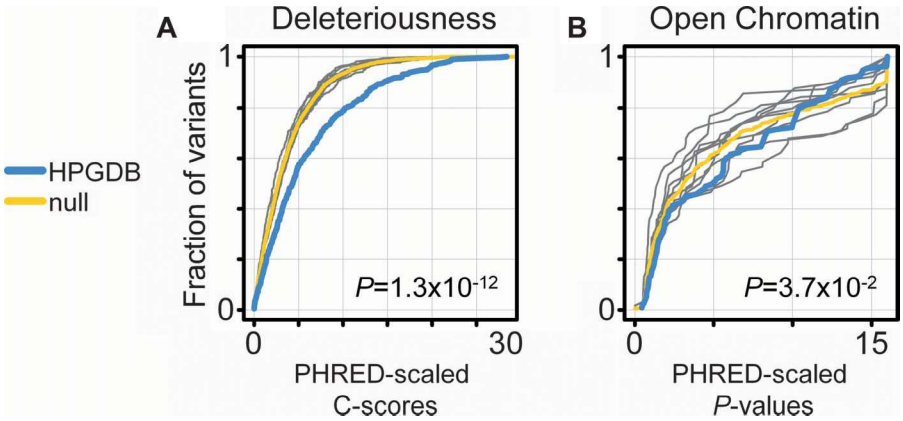

Figure 5. Cumulative distribution plots showing enrichment of the Human Pain Genetics Database variants for (A) deleterious variants and (B) variants located in regions of open chromatin. Cumulative distribution tracks of (A) Phred-scaled C-scores and (B) Phred-scaled *P* values are shown in blue. The null distribution (gold) was defined as the mean of 10 sets of randomly selected variants matching the Human Pain Genetics Database variants for minor allele frequency, number of variants in linkage disequilibrium (LD), distance to nearest gene, and gene density.

genes in human pain genetics and is mostly known for its contribution to migraine.[2,7,64] *TNF*, another frequently studied gene in human pain genetics, encodes a cytokine of pleiotropic actions that likely explains its implication in multiple pain phenotypes, such as neuropathic pain,[36] musculoskeletal pain,[35] and analgesia.[62] Completing this list of genes is *GCH1*, contributing to neuraxial pain,[39] nociception,[16] and pain in sickle cell anemia[10]; *ESR1*, contributing to neuraxial pain,[63] migraine,[43] analgesia,[27] and osteoarthritis[38]; *ABCB1*, contributing to analgesia[9] and nociception[68]; and *SCN9A*, contributing to fibromyalgia,[76] nociception,[31] postoperative pain and analgesia,[32] and neuraxial and neuropathic pain.[61]

Despite the design of association studies, the HPGDB seems to hold true signals of relatedness to pain because more than one-third of its variants were successfully replicated in at least one UKBB pain phenotype. Importantly, replicated variants were not only those originally included in the HPGDB for their associations with clinical pain phenotypes, but many were originally associated with analgesia- or nociception-related phenotypes, reaffirming that these are pathways of vulnerability and that studying them should help elucidate the pathophysiological mechanisms underlying pain conditions. The associations replicated in the UKBB pain phenotypes identified genes that robustly contribute to pain, such as *CYP3A4*, *ESR1*, and *KCNJ6*, associated with 6 phenotypes each (Supplementary Table 29, available online at http://links.lww.com/PAIN/A520). Notably, most HPGDB genes carrying the most variants associated with UKBB pain phenotypes (Supplementary Tables 10 and 21–28, available online at http://links.lww.com/PAIN/A520) are genes for which an association with a pain condition was reported in less than 1% of HPGDB studies, indicating that their contribution to pain warrants further investigations.

To further gauge whether the associations replicated in the UKBB are true, we showed that the distribution of association *P* values of HPGDB variants in 4 different pain phenotypes is significantly enriched for lower values when compared with random controls. Notably, even after removing GWAS hits from the pool of HPGDB variants, we still observed a significant enrichment for lower *P* values for headaches, once again suggesting that candidate gene studies are helpful in providing reliable hits.

The phenomenon by which a genetic variant affects different phenotypes is known as pleiotropy. Our findings that HPGDB variants associated with UKBB pain phenotypes correlated between multiple pairs of phenotypes and explained a considerable proportion of the variance within them align with the current prevailing theory that pain conditions share vulnerability pathways.[29,84] This high proportion of variance explained was largely driven by variants with strong individual effects, that have MAFs below 1 in 1000, exemplifying the genetic paradigm of high allele frequency, small effect size–low allele frequency, large effect size.[47] Although investigating the underpinnings of their direct contribution to pain may not be readily feasible because of large sample size requirements, they are important for our understanding of the biology of pain. Although the percent of variance explained is reduced substantially after exclusion of variants with strong individual effects, rank correlations that are not sensitive to statistical outliers remained significant. These results substantiate previous evidence that similar genetic pathophysiological mechanisms likely underlie the high comorbidity among different pain conditions[55,80] and indicate which variants are implicated in this shared genetic mechanism.

