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Genome-wide association study of pain sensitivity assessed by questionnaire and the cold pressor test

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Abstract

We deployed an online pain sensitivity questionnaire (PSQ) and an at-home version of the cold pressor test (CPT) in a large genotyped cohort. We performed genome-wide association studies on the PSQ score (25,321 participants) and CPT duration (6853). We identified one new genome-wide significant locus associated with the PSQ score, which was located in the *TSSC1* (also known as *EIPR1*) gene (rs58194899, OR = 0.950 [0.933-0.967], *P*-value = 1.9×10^{-8}). Although high pain sensitivity measured by both PSQ and CPT was associated with individual history of chronic and acute pains, genetic correlation analyses surprisingly suggested an opposite direction: PSQ score was inversely genetically correlated with neck and shoulder pain ($r_g = -0.71$), rheumatoid arthritis (−0.68), and osteoarthritis (−0.38), and with known risk factors, such as the length of working week (−0.65), smoking (−0.36), or extreme BMI (−0.23). Gene-based analysis followed by pathway analysis showed that genome-wide association studies results were enriched for genes expressed in the brain and involved in neuronal development and glutamatergic synapse signaling pathways. Finally, we confirmed that females with red hair were more sensitive to pain and found that genetic variation in the *MC1R* gene was associated with an increase in self-perceived pain sensitivity as assessed by the PSQ.

Keywords: Pain sensitivity, Pain sensitivity questionnaire, Cold pressor test, Genome-wide association study

1. Introduction

It has been established that pain sensitivity is predictive of acute postoperative pain, and of risk for the development of chronic pain conditions.[34] The precise assessment of pain sensitivity requires well-controlled experimental pain and emotional stimuli.[5] In general, such assessments are time-consuming in clinical settings because there is substantial interindividual variability in pain sensitivity and perception,[13,40] and they can only be deployed for modest cohort sizes. As a result, there have been few studies with large sample sizes, impeding progress in understanding the genetic architecture of pain sensitivity. Although hundreds of genes have been proposed to have associations with different types of pain,[53] most pain genetics studies analyzed small sample sizes, often using candidate gene or gene panel approaches. To date, the number of genome-wide association studies (GWAS) on pain phenotypes is still very limited. The largest pain GWAS have been performed in the UK Biobank and 23andMe, Inc cohorts for chronic pain,[49,56] knee pain,[45] neck and shoulder pain,[32] and migraine.[19] These studies have identified dozens of putative causal genes, which are primarily expressed within brain tissues and have been implicated in neurogenesis, neuronal development, neural connectivity, and cell-cycle processes. Pain phenotypes have been correlated with a range of psychiatric, personality, autoimmune, anthropometric, and circadian traits.[31] Only a couple of small GWAS studies have directly explored the genetic architecture of pain sensitivity.[38,52] They have found a small number of associations, but none of them have been replicated.

Several clinical and population-based studies have also reported that individuals who naturally have red hair tend to be more resistant to local anesthetics and more sensitive to thermal and dental pain.[24] Red hair, as well as fair skin and freckles, is associated with genetic variations of the melanocortin-1 receptor (MC1R), and it has been suggested that these mutations could directly modulate pain sensitivity, particularly in women.[9,14,18,55]

We recently validated an online version of the Pain Sensitivity Questionnaire (PSQ) and an at-home version of the cold pressor test (CPT), both of which are used in clinical assessments of pain.[29] The PSQ asks participants to imagine 14 painful situations and 3 nonpainful control situations. Subjects are asked to rate their painfulness on a 0 to 10 numeric scale. The CPT measures how long subjects can immerse one hand in ice water. The validation study demonstrated that these 2 pain sensitivity measures can be consistently collected online and allow pain sensitivity analyses in very large cohorts.

In this study, we performed GWAS on PSQ score and CPT duration in a large European ancestry cohort (31K) of genotyped individuals, followed by gene-based tests and enrichment analyses. We also attempted to replicate the association between hair color, *MC1R* genetic variants, and pain sensitivity.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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2. Materials and methods

2.1. Study sample

All participants included in the analyses were drawn from the research participant base of 23andMe, Inc, a personal genetics company. Participants provided informed consent and participated in the research online, under a protocol approved by the external AAHRPP-accredited IRB, Ethical & Independent Review Services (E&I Review) (http://www.eandireview.com; OHRP/FDA registration number IRB00007807, study number 10044-11). We restricted all analyses to a set of unrelated participants having >97% European ancestry, as determined through an analysis of local ancestry. Participants were labelled as related if they shared more than 700 cM of identity-by-descent.[53]

2.2. Pain sensitivity traits

For the assessment of the pain sensitivity, we used a PSQ and an at-home version of CPT on 2 subsets of 23andMe consented research customers who were invited to participate, without restrictions for the PSQ cohort and with some safety restrictions for the CPT cohort. The PSQ is an English-language version of the PSQ,[40,41] supplemented with additional questions about the participant's own memory of self-perceived painful experiences. The PSQ contains 14 questions in which participants should imagine themselves in certain situations. Participants should then grade how painful they would be, from 0 that stands for no pain to 10 that stands for the most severe pain that participants can imagine or consider possible. The total PSQ score is the mean of the 14 responses. We also computed 2 PSQ subscales: PSQ-minor score based on the least painful questions (#14, 3, 6, 12, 11, 10, and 7, ordered from least to most painful) and PSQ-moderate score (#8, 15, 2, 16, 17, 1, and 4). For the CPT, participants were asked to prepare their own bath of ice water at home and to keep their nondominant hand submerged to the wrist for no more than 150 seconds. A separate consent for the CPT was used: participants reporting neurological or temperature-triggered conditions (eg, migraine, history of syncope, or Raynaud phenomenon) or current injuries to their nondominant hands at the time of recruitment were ineligible. Two primary outcomes were assessed: cold pain threshold and cold pain tolerance. Cold pain threshold was the time to the first report of pain, and cold pain tolerance was the time to removal of the hand from the water. The 2 cold pain outcomes were partially correlated (Spearman $r_s = 0.64$). On the day of the test, 6.6% of the CPT participants reported using pain medication ("Did you take a medication to treat pain today?"). These participants logged a similar cold pain threshold (35.2 [32.1-38.3] vs 35.2 [34.3-36.0] seconds for participants who did not reported taking pain medication) and a significant lower cold pain tolerance (73.0 [68.4-77.7] vs 80.6 [79.4-81.9] seconds; general linear model *P*-value $= 9.6 \times 10^{-5}$, after rank-inverse normalizing and controlling for age, sex, and ancestry principal components). For the GWAS analysis, we used the cold pain tolerance as our main CPT duration, without excluding participants who reported taking pain medication on the day of test. For additional information on the PSQ and CPT, in particular the validity of these approaches for estimating pain sensitivity, see Ref. 29.

For all the participants in both cohorts, we also collected pain diagnosis and pain medication usage with the following 2 online questions: "Have you ever been diagnosed with, or treated for, any of the following conditions related to pain?" (16 pain traits, including chronic, acute, low-back, joint, complex regional pain syndrome, dental pain, …) and "In the past 5 years, have you taken any of these medications to relieve pain after injury or surgery that lasted more than 3 months?" (33 drug classes including celecoxib, codeine, oxycodone, fentanyl, duloxetine, …) (**Table 1**).

2.3. Genotyping and variant imputation

DNA extraction and genotyping were performed on saliva samples by LabCorp, Inc. Participants were genotyped on 1 of 5 Illumina genotyping platforms, containing between 550,000 and 950,000 variants, for a total of 1.6 million of genotyped variants. Samples that failed to reach 98.5% call rate were reanalyzed. Genotyping quality controls included discarding variants with a Hardy–Weinberg *P*-value $< 10^{-20}$, a call rate of <90%, or batch effects. About 57.5 M of variants were then imputed against a single unified imputation reference panel, combining the May 2015 release of the 1000 Genomes Phase 3 haplotypes with the UK10K imputation reference panel. Principal components were computed using ~65,000 high-quality genotyped variants present in all 5 genotyping platforms. For more details on genotyping, imputation process, and variant quality controls, see Ref. 47.

2.4. Genome-wide association study analysis

Imputed dosages and genotyped data were both tested for association with PSQ score or CPT duration (between 0 and 150 seconds). The PSQ scores were inverse normalized and analyzed using a Gaussian linear model. The association *P*-values were computed using a likelihood ratio test. The CPT duration was analyzed using a Cox proportional hazards model,[35] a survival model on the CPT time. We included covariates for age, sex, genotyping platform, and the top 5 principal components to account for residual population stratification. The PSQ association model did not include platform covariables because PSQ participants were all genotyped on platform v4. Results for the X chromosome were computed similarly, with males coded as if they were homozygous diploid for the observed allele. A total of 1.3 M genotyped and 25.5 M imputed variants passed the pre- and post-GWAS quality controls. We furthermore filtered out variants with MAF <0.1%, which are extremely sensitive to quantitative trait overdispersion, reducing to 13.7 M variants available for follow-up analyses. A detailed description of the variant quality control and GWAS methods can be found in Ref. 47. The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/dataset-access/ for more information and to apply to access the data.

2.5. Melanocortin-1 receptor and hair color

We defined 3 categories of *MC1R* variant carriers by combining 3 variants, rs1805007, rs1805008, and rs1805009: Noncarrier (0 *MC1R* alleles), Carrier1 (1 allele), and Carrier2+ (>1 alleles).[18] A self-reported hair color phenotype was available for 63% of the participants in the cohort.[33] It contained 6 hair color categories: red, light blond, dark blond, light brown, dark brown, and black. We also built a binary red hair variable from this categorical hair color phenotype. Association of MC1R carrier, hair color, and red variables were tested against PSQ score or CPT duration, using Gaussian linear and Cox proportional hazards models, respectively. To facilitate the visualization of the results, we also converted CPT duration to ranks, and tested associations using

Table 1

Description of pain sensitivity questionnaire and cold pressor test cohorts.

