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

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Abstract

Altered pain sensitivity is believed to play an important role in the development of chronic pain, a common debilitating condition affecting an estimated 1 in 5 adults. Pain sensitivity varies broadly between individuals, and it is notoriously difficult to measure at scale in large populations. Although pain sensitivity is known to be moderately heritable, only a small number of genetic studies have been published and they have had limited success in identifying the genetic architecture of pain sensitivity. In this study, 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 (GWAS) on the PSQ score (n = 25,321) and CPT duration (n = 6,853). Despite a reasonably large sample size, we identified only one genome-wide significant locus associated with the PSQ score, which was located in the *TSSC1* gene (rs58194899, OR = 0.950 [0.933-0.967], P-value = $1.9*10^{-8}$). This gene is responsible for intracellular cell trafficking and it could modulate pain sensitivity via changes in neuroplasticity. PSQ score is genetically correlated with chronic and acute pain, including chronic neck and shoulder pain (r_g = 0.71), fracture (0.71), rheumatoid arthritis (0.68), osteoarthritis (0.38), and pneumothorax (0.82). PSQ score was also genetically correlated with known risk factors, such as the length of working week (0.65), noisy workplace (0.41), smoking (0.36), or extreme BMI (0.23). Gene-based analysis followed by pathway analysis showed that GWAS results were enriched for genes expressed in the brain, predominantly in the frontal cortex and basal ganglia, and enriched for genes 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 pain sensitivity as assessed by the PSQ. Overall, we detailed the genetic background of pain sensitivity using scalable at-home procedures that may serve as a blueprint for larger genetic studies.

Author Summary

Despite decades of research and many advances in our understanding of the physical, emotional, and psychological aspects, the genetic contribution to pain sensitivity is still largely unknown. We administered a pain sensitivity questionnaire in parallel with an experimental cold pressor test in a large, genotyped cohort of 31,000 research participants, and found a novel genome-wide genetic association in *TSSC1*. This gene is responsible for intracellular cell trafficking and it could modulate pain sensitivity via changes in neuroplasticity. Overall, the pain sensitivity measurements revealed strong positive genetic correlations with chronic and acute pain conditions, as well as with unhealthy traits and behaviors, such as obesity, smoking, or difficult working conditions. The genetic association results also suggested, perhaps unsurprisingly, that only the brain tissues could be relevant for pain sensitivity, and identified enrichments for glutamatergic synapse pathways. Despite the complexity of pain sensitivity, our study demonstrated that it is now possible to deploy sophisticated pain questionnaires online and perform experimental medicine tests in at-home settings to further study the genetic architecture of pain sensitivity.

Introduction

It has been established that pain sensitivity is predictive of acute postoperative pain, and of risk for future chronic pain conditions. The assessment of pain sensitivity requires well-controlled experimental pain and emotional stimuli [1]. In general, such assessments are time-consuming in clinical settings because there is substantial inter-individual variability in pain sensitivity and perception [2][3]. 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 [4], 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 [5][6], knee pain [7], neck and shoulder pain [8], and migraine [9][10]. 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. Only a couple of small GWAS studies have directly explored the genetic architecture of pain sensitivity [11][12]. 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 [13]. 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 [14–16].

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 [17]. The PSQ asks participants to imagine 14 painful situations and 3 non-painful control situations. Subjects are asked to rate their painfulness on a 0–10 numeric scale. The CPT measures how long subjects can immerse one hand in cold ice water. The validation study demonstrated that these two 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 (n = 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.

Results

A total of 25,321 and 6,853 research participants of European ancestry were included in the PSQ and CPT GWAS analyses, respectively (Table 1). The two cohorts were largely independent; only 1,534 participants were included in both analyses. The sex ratio was unbalanced in both cohorts, with 71% and 63% of females, respectively. On average, females reported a higher pain sensitivity with a mean PSQ score of 3.23±0.01 versus 3.11±0.01 in males, as well as a lower tolerance to the CPT with 72±0.7 vs. 94±1.0 seconds of hand immersion in ice water. The PSQ and CPT distributions are presented in S1 Fig, and a full description of the overlapping participants has been published elsewhere [18].

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, which is also known as *EIPR1* (Fig 1A and Fig 2B). The

associated haplotype was relatively small (54 variants in the 99% credible set), and entirely located within the *TSSC1* gene boundary (Figure 2A). To date, no studies have reported a phenotypic association with this haplotype (https://genetics.opentargets.org/). However, a cognitive decline GWAS reported an independent association in the *TSSC1* gene (lead variant rs75365287) [19]. TSSC1 is a component of the endosomal retrieval pathway. It plays a critical role as a regulator of both Golgi-associated retrograde protein (GARP) and endosome-associated recycling protein (EARP) functions, as well as the transport of internalized proteins to the plasma membrane [20,21]. TSSC1 and the GARP/EARP 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 < 1.0×10^{-4} (Table 3). These genes were over-expressed in the brain, especially in the frontal cortex, basal ganglia, amygdala, and hypothalamus (S5 Fig). They were significantly enriched for genes involved in brain development and synaptic signaling pathways (Table 3 and S6 Table). 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*; S6 Fig). 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 highest genetic correlations with PSQ, as computed on LD Hub, are listed in Table 4. PSQ score was strongly 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), and with acute pain phenotypes, such

as pneumothorax (0.82) or fracture (0.71). It was also strongly 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 strong positive genetic correlation between the PSQ score and ADHD (0.67), but negative correlations with schizophrenia (-0.21), bipolar disorder (-0.25), and neuroticism (-0.22).