An assessment of the phenotypes in the NHGRI-EBI catalog has identified that up to 18.6% of genes and 7.8% of variants can be defined as pleiotropic.[66] It is also known that risk-associated variants are enriched for functionality.[21] Thus, being associated with other nonprimarily painful phenotypes provides additional evidence of variant functionality. Notably, the HPGDB data set is robustly enriched with pleiotropic variants. This finding indicates that pain conditions share common genetic mechanisms not only with each other[29] but also with other non–pain-related phenotypes. In specific, the nonprimarily painful phenotypes most commonly associated with HPGDB variants were those related to the cardiac system, measures of cholesterol, asthma, ulcerative colitis, height, and age-related macular degeneration.

Finally, we showed that *cis*-eQTLs are abundantly present among the HPGDB variants in tissues that are relevant to pain, such as the anterior cingulate and frontal cortices,[13] nerve, and whole blood, an accessible tissue whose gene expression shares similarities with multiple brain tissues.[70] In opposition, DRGs carry a unique transcriptome that stands apart from brain tissues,[58] and fewer variants were identified as *cis*-eQTL in this tissue. It may be the case that most variants associated thus far with pain conditions have stronger central rather than peripheral implications. Such disparity may also be attributed to the difference in MAFs used to filter variants in the GTEx project and in the

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DRG-eQTL data set, which are 1% and 5%, respectively. Regardless, by providing access to relevant eQTL information, the HPGDB facilitates identification of the variants that should be investigated in future studies for their contribution to the regulation of pain-related transcriptional networks. For instance, *GCH1* is among the genes whose expression is strongly regulated by HPGDB *cis*-eQTL, and the mechanisms underlying its contribution to pain have been at least in part identified.[74] However, HPGDB *cis*-eQTLs in *MTRR*, *IL10RB*, *ELAC2*, and *ZNF55* are novel findings.

Additional evidence of functionality among HPGDB variants comes from their C-score, a measure of deleteriousness that strongly correlates with molecular functionality and pathogenicity.[40] The set of HPGDB variants is heavily enriched for higher C-scores when compared with appropriate sets of random controls. Furthermore, HPGDB variants also seem to lie in genomic regions of open chromatin, providing complementary evidence of enrichment for gene regulatory elements among the variants included in our database, such as transcriptional start sites, distal enhancers, transcription factor binding sites, and active histone marks.[54]

We also investigated whether the genes contributing to pain phenotypes are enriched in any specific biological pathway. The sensory perception of pain pathway presented the highest fold-enrichment. Most genes in this pathway have established roles pain processing mechanisms, such as serotonin receptors,[8] enzymes of the catecholaminergic system,[37] opioid receptors,[59] ion channels,[49] and cytokines.[28] As most HPGDB variants came from candidate gene studies, this finding is not surprising and may serve a positive control for the pathway analysis approach used. Negative regulation of blood pressure, though, was an unexpected finding, and its high fold-enrichment ranking may be a reflection of the underestimated importance of the contribution of blood pressure to the regulation of pain pathogenesis.

The HPGDB has its limitations, including perhaps the need to broaden search terms to capture more studies that may be eligible for inclusion in the database. Nonetheless, it also has useful features that are absent in other databases and make it a valuable resource for pain researchers: the display of direction of association between genotypes and phenotypes, the ability to extract data from the database, and links to key complementary resources.

In summary, the database presented here is an up-to-date repository of the relevant literature pertaining to the genetic contributions to pain in humans. Many variants contained in the HPGDB database were replicated in multiple pain phenotypes from a large independent cohort, and the data set demonstrated to be enriched for deleterious functional variants with pleiotropic associations. Importantly, the HPGDB provides direct access to this evidence of functionality, and the ability to look at these pain-related variants through various functional lenses will provide important leads to foster future research on unexplored aspects of human pain.

Conflict of interest statement

C. Beraldo Meloto reports receiving the Catherine Bushnell Fellowship in Pain Research during the conduct of the study. L. Diatchenko reports a grant from the Canadian Excellence Research Chairs (CERC) Program (http://www.cerc.gc.ca/home-accueil-eng.aspx, CERC09) during the conduct of the study; personal fees from Proove Biosciences and from Algynomics outside the submitted work; in addition, L. Diatchenko has a patent related to genetic variants of *COMT* associated with pain, pending to Proove Biosciences. The remaining authors have no conflicts of interest to declare.

Preliminary data have been presented by L. Diatchenko at the 6th International Congress on Neuropathic Pain (NeuPSIG 2017), invited presentation (Keynote speaker), in Gothenburg, Sweden, June 15-18, 2017; by X. Wen in the form of poster at the 19th Annual McGill Pain Day, in Montreal, Canada, on January 2015; and by R.N. Lichtenwalter in the form of poster at the International Association for the Study of Pain/15th World Congress on Pain in Buenos Aires, Argentina, on October 2014.

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A520.

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