	PSQ cohort		CPT cohort	
	Females	Males	Females	Males
No. of participants	18,060	7261	4343	2510
Age (y)	50.2 (0.4)	50.9 (0.4)	42.5 (0.3)	42.1 (0.2)
Reported pain				
Chronic	28.7%	24.0%	19.0%	18.6%
Acute	56.3%	52.5%	42.8%	48.7%
Low back	39.6%	35.7%	28.5%	32.9%
Db neuropathy	1.0%	1.7%	0.9%	0.4%
CRPS	4.8%	2.8%	1.4%	3.6%
Migraine	35.6%	16.1%	8.8%	12.4%
Dental	59.0%	55.1%	54.0%	52.1%
Shingles	21.9%	15.7%	15.6%	16.6%
Medication usage				
OTC NSAID	95.3%	92.0%	92.6%	94.2%
Opioids	53.9%	49.0%	47.3%	47.4%
Antiepileptics	17.9%	10.1%	8.7%	6.7%
Antidepressants	13.7%	5.9%	6.2%	7.0%
PSQ score and CPT duration				
All	3.23 (0.01)	3.11 (0.01)	72.39 (0.75)	93.9 (1.01)
Per age classes (y)				
<40	3.16 (0.018)	3.04 (0.028)	73.38 (1.00)	94.67 (1.36)
40-60	3.27 (0.017)	3.12 (0.026)	68.95 (1.42)	94.72 (1.81)
>60	3.25 (0.017)	3.16 (0.024)	74.83 (1.91)	89.46 (2.71)
MC1R carriers				
Noncarrier	3.22 (0.012)	3.10 (0.018)	71.98 (0.91)	92.70 (1.19)
Carrier1	3.26 (0.019)	3.14 (0.029)	73.51 (1.42)	96.98 (2.01)
Carrier2+	3.33 (0.062)	3.18 (0.093)	72.36 (4.39)	100.35 (6.18)
Hair color sub-cohort				
No. of participants with hair color information	10,928	4239	3375	1868
Proportion of redhead	5.1%	3.2%	5.0%	3.8%
Not redhead	3.22 (0.013)	3.13 (0.02)	72.12 (0.88)	92.22 (1.20)
Redhead	3.37 (0.060)	3.01 (0.10)	71.69 (3.77)	100.43 (5.77)

Pain phenotypes were collected with the following question: "Have you ever been diagnosed with, or treated for, any of the following conditions related to pain?" Medication usage was collected with "In the past 5 years, have you taken any of these medications to relieve pain after injury or surgery that lasted more than 3 months." Chronic and acute are defined as pain after injury or surgery that lasted >3 and <3 months, respectively. CPT duration (mean and SE) is reported in seconds.

Antiepileptics, antiepileptic or anticonvulsant drugs; CPT, cold pressor test; CRPS, complex regional pain syndrome; Db neuropathy, diabetic neuropathy; MC1R, melanocortin-1 receptor; OTC NSAID, over-the-counter nonsteroidal anti-inflammatory drugs; PSQ, pain sensitivity questionnaire; Shingles, included pain related to shingles, cold sores, or herpes.

analysis of variances. The same set of covariables used in GWAS was included in these models.

2.6. Genetic correlation, Mendelian randomization, gene-based and pathway analyses

Genetic correlations between PSQ and a broad list of 832 diseases and traits were estimated with LD Hub v1.9.1,[54] using the default analysis parameters. In addition, we computed genetics correlations with 8 published pain[31] and a derived multisite chronic pain (MCP) GWAS[21] from UK Biobank. The UK Biobank participants were offered a pain-related questionnaire, which included the question: "In the last month have you experienced any of the following that interfered with your usual activities?" The options were: (1) headache (74,761 cases); (2) facial pain (2610); (3) neck or shoulder pain (53,994); (4) back pain (43,991); (5) stomach or abdominal pain (8217); (6) hip pain (10,116); (7) knee pain (22,204); and (8) pain all over the body (5670). From the same question, MCP was defined as the sum of body sites at which chronic pain (at least 3 months' duration) was reported. A 2-sample Mendelian randomization (MR) between PSQ score and the 9 published pain traits were conducted in accordance with published methods.[6] The gene-based analysis was performed on MAGMA (v1.07).[11] After correction with a Benjamini–Hochberg procedure, genes with adjusted P-values $< 10^{-4}$ were selected and used in pathway analyses performed on FUMA (GENE2FUNC, v1.3.5; https://fuma.ctglab.nl/). Pathway enrichment was tested for different gene sets, including canonical pathways, Reactome, and GO biological processes. Tissue specificity for the set of selected genes was tested using an enrichment analysis of differentially expressed genes.

3. Results

A total of 25,321 and 6853 research participants of European ancestry were included in the PSQ and CPT GWAS analyses, respectively (**Table 1**). For the PSQ cohort, no specific selection criteria were used; all participants from the 23andMe research database were invited to contribute. However, for the CPT cohort, research participants with a history of severe migraine and a number of other chronic conditions that might be directly exacerbated by the cold stress were not invited to participate to ensure their safety. Consequently, the proportion of participants reporting migraine but also any types of pain was lower in the CPT

than the PSQ cohort. Overall, the 2 cohorts included between 18.8% to 27.3% and 44.9% to 55.2% of participants having been diagnosed or treated for chronic and acute pain, respectively. In the PSQ cohort, a higher proportion of females than males reported been diagnosed or treated for pain whereas, in the CPT cohort, the proportion was more balanced or slightly higher in males. The 2 cohorts were largely independent; only 1534 participants were included in both analyses. The sex ratio was unbalanced in both cohorts, with 71% and 63% of females, respectively. A more detailed description of the 2 cohorts has been published elsewhere.[29]

On average, females reported a higher pain sensitivity with a mean PSQ score of 3.23 ± 0.01 vs 3.11 ± 0.01 in males, as well as a lower tolerance to the CPT with 72.4 +/- 0.8 vs 94 ± 1.0 seconds of hand immersion in ice water (**Table 1**; and Supplementary Fig. 1, available at http://links.lww.com/PAIN/B557). A higher PSQ score (higher pain sensitivity) was systematically associated with pain diagnosis, reported multiple pain conditions, and with medication usage (**Table 1**; Supplementary Table 1, and Supplementary Figs. 2 and 3, available at http://links.lww.com/PAIN/B557). A lower CPT duration (higher pain sensitivity) also tended to be associated with pain diagnosis and with medication usage. However, many of these associations were not significant (Supplementary Fig. 4, available at http://links.lww.com/PAIN/B557).

The GWAS on PSQ score produced one locus that reached genome-wide significance. The lead SNP (rs58194899: OR = 0.950 [95% CI 0.933-0.967], P-value = 1.9×10^{-8}, MAF = 0.47; **Table 2**) was located in the *TSSC1* gene (**Figs. 1A and 2**). The associated haplotype was relatively small (54 variants in the 99% credible set) and entirely located within the *TSSC1* gene boundary (**Fig. 2A**). To date, no studies have reported a phenotypic or eQTL association with this haplotype (https://genetics.opentargets.org/). However, a cognitive decline GWAS reported an independent association in the *TSSC1* gene (lead variant rs75365287).[26] *TSSC1* is a component of the endosomal retrieval pathway. It plays a critical role as a regulator of both Golgi-associated retrograde protein and endosome-associated recycling protein functions, as well as the transport of internalized proteins to the plasma membrane.[3,17] *TSSC1* and the Golgi-associated retrograde protein/endosome-associated recycling protein complexes are not known to be involved in pain traits. However, *TSSC1* is overexpressed in the brain, particularly in the hypothalamus, and in the frontal and interior cingulate cortices (GTEx v8).

Using the PSQ GWAS summary statistics, we performed a gene-based association analysis in MAGMA (v1.07), followed by a gene-set enrichment analysis in FUMA (GENE2FUNC, v1.3.5). A total of 58 genes were identified with an adjusted P-value $< 1.0 \times 10^{-4}$ (**Table 3**). These genes were overexpressed in the brain, especially in the frontal cortex, basal ganglia, amygdala, and hypothalamus (Supplementary Fig. 5, available at http://links.lww.com/PAIN/B557). They were significantly enriched for genes involved in brain development and synaptic signaling pathways (**Table 3**). Like *TSSC1*, many of these genes are involved in Golgi apparatus function (*RBFOX1*, *PARK2*, *WWOX*, *PRKG1*, *LARGE*, *GPC6*, *PCSK6*, *HS3ST4*, *WWOX*, *SLC39A11*, *PRKCE*, *TENM2*, and *CNTNAP2*; Supplementary Table 2 and Supplementary Fig. 6, available at http://links.lww.com/PAIN/B557). Several genes identified by MAGMA are active in glutamatergic synapses, which are involved in pain sensation and transmission (*PTPRD*, *NRG1*, *NRG3*, *DLG2*, *GPC6*, and *GRID2*). Finally, among the genes that were most strongly associated with PSQ score, *CSMD1*, *LRP1B*, and *DMD* were not known to be directly involved in pain sensitivity but were linked to neurological diseases like bipolar disorder. The traits with the strongest genetic correlations with PSQ, as computed on LD Hub (bivariate linkage disequilibrium score regression [LDSC] on 832 tested traits), are listed in **Table 4**. Pain sensitivity questionnaire score was negatively genetically correlated with chronic pain related phenotypes, such as neck-and-shoulder pain ($r_g = -0.71$), rheumatoid arthritis (0.68), or mononeuropathies (0.53), but positively correlated with acute pain phenotypes, such as pneumothorax (0.82) or fracture (0.71). It was also negatively correlated with health risk factors and behaviors, such as the length of working week (−0.65), working in a noisy environment (−0.42), shift work (−0.41), smoking (−0.36), and extreme BMI (−0.23). We also observed a negative genetic correlation between the PSQ score and ADHD (−0.67), but positive correlations with schizophrenia (0.21), bipolar disorder (0.25), and neuroticism (0.22). Many of these genetic correlations were marginally significant and none of them passed the multiple testing discovery threshold ($0.05/832 = 6 \times 10^{-5}$). The genetic correlations with the 8 additional published pain susceptibility traits[31] and the MCP[21] also suggested a negative genetic correlation with PSQ score but none of them were significant. The causality between pain sensitivity, measured by PSQ score, and pain susceptibility traits was assessed by MR analyses (Supplementary Fig. 7, available at http://links.lww.com/PAIN/B557). Although we observed few significant MR

Table 2

Top genome-wide association study variants and melanocortin-1 receptor association results for pain sensitivity questionnaire and cold pressor test traits.

CHR	POS	ID	Alleles	MAF	Gene context	PSQ GWAS			CPT GWAS		
						Effect	SE	P	Effect	SE	P
Top PSQ GWAS variants											
2	3280983	rs58194899	G/A	0.47	TSSC1	***−0.051***	***0.009***	***1.90E-08***	−0.01	0.018	5.80E-01
18	10008669	rs142738119	T/C	0.001	VAPA, APCDD1	0.696	0.138	4.70E-07	−0.035	0.275	9.00E-01
13	1.02E+08	rs12583902	G/A	0.166	NALCN	0.059	0.012	7.60E-07	−0.025	0.023	2.90E-01
Top CPT GWAS variants											
17	65520139	rs141828201	G/A	0.027	PITPNC1	0.036	0.031	2.60E-01	0.356	0.066	2.40E-07
MC1R variants											
16	89986144	rs1805008	C/T	0.071	MC1R	0.031	0.017	6.80E-02	−0.032	0.034	3.50E-01
16	89986117	rs1805007	C/T	0.075	MC1R	0.047	0.017	5.10E-03	−0.039	0.033	2.40E-01
16	89986546	rs1805009	G/C	0.019	MC1R	0.041	0.032	2.00E-01	−0.089	0.063	1.50E-01

PSQ score was analyzed using a linear model. CPT duration (in seconds) was analyzed using a survival model. Bold-italic indicates genome-wide significant association.

CHR, chromosome; CPT, cold pressor test; GWAS, genome-wide association studies; MAF, minor allele frequency; MC1R, melanocortin-1 receptor; POS, genomic position; PSQ, pain sensitivity questionnaire.

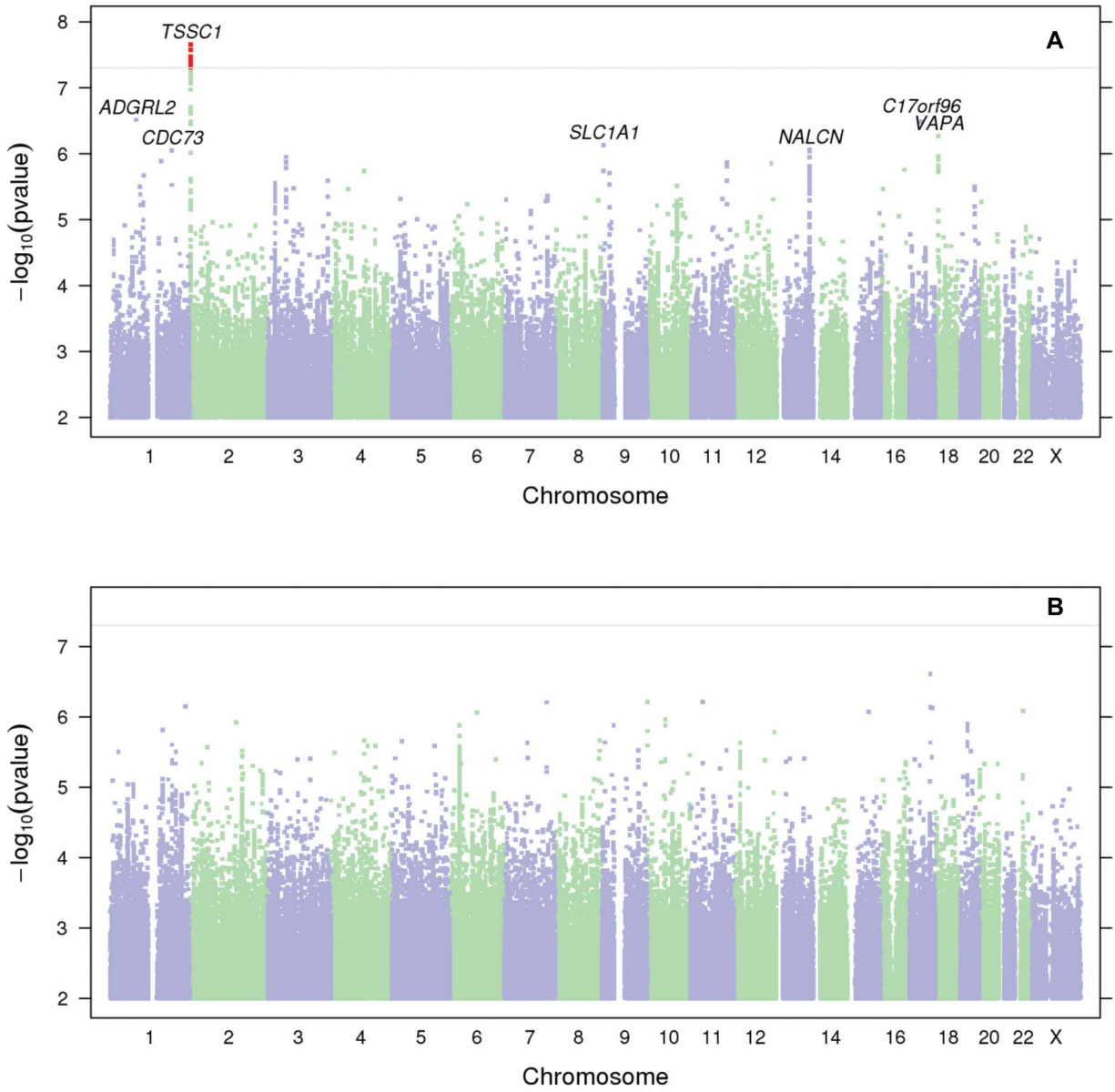

Figure 1. Manhattan plots for PSQ and CPT GWAS (panel A and B, respectively). CPT, cold pressor test; GWAS, genome-wide association studies; PSQ, pain sensitivity questionnaire.

results, mainly from the inverse-variance weighted model, the directions of effect were inconsistent. The comparison between PSQ score and the strongest published pain susceptibility trait (MCP) showed no evidence of directionality.

The GWAS on CPT duration was underpowered and did not produce any significant genome-wide associations (**Fig. 1B**; Supplementary Fig. 8 and Supplementary Table 1, available at http://links.lww.com/PAIN/B557). The genetic correlation between PSQ score and CPT duration was $r_g = -0.73 \pm 0.38$ (P-value = 0.054), and the results from the CPT gene-based analysis and pathway analysis were consistent with the PSQ results. Among the 50 CPT genes identified by MAGMA, 21 were also identified in the PSQ analysis (**Table 3**). Similarly, these 50 genes were overexpressed in brain (Supplementary Fig. 9, available at http://links.lww.com/PAIN/B557) and enriched for brain development and synaptic signaling pathways (Supplementary Table 3, available at http://links.lww.com/PAIN/B557). Despite the shared genetic architecture between PSQ and CPT, the genome-wide significant association in *TSSC1* (lead variant rs75365287) identified in the PSQ GWAS was not replicated in the CPT GWAS (P-value $= 5.8 \times 10^{-1}$; **Table 2**).

We also specifically focused on the association results for genes reported to be associated with nociception. We obtained a list of 21 nociception genes from the Human Pain Genes Database (HPGDB, https://humanpaingenetics.org/hpgdb/).[30] None of the 21 nociception genes showed evidence of association with PSQ and CPT (Supplementary Table 4, MAGMA analysis, available at http://links.lww.com/PAIN/B557).

Finally, we assessed the relationship between both pain sensitivity measures, PSQ and CPT, and hair color on a subset of the PSQ and CPT cohorts with available hair color data (**Table 1**). A total of 15,167 and 5243 participants were included in the analyses for PSQ score and CPT duration, respectively. Using a Gaussian linear model including age, sex, and the first 5 genetic principal components as covariables, we showed that participants with red hair reported significantly higher PSQ scores than participants with light blond, dark blond, light brown, dark brown, or black hair (**Fig. 3**). Furthermore, females with red hair

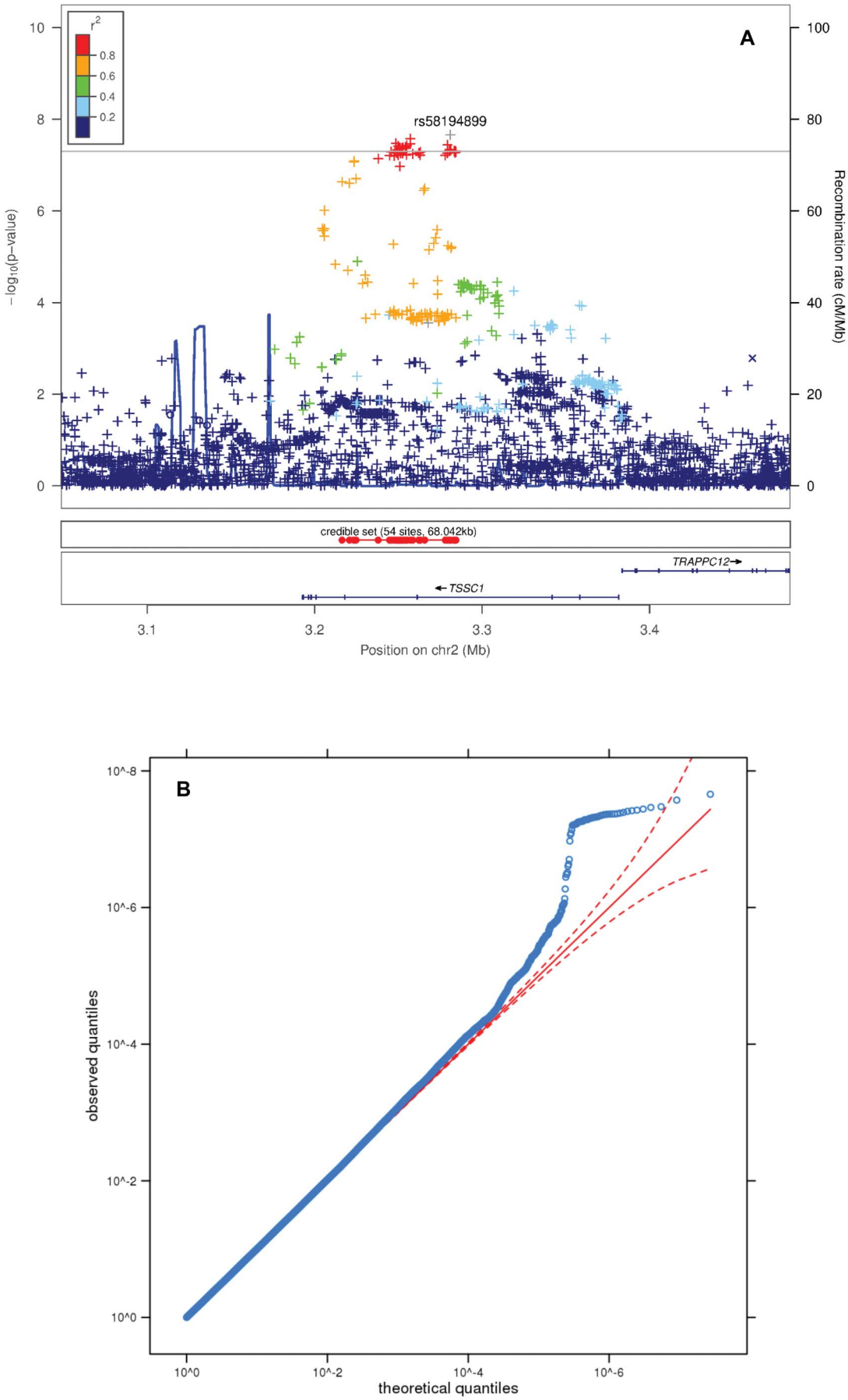

Figure 2. Results of MC1R and hair color association with PSQ score. MC1R, melanocortin-1 receptor; PSQ, pain sensitivity questionnaire.

Table 3

Gene-based (MAGMA) and pathway analysis results for pain sensitivity questionnaire and cold pressor test.

Gene	Position	PSQ		CPT		Pathways
		No. of variants	*P*	No. of variants	*P*	
ADARB2	10:1223253-1779670	296	8.86E-03	219	***9.37E-05***	
ASIC2	17:31340105-32483825	486	***5.61E-07***	431	***3.29E-09***	GO synaptic signalling
C10orf11	10:77191217-78317133	187	1.04E-01	241	***6.35E-07***	
CACNA2D3	3:54156620-55108584	250	1.39E-03	358	***6.66E-06***	
CADPS	3:62384021-62861064	79	1.04E-01	85	***1.53E-05***	GO neurogenesis
CAMTA1	1:6845384-7829766	379	***6.46E-05***	349	1.49E-02	
CDH13	16:82660399-83830215	321	***2.39E-07***	797	***8.53E-12***	
CDH23	10:73156691-73575704	161	3.10E-03	205	***6.32E-05***	GO synaptic signalling
CDH4	20:59827482-60515673	330	***1.20E-06***	121	***3.90E-07***	GO neurogenesis
CNTN4	3:2140550-3099645	469	***7.03E-07***	482	***1.20E-06***	GO neurogenesis; GO synaptic signalling
CNTN5	11:98891706-100229616	679	9.18E-03	600	***1.14E-06***	
CNTNAP2	7:145813453-148118090	706	***5.37E-06***	1294	***2.47E-08***	GO neurogenesis; GO Golgi apparatus
COL23A1	5:177664617-178017573	156	***4.91E-05***	133	6.87E-03	
CSMD1	8:2792875-4852328	1918	***1.54E-17***	1968	***3.45E-30***	
CTNNA3	10:67672276-69455949	712	***3.08E-05***	612	9.32E-02	
DAB1	1:57460453-58716211	424	***3.73E-05***	226	1.07E-02	GO neurogenesis
DLG2	11:83166055-85338314	781	***1.21E-06***	368	***1.35E-05***	GO synaptic signalling; GO glutamatergic synapse
DLGAP1	18:3496030-4455310	363	6.23E-04	506	1.01E-10	GO neurogenesis
DLGAP2	8:877021-1656642	272	9.90E-02	618	***1.83E-09***	GO neurogenesis
DMD	X:31137345-33357726	653	***1.18E-11***	532	***1.64E-06***	
DSCAM	21:41384343-42219039	279	***2.97E-05***	282	2.04E-04	GO neurogenesis
EGFR	7:55086678-55279262	34	1.04E-01	59	***1.21E-05***	GO neurogenesis; GO synaptic signalling
FAM155A	13:107820879-108519460	311	***1.98E-08***	250	5.93E-03	
FHIT	3:59735036-61237133	693	***5.08E-07***	757	***9.88E-11***	
FRMD4A	10:13685706-14372866	194	1.36E-04	372	1.01E-07	
FSTL4	5:132532152-132948223	122	***3.04E-05***	75	6.75E-03	GO neurogenesis
GALNT18	11:11292421-11643561	82	1.04E-01	163	***2.78E-05***	
GLIS3	9:3824128-4300036	151	***7.30E-06***	128	2.81E-04	
GPC6	13:93879078-95060274	323	***1.91E-05***	96	3.87E-02	GO Golgi apparatus; GO glutamatergic synapse
GRID2	4:93225453-94695707	736	***7.90E-06***	402	8.30E-03	GO neurogenesis; GO synaptic signalling; GO glutamatergic synapse
GSE1	16:85203152-85709812	134	***3.40E-06***	131	6.68E-02	
HHAT	1:210501596-210849638	123	***5.90E-05***	109	1.05E-01	
HS3ST4	16:25703347-26149009	137	***2.41E-05***	127	4.77E-04	GO Golgi apparatus
KAZN	1:14219646-15444544	314	***2.28E-08***	512	***7.58E-10***	
KCNIP4	4:20730234-21950424	950	***7.40E-06***	617	1.05E-01	
KCNQ3	8:133133105-133493004	169	***9.40E-06***	162	4.99E-03	GO synaptic signalling
KIRREL3	11:126293388-126870766	169	4.91E-03	272	***2.31E-06***	GO synaptic signalling
LARGE	22:33668509-34316464	259	***1.58E-05***	258	***3.86E-05***	GO Golgi apparatus
LRP1B	2:140988996-142889270	1070	***1.14E-12***	1374	***7.01E-08***	
MACROD2	20:13976146-16033842	621	***2.93E-06***	623	***7.14E-12***	
MAGI1	3:65339200-66024511	445	***2.72E-05***	252	4.21E-03	
MAGI2	7:77646374-79083121	423	1.10E-04	551	***1.64E-05***	GO synaptic signalling
MAML2	11:95711440-96076344	267	***1.50E-08***	66	1.05E-01	

(*continued on next page*)

Table 3 (*continued*)

Gene	Position	PSQ		CPT		Pathways
		No. of variants	*P*	No. of variants	*P*	
MCTP1	5:94038280-94620279	265	***6.98E-08***	252	4.93E-03	GO synaptic signalling
NAV2	11:19372271-20143147	205	6.74E-04	122	***4.61E-05***	GO synaptic signalling
NCKAP5	2:133429361-134399118	125	***1.79E-05***	274	1.33E-03	
NELL1	11:20691117-21597232	294	7.47E-03	380	***7.64E-06***	
NKAIN2	6:124124991-125146786	409	***8.77E-05***	87	1.87E-02	
NPAS3	14:33404115-34273382	260	***7.74E-11***	469	***1.25E-07***	
NRG1	8:31496820-32622558	604	***5.38E-05***	340	1.05E-01	GO neurogenesis; GO synaptic signalling; GO glutamatergic synapse; ERBB2/4 pathway
NRG3	10:83635070-84746935	309	***2.33E-05***	405	***2.95E-05***	GO neurogenesis; GO synaptic signalling; GO glutamatergic synapse; ERBB2/4 pathway
NRXN3	14:78636716-80334633	381	***3.99E-07***	426	3.56E-04	GO neurogenesis
NTM	11:131240371-132206716	395	***3.29E-08***	302	1.47E-03	GO neurogenesis
OPCML	11:132284875-133402403	326	***1.52E-06***	322	2.30E-04	GO neurogenesis
PALM2-AKAP2	9:112542577-112934792	146	***2.72E-05***	334	1.35E-03	
PARK2	6:161768590-163148834	1097	***5.06E-10***	648	7.70E-03	GO neurogenesis; GO synaptic signalling; GO Golgi apparatus
PCSK5	9:78505560-78977255	182	3.45E-04	201	***4.34E-07***	
PCSK6	15:101844133-102030187	36	***2.07E-05***	82	2.25E-02	GO Golgi apparatus
PDZD2	5:31639345-32111038	178	***9.69E-05***	253	1.58E-04	
PLCB1	20:8112912-8865547	217	1.03E-04	296	4.06E-05	GO neurogenesis
PPP2R2C	4:6322305-6565327	82	1.04E-01	69	1.31E-05	
PRKCE	2:45878454-46415129	278	***2.19E-07***	253	3.58E-04	GO synaptic signalling; GO Golgi apparatus; ERBB2/4 pathway
PRKG1	10:52750911-54058110	493	***2.27E-06***	822	***3.93E-07***	GO neurogenesis; GO Golgi apparatus
PTPRD	9:8314246-10612723	943	***1.24E-11***	1232	***6.80E-15***	GO neurogenesis; GO synaptic signalling; GO glutamatergic synapse
PTPRG	3:61547243-62280573	123	3.71E-02	137	***4.35E-06***	GO synaptic signalling
PTPRN2	7:157331750-158380482	611	1.08E-04	427	***1.35E-05***	GO neurogenesis
PTPRT	20:40701392-41818557	227	***5.55E-05***	268	2.39E-02	
RBFOX1	16:5289469-7763342	1457	***6.38E-24***	1632	2.09E-25	GO Golgi apparatus
RBFOX3	17:77085427-77512230	233	***4.28E-05***	100	1.27E-02	
ROBO2	3:75955845-77699115	846	***3.05E-05***	407	***5.49E-05***	GO neurogenesis
SDK1	7:3341080-4308632	170	***3.34E-05***	231	1.36E-03	GO neurogenesis
SGCZ	8:13947373-15095792	564	1.04E-01	348	***1.08E-05***	
SLC39A11	17:70642085-71088853	172	***5.62E-05***	191	1.05E-01	GO Golgi apparatus
SNX29	16:12070602-12668146	316	1.04E-01	328	***4.75E-05***	
SORCS2	4:7194374-7744564	452	***2.36E-10***	290	***4.41E-05***	
SOX5	12:23682438-24715383	373	***7.65E-08***	207	6.39E-03	GO neurogenesis
TENM2	5:166406083-167691162	124	***1.45E-06***	487	7.62E-04	GO neurogenesis; GO Golgi apparatus
TENM3	4:183065112-183724177	331	1.24E-03	268	***1.03E-07***	GO synaptic signalling
TENM4	11:78364328-79152014	178	6.73E-02	283	***3.06E-05***	GO synaptic signalling
TMEM132D	12:129556271-130388212	190	2.52E-03	325	***7.91E-06***	
TRA	14:22090057-23021075	409	1.04E-01	332	***1.81E-05***	
TSHZ2	20:51588946-52111869	200	1.04E-01	222	***3.38E-05***	
TSSC1	2:3192741-3381653	631	***6.35E-05***	120	1.05E-01	GO Golgi apparatus
USH2A	1:215796236-216596738	197	***4.32E-05***	341	1.86E-03	GO neurogenesis

(*continued on next page*)

Table 3 (*continued*)

Gene	Position	PSQ		CPT		Pathways
		No. of variants	*P*	No. of variants	*P*	
WWOX	16:78133310-79246567	687	***1.19E-09***	667	***4.57E-07***	GO Golgi apparatus; ERBB2/4 pathway
ZNF385B	2:180306709-180726232	197	1.04E-01	327	***7.94E-05***	
ZNF385D	3:21459911-22414132	497	3.33E-04	369	***2.87E-06***	

A total of 58 and 50 significantly associated genes (*P*-value $< 10^{-4}$, highlighted in bold-italic) for PSQ and CPT, respectively, including 21 genes identified in both pain sensitivity measures.
CPT, cold pressor test; PSQ, pain sensitivity questionnaire.

reported on average higher PSQ scores than nonred hair females and red hair or nonred hair males (sex-by-red hair interaction *P*-value = 0.046; Supplementary Table 5, available at http://links.lww.com/PAIN/B557). We did not observe significant PSQ differences between red hair and nonred hair males. For CPT, we did not observe any significant associations using a Cox proportional hazards model (Supplementary Table 6 and Supplementary Fig. 10, available at http://links.lww.com/PAIN/B557) or an analysis of variance on CPT duration converted in ranks (Supplementary Table 7 and Supplementary Fig. 11, available at http://links.lww.com/PAIN/B557). Because red hair color is partially determined by recessive genetic polymorphism in the *MC1R* gene, we explored the association of the 3 main variants rs1805009 (D294H), rs1805008 (R160W) and rs1805007 (R151C) in the PSQ and CPT GWAS (**Table 2**; and Supplementary Table 5, available at http://links.lww.com/PAIN/B557). None of these individual variants passed the genome-wide significant threshold in the GWAS analyses (*P*-values $> 5.1 \times 10^{-3}$). We combined these 3 variants and defined 3 categories of *MC1R* variant carriers, Noncarrier (0 *MC1R* recessive allele), Carrier1 (1 allele), and Carrier2+ (>1 alleles), and tested their association with PSQ score and CPT duration. *MC1R* carriers reported significantly higher PSQ score that noncarriers (*P*-value $= 6.8 \times 10^{-3}$ and 1.5×10^{-2}, respectively). However, we did not observe any significant associations between *MC1R* carriers and CPT duration (Supplementary Tables 6 and 7, and Supplementary Figs. 10 and 11, available at http://links.lww.com/PAIN/B557).

4. Discussion

The purpose of this study was to identify genetic factors contributing to the individual perception of pain.[51] We used 2 self-administered pain sensitivity measurements, the PSQ and CPT, and performed, for the first time, a comprehensive genetic association analysis on these pain sensitivity metrics. Pain sensitivity questionnaire score and CPT duration have been previously shown to be only moderately phenotypically correlated ($r = -0.22$ [-0.27, -0.17]),[29] and we showed that they are also genetically correlated ($r_g = -0.73$ [-1.49, -0.02]). The PSQ is a self-perceived pain intensity rating of imagined painful situations occurring in daily life, whereas the CPT directly measures pain tolerance. Although the PSQ was not designed to cover all dimensions of pain experience, it explicitly incorporates some emotional and cognitive components of the pain sensitivity.[23]

Most of the genes identified by the association analyses are overexpressed in the brain. This is especially true for the amygdala, the emotional pain processing center, but other components of the pain matrix (eg, frontal cortex, basal ganglia, and hypothalamus) also showed significant enrichment.[46,50] We found that no brain area seemed to be selectively and exclusively associated with pain sensitivity. These genetic results were in line with recent brain imaging studies that suggested that the individual variability in pain sensitivity is most probably produced by the connectivity of multiple brain areas.[28,43] It is interesting to notice the absence of association enrichments in nonbrain tissues. According to the current multifaceted experience concept, pain results from the integration of nociception and the cognitive–emotional state of the individual. Nociception occurs with the activation of nociceptors, found in skin and mucosa, as well as in a variety of organs, such as the digestive tract, the bladder, the gut, and muscles, followed by the propagation and modulation of the nociceptive signal through the peripheral nervous system. However, our analysis of 21 genes previously reported involved in nociception showed no evidence that genetic polymorphism near these genes were associated with PSQ score or CPT duration. The CPT was designed to quantify the evoked pain and signal sensory responses of cold pain sensitivity. Although underpowered, analyses showed very similar enrichment patterns than the PSQ. Among the combined 87 genes identified by the PSQ and CPT gene-based analyses, 10 of them were present in HPGDB (*ADARB2*, *CACNA2D3*, *CTNNA3*, *DMD*, *KCNQ3*, *PARK2*, *PCSK6*, *PRKG1*, *PTPRD*, and *TRA*). The vast majority were identified in various migraine GWAS.[19] Pathway enrichment analyses showed that many of these 87 genes are involved in neuron and brain development, and neuron signaling. In particular, they highlighted genes active in glutamatergic synapses. Glutamate receptors have a leading role in pain signal transmission and are often considered promising targets for the treatment of chronic pain.[37] Among the 6 genes identified in this pathway by PSQ associations, *NGR1* and *NGR3* were already known to be linked to pain sensitivity. Both are involved in the ErbB2 and ErbB4 signaling pathways, which have repeatedly been demonstrated to directly contribute to the development of neuropathic pain.[7] To our knowledge, however, our study is the first where genetic polymorphism in these 2 genes has been associated with pain sensitivity measurements. However, the 4 other genes in the pathway, *PTPRD*, *DLG2*, *GPC6*, and *GRID2*, have been all associated with diverse neurological disorders.[1,4,20,48] This observation supports earlier findings that neurological disorders and pain sensitivity are intimately linked.[8] The genome-wide significant locus, *TSSC1*, has not previously been associated with pain traits. Some suggestive associations (*P*-value $= 1.1 \times 10^{-7}$) were recently identified in small fiber neuropathy or joint disorders (http://r4.finngen.fi/gene/TSSC1). It has been also associated with psychiatric disorders,[25] cognitive decline,[26] and was recently identified in a study that examined alterations in the postsynaptic protein profile as a consequence of prolonged exposure to morphine.[44] Finally, we confirmed that women with red hair are more sensitive to pain, but we did not observe this relationship in men. However, the increased sensitivity in red hair women was only detected by the PSQ, and the lack of association with CPT duration was surprising because it is generally accepted that red hair women are more sensitive to cold and hot stimuli.[15,27] However, more recent publications did not confirm this

Table 4

Genetic correlations between pain sensitivity questionnaire and published genome-wide association study traits.

Trait	r_g	r_g SE	z	P	h2	h2 SE
CPT	−0.7336	0.3808	−1.9264	0.0541	0.129	0.0686
Neck/shoulder pain for 3+ mo	−0.7118	0.3416	−2.0835	0.0372	0.0214	0.0064
Rheumatoid arthritis	−0.6804	0.3244	−2.0974	0.036	0.0044	0.0014
ADHD	−0.6738	0.3204	−2.1031	0.0355	0.2428	0.0988
Length of working week for main job	−0.6473	0.2681	−2.4141	0.0158	0.0191	0.0029
Complications of internal orthopaedic prosthetic devices	−0.614	0.3208	−1.9138	0.0557	0.0052	0.0017
Cholelithiasis/gall stones	−0.5583	0.2568	−2.1743	0.0297	0.0083	0.0015
Mononeuropathies of upper limb	−0.5271	0.2086	−2.5264	0.0115	0.0131	0.0018
Internal derangement of knee	−0.4772	0.231	−2.0662	0.0388	0.0081	0.002
Hip pain	−0.4486	0.3536	−1.2687	0.2046	0.0071	0.0029
Duration of walks	−0.4281	0.1406	−3.0447	0.0023	0.0463	0.0029
Heavy physical activity (eg, weeding, carpentry)	−0.4168	0.1579	−2.6392	0.0083	0.0343	0.002
Noisy workplace	−0.4153	0.1782	−2.3303	0.0198	0.062	0.006
Job involves shift work	−0.4098	0.1847	−2.2185	0.0265	0.03	0.0029
Osteoarthritis	−0.3844	0.1659	−2.3169	0.0205	0.0186	0.0018
Disability diagnosed by doctor	−0.3595	0.1612	−2.23	0.0257	0.025	0.0019
Number of cigarettes previously smoked daily	−0.3557	0.165	−2.1554	0.0311	0.0996	0.0143
Falls in the last year	−0.3544	0.1513	−2.3416	0.0192	0.0321	0.002
Getting up in morning	−0.3128	0.1151	−2.7181	0.0066	0.0686	0.0031
Mouth/teeth dental problems	−0.3069	0.1363	−2.2521	0.0243	0.048	0.0028
Pain all over body	−0.2673	0.1397	−1.9136	0.0557	0.0309	0.0034
Pack years of smoking	−0.2538	0.1376	−1.8445	0.0651	0.1086	0.0104
Risk taking	−0.247	0.1176	−2.1013	0.0356	0.0561	0.0028
Extreme BMI	−0.2348	0.1271	−1.8468	0.0648	0.6852	0.0576
Disability or infirmity	−0.2339	0.1252	−1.8685	0.0617	0.0496	0.0025
Overweight	−0.221	0.1114	−1.9843	0.0472	0.1093	0.0068
MCP	−0.1479	0.092	−1.6075	0.1079	0.0783	0.0029
Neck or shoulder pain	−0.0909	0.119	−0.7634	0.4452	0.0491	0.0032
Headache	−0.0584	0.0959	−0.6088	0.5427	0.0867	0.0043
Back pain	−0.0476	0.1474	−0.3228	0.7468	0.0326	0.0032
Knee pain	0.0283	0.18	0.1575	0.8748	0.0187	0.0039
Stomach or abdominal pain	0.0496	0.286	0.1733	0.8624	0.0109	0.0029
Facial pain	0.0966	0.2035	0.475	0.6348	0.0165	0.0034
Morning/evening person	0.1871	0.1022	1.83	0.0672	0.1157	0.0046
Schizophrenia	0.2109	0.0886	2.3805	0.0173	0.462	0.0192
Neuroticism score	0.2165	0.1132	1.9126	0.0558	0.1191	0.0064
Guilty feelings	0.2183	0.122	1.7895	0.0735	0.052	0.0028
Coronary artery disease	0.2341	0.1325	1.7672	0.0772	0.0792	0.0058
Bipolar disorder	0.2473	0.1442	1.7147	0.0864	0.4282	0.0362
Suffer from nerves	0.2499	0.1295	1.9297	0.0536	0.046	0.0031
Narcolepsy	0.2509	0.1315	1.9081	0.0564	0.049	0.0027
Time spent watching television	0.2701	0.1257	2.148	0.0317	0.0991	0.004
Nervous feelings	0.2843	0.1283	2.2158	0.0267	0.0669	0.0042
Psoriasis	0.4522	0.2555	1.7702	0.0767	0.0075	0.0017

(*continued on next page*)

Table 4 (*continued*)

Trait	r_g	r_g SE	z	P	h2	h2 SE
Triglycerides in medium VLDL	0.5529	0.2981	1.8549	0.0636	0.0946	0.0301
Celiac disease	0.5604	0.2962	1.8918	0.0585	0.2959	0.0502
Acetate	0.5861	0.3195	1.8344	0.0666	0.0556	0.0192
Nasal polyps	0.6148	0.3046	2.0187	0.0435	0.0053	0.0016
Fracture resulting from simple fall	0.7123	0.3442	2.0692	0.0385	0.0518	0.0154
Pneumothorax	0.8217	0.4166	1.9722	0.0486	0.0031	0.0014

Genetic correlation (r_g) and heritability estimates (h2), as well as the standard errors (SEs) and test statistics were computed with the linkage disequilibrium score regression (LDSC) method. PSQ genetic correlations with 832 traits were computed on LD Hub. We also computed PSQ r_g with CPT, and an additional 9 pain phenotypes (in italic) from Refs. 21, 31 The table includes 49 entries (top 40 traits from LD hub, based on *P* values and after excluding related phenotypes, + CPT + 8 additional pain phenotypes). None of the r_g *P* values passed the discovery threshold *P* values ($0.05/832 = 6 \times 10^{-5}$).
ADHD, attention deficit hyperactivity disorder; BMI, body mass index; CPT, cold pressor test; GWAS, genome-wide association studies; MCP, multisite chronic pain; PSQ, pain sensitivity questionnaire.

association,[2] and the CPT was never used as cold stimuli in these published studies. None of the 3 main variants in *MC1R* that control red hair color showed genome-wide significant associations with PSQ score or CPT duration. Nevertheless, in combination, individuals carrying one or more copies of these 3 variants reported a significant higher self-perceived pain sensitivity.

The opposite direction of the genetic and phenotypic correlations between pain sensitivity and susceptibility traits was unexpected. Although higher pain sensitivity, measured by the PSQ and CPT, was consistently associated with higher pain susceptibility and medication usage, the bivariate LDSC analyses suggested that the genetic architectures of pain sensitivity and pain susceptibility were inversely correlated. It is an unusual result because empirical evidence across plant and animal species, including human, had supported the Cheverud Conjecture, which states that genetic correlations usually mirror phenotypic correlations.[42] The opposite direction suggests a strong and positive environmental correlation between pain sensitivity and susceptibility traits. It is generally recognized that chronic exposure to pain increases pain sensitivity through central sensitization, an excessive responsiveness to pain in the nociceptive pathway.[22,34] Our results seemed to support this relationship with a higher measured sensitivity pain in participants reporting pain and pain medication usage, but also in older participants and participants reporting multiple pains. However, it has also been reported that pain tolerance in patients with acute pain could increase via self-induced positive expectancies or cognitive–behavioral therapy.[34,36] It may explain the observed positive genetic correlations between the PSQ score and acute pain traits, such as pneumothorax and fracture resulting from a simple fall, and the apparent decrease of pain sensitivity for participants reporting only one pain condition (Supplementary Fig. 3, available at http://links.lww.com/PAIN/B557). We explored the causal relationship between pain sensitivity and susceptibility with an MR

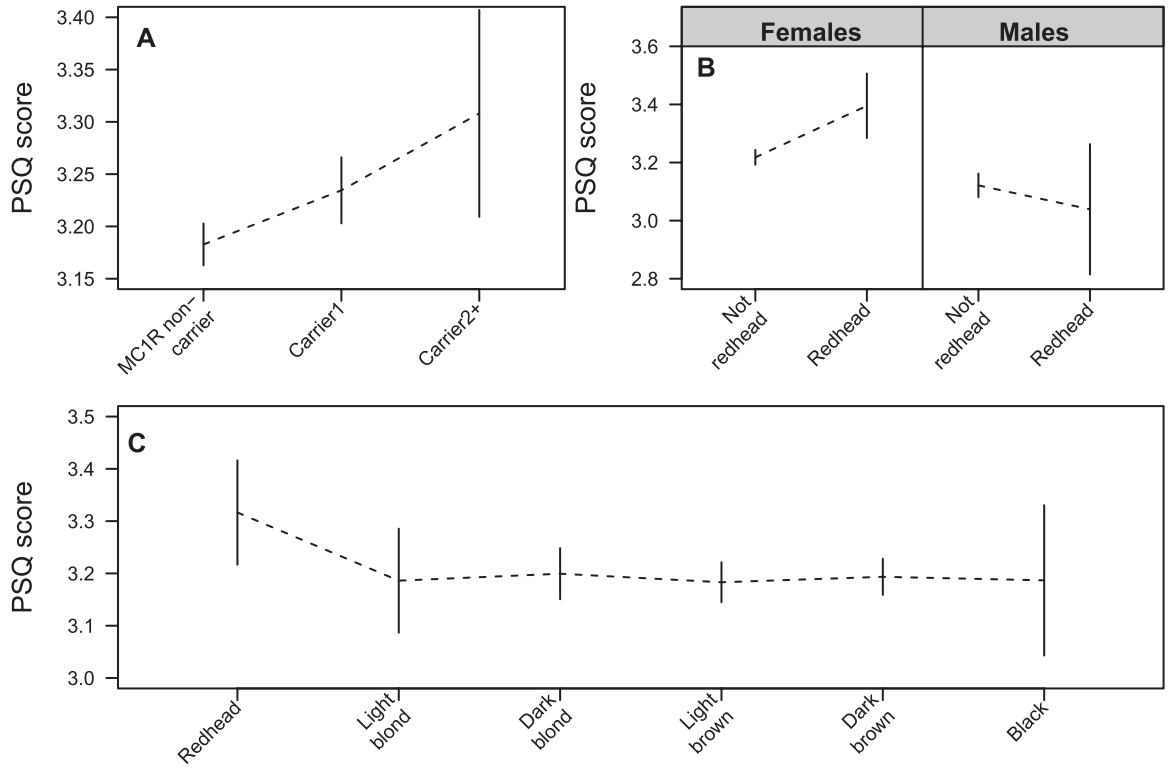

Figure 3. Regional association plot for TSSC1 locus and QQ plot from the PSQ GWAS. GWAS, genome-wide association studies; PSQ, pain sensitivity questionnaire.

approach, but the results were inconclusive, probably because of the lack of statistical power in the PSQ GWAS. It was reassuring to also observe negative genetic correlations between pain sensitivity and disability phenotypes, including complications associated with prosthetic devices, and with unhealthy behaviors such as smoking, extreme BMI, length of working week, shift work, or working in a noisy environment.[10] Finally, we also observed significant genetic correlations between pain sensitivity and neurological disorders or personality traits. Notably, pain sensitivity showed a strong negative genetic correlation with ADHD.[12] However, we observed positive genetic correlations between pain sensitivity and schizophrenia, neuroticism, and bipolar disorder. It is also interesting to note the absence of genetic correlation with migraine and depression despite the fact that migraine, in particular, is associated with intense pain.

Even with the largest PSQ score and CPT duration datasets collected to date in a general population, the results of this study should be interpreted with caution. Uncovering the genetic architecture of complex traits requires generally large datasets, and our results confirmed that pain sensitivity will not be an exception. Because of the limited statistical power of the PSQ and CPT GWAS, many of the highlighted genes, pathways, and genetic correlations were marginally significant and will require validation in larger datasets or with functional experiments. The study focused on only 2 measures of pain sensitivity, but because the interindividual and temporal variation of pain sensitivity depends on the integration of the sensory pathways and the emotional–cognitive states of individuals, it is unlikely that the PSQ and CPT captured the total variability of pain sensitivity within the 23andMe cohort. The PSQ hinges on the participant's own memory of painful experiences, whereas the CPT measures sensitivity to cold temperatures. More advanced or combination of measures covering the full pain sensitivity spectrum, such as quantitative sensory tests but also self-perceived pain sensitivity instruments, will be required to identify and disentangle the genetic architecture of pain sensitivity.[16,39] The lack of statistical power also limited our ability to fully characterize the home-based CPT used in this study. Larger datasets and CPT measured in more traditional clinical setups will certainly be required. Nevertheless, despite these limitations, we demonstrated that it is now possible to study the genetics of pain sensitivity at a population scale, by deploying and collecting relevant pain sensitivity data for fairly sophisticated instruments such as the CPT. We showed that the genetic architecture of pain sensitivity is related to the genetic architecture of pain susceptibility, but the relationship is probably more complex than it was initially perceived. Our results also provided some support to previous reports, suggesting that people with red hair are more sensitive to certain types of pain and that the higher pain sensitivity could be modulated by genetic polymorphism in *MC1R*. However, the bulk of the genetic architecture identified in this study implicated the brain, and not the peripheral nociception system, as the main modulator of pain sensitivity.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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P. Fontanillas and J. Y. Tung are employed by and hold stock or stock options in 23andMe, Inc.

Data availability: The complete summary statistics for the PSQ and CPT GWAS can be requested at https://research.23andme.com/dataset-access/.

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B557.

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References

[1] Allen JD, Bishop JR. A systematic review of genome-wide association studies of antipsychotic response. Pharmacogenomics 2019;20:291–306.
[2] Andresen T, Lunden D, Drewes AM, Arendt-Nielsen L. Pain sensitivity and experimentally induced sensitisation in red haired females. Scand J Pain 2011;2:3–6.
[3] Battle A, Mostafavi S, Zhu X, Potash JB, Weissman MM, McCormick C, Haudenschild CD, Beckman KB, Shi J, Mei R, Urban AE, Montgomery SB, Levinson DF, Koller D. Characterizing the genetic basis of transcriptome diversity through RNA-sequencing of 922 individuals. Genome Res 2014;24:14–24.
[4] BIOS Consortium, Social Science Genetic Association Consortium, Baselmans BML, Jansen R, Ip HF, van Dongen J, Abdellaoui A, van de Weijer MP, Bao Y, Smart M, Kumari M, Willemsen G, Hottenga JJ, Boomsma DI, de Geus EJC, Boomsma DI, de Geus EJC, Nivard MG, Bartels M. Multivariate genome-wide analyses of the well-being spectrum. Nat Genet 2019;51:445–51.
[5] Borsook D, Youssef AM, Simons L, Elman I, Eccleston C. When pain gets stuck: the evolution of pain chronification and treatment resistance. PAIN 2018;159:2421–36.
[6] Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512–25.
[7] Calvo M, Zhu N, Tsantoulas C, Ma Z, Grist J, Loeb JA, Bennett DL. Neuregulin-ErbB signaling promotes microglial proliferation and chemotaxis contributing to microgliosis and pain after peripheral nerve injury. J Neurosci 2010;30:5437–50.
[8] Cao S, Fisher DW, Yu T, Dong H. The link between chronic pain and Alzheimer's disease. J Neuroinflammation 2019;16:204.
[9] Chen X, Chen H, Cai W, Maguire M, Ya B, Zuo F, Logan R, Li H, Robinson K, Vanderburg CR, Yu Y, Wang Y, Fisher DE, Schwarzschild MA. The melanoma-linked "redhead" MC1R influences dopaminergic neuron survival. Ann Neurol 2017;81:395–406.
[10] Dean E, Söderlund A. What is the role of lifestyle behaviour change associated with non-communicable disease risk in managing musculoskeletal health conditions with special reference to chronic pain? BMC Musculoskelet Disord 2015;1316:87.

[11] de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput Biol 2015;11:e1004219.
[12] Demontis D, Walters RK, Martin J, Mattheisen M, Als TD, Agerbo E, Baldursson G, Belliveau R, Bybjerg-Grauholm J, Bækvad-Hansen M, Cerrato F, Chambert K, Churchhouse C, Dumont A, Eriksson N, Gandal M, Goldstein JI, Grasby KL, Grove J, Gudmundsson OO, Hansen CS, Hauberg ME, Hollegaard MV, Howrigan DP, Huang H, Maller JB, Martin AR, Martin NG, Moran J, Pallesen J, Palmer DS, Pedersen CB, Pedersen MG, Poterba T, Poulsen JB, Ripke S, Robinson EB, Satterstrom FK, Stefansson H, Stevens C, Turley P, Walters GB, Won H, Wright MJ, ADHD Working Group of the Psychiatric Genomics Consortium (PGC); Early Lifecourse & Genetic Epidemiology (EAGLE) Consortium; 23andMe Research Team, Andreassen OA, Asherson P, Burton CL, Andreassen OA, Asherson P, Burton CL, Boomsma DI, Cormand B, Dalsgaard S, Franke B, Gelernter J, Geschwind D, Hakonarson H, Haavik J, Kranzler HR, Kuntsi J, Langley K, Lesch KP, Middeldorp C, Reif A, Rohde LA, Roussos P, Schachar R, Sklar P, Sonuga-Barke EJS, Sullivan PF, Thapar A, Tung JY, Waldman ID, Medland SE, Stefansson K, Nordentoft M, Hougaard DM, Werge T, Mors O, Mortensen PB, Daly MJ, Faraone SV, Børglum AD, Neale BM. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat Genet 2019;51:63–75.
[13] Fillingim RB, Loeser JD, Baron R, Edwards RR. Assessment of chronic pain: domains, methods, and mechanisms. J Pain 2016;17:T10–20.
[14] Flegr J, Sýkorová K. Skin fairness is a better predictor for impaired physical and mental health than hair redness. Sci Rep 2019;9:18138.
[15] Frost P, Kleisner K, Flegr J. Health status by gender, hair color, and eye color: red-haired women are the most divergent. PLoS One 20172017;12:e0190238.
[16] Garland EL. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim Care 2012;39:561–71.
[17] Gershlick DC, Schindler C, Chen Y, Bonifacino JS. TSSC1 is novel component of the endosomal retrieval machinery. Mol Biol Cell 2016;27:2867–78.
[18] Healy E, Jordan SA, Budd PS, Suffolk R, Rees JL, Jackson IJ. Functional variation of MC1R alleles from red-haired individuals. Hum Mol Genet 2001;10:2397–402.
[19] Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers TH, Farh KH, Cuenca-Leon E, Muona M, Furlotte NA, Kurth T, Ingason A, McMahon G, Ligthart L, Terwindt GM, Kallela M, Freilinger TM, Ran C, Gordon SG, Stam AH, Steinberg S, Borck G, Koiranen M, Quaye L, Adams HH, Lehtimäki T, Sarin AP, Wedenoja J, Hinds DA, Buring JE, Schürks M, Ridker PM, Hrafnsdottir MG, Stefansson H, Ring SM, Hottenga JJ, Penninx BW, Färkkilä M, Artto V, Kaunisto M, Vepsäläinen S, Malik R, Heath AC, Madden PA, Martin NG, Montgomery GW, Kurki MI, Kals M, Mägi R, Pärn K, Hämäläinen E, Huang H, Byrnes AE, Franke L, Huang J, Stergiakouli E, Lee PH, Sandor C, Webber C, Cader Z, Muller-Myhsok B, Schreiber S, Meitinger T, Eriksson JG, Salomaa V, Heikkilä K, Loehrer E, Uitterlinden AG, Hofman A, van Duijn CM, Cherkas L, Pedersen LM, Stubhaug A, Nielsen CS, Männikkö M, Mihailov E, Milani L, Göbel H, Esserlind AL, Christensen AF, Hansen TF, Werge TInternational Headache Genetics Consortium, International Headache Genetics Consortium, , Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers TH, Farh KH, Cuenca-Leon E, Muona M, Furlotte NA, Kurth T, Ingason A, McMahon G, Ligthart L, Terwindt GM, Kallela M, Freilinger TM, Ran C, Gordon SG, Stam AH, Steinberg S, Borck G, Koiranen M, Quaye L, Adams HH, Lehtimäki T, Sarin AP, Wedenoja J, Hinds DA, Buring JE, Schürks M, Ridker PM, Hrafnsdottir MG, Stefansson H, Ring SM, Hottenga JJ, Penninx BW, Färkkilä M, Artto V, Kaunisto M, Vepsäläinen S, Malik R, Heath AC, Madden PA, Martin NG, Montgomery GW, Kurki MI, Kals M, Mägi R, Pärn K, Hämäläinen E, Huang H, Byrnes AE, Franke L, Huang J, Stergiakouli E, Lee PH, Sandor C, Webber C, Cader Z, Muller-Myhsok B, Schreiber S, Meitinger T, Eriksson JG, Salomaa V, Heikkilä K, Loehrer E, Uitterlinden AG, Hofman A, van Duijn CM, Cherkas L, Pedersen LM, Stubhaug A, Nielsen CS, Männikkö M, Mihailov E, Milani L, Göbel H, Esserlind AL, Christensen AF, Hansen TF, Werge T, Kaprio J, Kaprio J, Aromaa AJ, Raitakari O, Ikram MA, Spector T, Järvelin MR, Metspalu A, Kubisch C, Strachan DP, Ferrari MD, Belin AC, Dichgans M, Wessman M, van den Maagdenberg AM, Zwart JA, Boomsma DI, Smith GD, Stefansson K, Eriksson N, Daly MJ, Neale BM, Olesen J, Chasman DI, Nyholt DR, Palotie A. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat Genet 2016;48:856–66.
[20] Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, van der Brug M, Cai FInternational Parkinson's Disease Genomics Consortium, 23andMe Research Team, International Parkinson's Disease Genomics Consortium; 23andMe Research Team, , Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, van der Brug M, Cai F, Kerchner GA, Ayalon G, Bingol B, Sheng M, Hinds D, Behrens TW, Singleton AB, Bhangale TR, Graham RR. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat Genet 2017;49:1511–16.
[21] Johnston KJA, Adams MJ, Nicholl BI, Ward J, Strawbridge RJ, Ferguson A, McIntosh AM, Bailey MES, Smith DJ. Genome-wide association study of multisite chronic pain in UK Biobank. PLoS Genet 2019;15:e1008164.
[22] Kim D, Chae Y, Park HJ, Lee IS. Effects of chronic pain treatment on altered functional and metabolic activities in the brain: a systematic review and meta-analysis of functional neuroimaging studies. Front Neurosci 2021;15:684926.
[23] Kuperman P, Granovsky Y, Bahouth H, Fadel S, Ben Lulu H, Bosak N, Buxbaum C, Sprecher E, Crystal S, Granot M. Explaining very early acute mild traumatic brain injury after motor vehicle collision pain variability: additive value of pain sensitivity questionnaire. Pain Rep 2020;5:e821.
[24] Li WQ, Gao X, Tworoger SS, Qureshi AA, Han J. Natural hair color and questionnaire-reported pain among women in the United States. Pigment Cell Melanoma Res 2016;29:239–42.
[25] Li Q, Wang Z, Zong L, Ye L, Ye J, Ou H, Jiang T, Guo B, Yang Q, Liang W, Zhang J, Long Y, Zheng X, Hou Y, Wu F, Zhou L, Li S, Huang X, Zhao C. Allele-specific DNA methylation maps in monozygotic twins discordant for psychiatric disorders reveal that disease-associated switching at the EIPR1 regulatory loci modulates neural function. Mol Psychiatry 2021. doi: 10.1038/s41380-021-01126-w [Epub ahead of print].
[26] Li QS, Parrado AR, Samtani MN, Narayan VA; Alzheimer's Disease Neuroimaging Initiative. Variations in the FRA10AC1 fragile site and 15q21 are associated with cerebrospinal fluid Aβ1-42 level. PLoS One 2015;10:e0134000.
[27] Liem EB, Joiner TV, Tsueda K, Sessler DI. Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. Anesthesiology 2005;102:509–14.
[28] Mano H, Seymour B. Pain: a distributed brain information network? PLoS Biol 2015;13:e1002037.
[29] McIntyre MH, Team 23andMe Research, Kless A, Hein P, Field M, Tung JY. Validity of the cold pressor test and pain sensitivity questionnaire via online self-administration. PLoS One 2020;15:e0231697.
[30] Meloto CB, Benavides R, Lichtenwalter RN, Wen X, Tugarinov N, Zorina-Lichtenwalter K, Chabot-Doré AJ, Piltonen MH, Cattaneo S, Verma V, Klares R III, Khoury S, Parisien M, Diatchenko L. Human pain genetics database: a resource dedicated to human pain genetics research. PAIN 2018;159:749–63.
[31] Meng W, Adams MJ, Reel P, Rajendrakumar A, Huang Y, Deary IJ, Palmer CNA, McIntosh AM, Smith BH. Genetic correlations between pain phenotypes and depression and neuroticism. Eur J Hum Genet 2020;28:358–66.
[32] Meng W, Chan BW, Harris C, Freidin MB, Hebert HL, Adams MJ, Campbell A, Hayward C, Zheng H, Zhang X, Colvin LA, Hales TG, Palmer CNA, Williams FMK, McIntosh A, Smith BH. A genome-wide association study finds genetic variants associated with neck or shoulder pain in UK Biobank. Hum Mol Genet 2020;29:1396–404.
[33] Morgan MD, Pairo-Castineira E, Rawlik K, Canela-Xandri O, Rees J, Sims D, Tenesa A, Jackson IJ. Genome-wide study of hair colour in UK Biobank explains most of the SNP heritability. Nat Commun 2018;9:5271.
[34] Nijs J, George SZ, Clauw DJ, Fernández-de-las-Peñas C, Kosek E, Ickmans K, Fernández-Carnero J, Polli A, Kapreli E, Huysmans E, Cuesta-Vargas AI, Mani R, Lundberg M, Leysen L, Rice D, Sterling M, Curatolo M. Central sensitisation in chronic pain conditions: latest discoveries and their potential for precision medicine. Lancet Rheumatol 2021;3:e383–92.
[35] Patanwala AE, Norwood C, Steiner H, Morrison D, Li M, Walsh K, Martinez M, Baker SE, Snyder EM, Karnes JH. Psychological and genetic predictors of pain tolerance. Clin Transl Sci 2019;12:189–95.
[36] Peerdeman KJ, van Laarhoven AI, Peters ML, Evers AW. An integrative review of the influence of expectancies on pain. Front Psychol 2016;7:1270.
[37] Pereira V, Goudet C. Emerging trends in pain modulation by metabotropic glutamate receptors. Front Mol Neurosci 2019;11:464.
[38] Randall CL, Wright CD, Chernus JM, McNeil DW, Feingold E, Crout RJ, Neiswanger K, Weyant RJ, Shaffer JR, Marazita ML. A preliminary genome-wide association study of pain-related fear: implications for orofacial pain. Pain Res Manag 2017;2017:7375468.
[39] Rolke R, Baron R, Maier C, Tölle TR, Treede DR, Beyer A, Binder A, Birbaumer N, Birklein F, Bötefür IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihöfner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. PAIN 2006;123:231–43.

[40] Ruscheweyh R, Marziniak M, Stumpenhorst F, Reinholz J, Knecht S. Pain sensitivity can be assessed by self-rating: development and validation of the Pain Sensitivity Questionnaire. PAIN 2009;146:65–74.
[41] Ruscheweyh R, Verneuer B, Dany K, Marziniak M, Wolowski A, Colak-Ekici R, Schulte TL, Bullmann V, Grewe S, Gralow I, Evers S, Knecht S. Validation of the pain sensitivity questionnaire in chronic pain patients. PAIN 2012;153:1210–8.
[42] Sodini SM, Kemper KE, Wray NR, Trzaskowski M. Comparison of genotypic and phenotypic correlations: Cheverud's conjecture in humans. Genetics 2018;209:941–8.
[43] Spisak T, Kincses B, Schlitt F, Zunhammer M, Schmidt-Wilcke T, Kincses ZT, Bingel U. Pain-free resting-state functional brain connectivity predicts individual pain sensitivity. Nat Commun 2020;11:187.
[44] Stockton SD Jr, Gomes I, Liu T, Moraje C, Hipólito L, Jones MR, Ma'ayan A, Morón JA, Li H, Devi LA. Morphine regulated synaptic networks revealed by integrated proteomics and network analysis. Mol Cell Proteomics 2015;14:2564–76.
[45] The 23andMe Research Team, Meng W, Adams MJ, Palmer CNA, Shi J, Shi J, Auton A, Ryan KA, Jordan JM, Mitchell BD, Jackson RD, Yau MS, McIntosh AM, Smith BH. Genome-wide association study of knee pain identifies associations with GDF5 and COL27A1 in UK Biobank. Commun Biol 2019;2:321.
[46] Thompson JM, Neugebauer V. Amygdala plasticity and pain. Pain Res Manag 2017;2017:8296501.
[47] Tian C, Hromatka BS, Kiefer AK, Eriksson N, Noble SM, Tung JY, Hinds DA. Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nat Commun 2017;8:599.
[48] Tomita H, Cornejo F, Aranda-Pino B, Woodard CL, Rioseco CC, Neel BG, Alvarez AR, Kaplan DR, Miller FD, Cancino GI. The protein tyrosine phosphatase receptor delta regulates developmental neurogenesis. Cell Rep 2020;30:215–28. e5.
[49] Tsepilov YA, Freidin MB, Shadrina AS, Sharapov SZ, Elgaeva EE, Zundert JV, Karssen LC, Suri P, Williams FMK, Aulchenko YS. Analysis of genetically independent phenotypes identifies shared genetic factors associated with chronic musculoskeletal pain conditions. Commun Biol 2020;3:329.
[50] Veinante P, Yalcin I, Barrot M. The amygdala between sensation and affect: a role in pain. J Mol Psychiatry 2013;1:9.
[51] Wandner LD, Scipio CD, Hirsh AT, Torres CA, Robinson ME. The perception of pain in others: how gender, race, and age influence pain expectations. J Pain 2012;13:220–7.
[52] Williams FM, Scollen S, Cao D, Memari Y, Hyde CL, Zhang B, Sidders B, Ziemek D, Shi Y, Harris J, Harrow I, Dougherty B, Malarstig A, McEwen R, Stephens JC, Patel K, Menni C, Shin SY, Hodgkiss D, Surdulescu G, He W, Jin X, McMahon SB, Soranzo N, John S, Wang J, Spector TD. Genes contributing to pain sensitivity in the normal population: an exome sequencing study. PLoS Genet 2012;8:e1003095.
[53] Young EE, Lariviere WR, Belfer I. Genetic basis of pain variability: recent advances. J Med Genet 2012;49:1–9.
[54] Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, Hemani G, Tansey K, Laurin C, Early Genetics and Lifecourse Epidemiology (EAGLE) Eczema Consortium, Pourcain BS, Pourcain BS, Warrington NM, Finucane HK, Price AL, Bulik-Sullivan BK, Anttila V, Paternoster L, Gaunt TR, Evans DM, Neale BM. LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. Bioinformatics 2017;33:272–9.
[55] Zorina-Lichtenwalter K, Maixner W, Diatchenko L. Detangling red hair from pain: phenotype-specific contributions from different genetic variants in melanocortin-1 receptor. PAIN 2020;161:938–48.
[56] Zorina-Lichtenwalter K, Meloto CB, Khoury S, Diatchenko L. Genetic predictors of human chronic pain conditions. Neuroscience 2016;338:36–62.