The GWAS on CPT duration was underpowered and did not produce any significant genome-wide associations (Fig. 1b, S2 Fig., and S1 Table). Nevertheless, the genetic correlation between PSQ score and CPT duration was r_g = -0.64 ± 0.37 (P-value = 0.088), 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 (S7 Fig.) and enriched for brain development and synaptic signaling pathways (S6 Table).

We also specifically focused on the association results for genes involved in nociception. We obtained a list of 26 nociception genes from the Human Pain Genes Database ([22], https://humanpaingenetics.org/hpgdb/). None of the 21 nociception genes showed evidence of association with PSQ and CPT (S7 Table, MAGMA analysis).

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 5,243 participants were included in the analyses for PSQ score and CPT duration, respectively. Using a Gaussian linear model including age, sex, and the first five 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. 3c and S2 Table). Furthermore, females with red hair reported on average higher PSQ scores than non-red hair females, and red or non-red hair males (sex-by-red hair interaction P-value = 0.046; S2 Table and Fig. 3b). We did not observe significant PSQ

differences between red and non-red hair males. For CPT, we did not observe any significant associations using a Cox proportional hazards model (S3 Table and S4 Fig.) or an ANOVA on CPT duration converted in ranks (S4 Table). Since red hair color is partially determined by recessive genetic polymorphism in the *MC1R* gene, we explored the association of the three main variants rs1805009 (D294H), rs1805008 (R160W) and rs1805007 (R151C) in the PSQ and CPT GWAS (Table 2 and S1 Table). None of these individual variants passed the genome-wide significant threshold in the GWAS analyses (P-values > 5.1×10^{-3}). We combined these three variants and defined three categories of *MC1R* variant carriers, Non-carrier (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 non-carriers (P-value = 6.8×10^{-3} and 1.5×10^{-2}, respectively; S2 Table and Fig. 3a). On the other hand, we did not observe any significant associations between *MC1R* carriers and CPT duration (S3 and S4 Tables, and S3 Fig.)

Discussion

The purpose of this study was to identify genetic factors contributing to the individual perception of pain [23]. We used two self-administered pain sensitivity measurements, the PSQ and CPT, and established that they could be reliable phenotypes for evaluating the genetic architecture of pain sensitivity. PSQ score and CPT duration have been previously shown to be only moderately phenotypically correlated (r = -0.22 [-0.27, -0.17]) [8]. The PSQ is a 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 incorporates the emotional and cognitive components of the pain sensitivity [24].

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 (e.g. frontal cortex, basal ganglia, and hypothalamus) also showed significant enrichment [25,26]. 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 [27,28]. It is interesting to notice the absence of association enrichments in non-brain tissues. According to the current multifaceted experience concept, pain results from the integration of nociception and the cognitive-emotional state of the individual. Nociceptors are found in skin and mucosa, as well as in a variety of organs, such as the digestive tract, the bladder, the gut, and muscles. However, our analysis of the 21 nociceptor genes identified in HPGDB 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, ten 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 [9]. 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 [29]. Among the six 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 [30]. To our knowledge, however, our study is the first where genetic polymorphism in these two genes has been associated with pain sensitivity measurements. On the other hand, the four other genes in the pathway, *PTPRD, DLG2, GPC6*, and *GRID2*, have

been all associated with diverse neurological disorders [31–34]. This observation supports earlier findings that neurological disorders and pain sensitivity are intimately linked [35]. The genome-wide significant locus, *TSSC1,* has not previously been associated with pain traits. However, it has been associated with cognitive decline [19], and was recently identified in a study that examined alterations in the postsynaptic protein profile as a consequence of prolonged exposure to morphine [36].

Finally, we confirmed that women with red hair are more sensitive to pain, but we did not observe this relationship in men. None of the three 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 three variants reported a significant higher pain sensitivity.

Overall, the PSQ score and CPT duration had similar genetic correlation profiles — both were correlated with general pain phenotypes such as neck-and-shoulder pain, rheumatoid arthritis, osteoarthritis, neuropathy, and fracture. We also observed positive genetic correlations between pain sensitivity and disability phenotypes, including complications associated with prosthetic devices. Given that epidemiological studies have regularly reported that unhealthy lifestyle practices accentuate pain sensitivity and chronic pain [37], it was encouraging to observe that the pain sensitivity phenotypes were genetically correlated with unhealthy behaviors such as smoking, extreme BMI, length of working week, shift work, or working in a noisy environment. As expected, we also observed significant genetic correlations between pain sensitivity and neurological disorders or personality traits. Notably, pain sensitivity showed a strong positive genetic correlation with ADHD. This was somewhat unexpected based on the observed genetic architectures described in recent pain and ADHD GWAS studies [38]. On the other hand, we observed negative 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.

Because the inter-individual variation of pain sensitivity depends on the integration of the sensory pathways and the emotional-cognitive states of individuals, it will require more advanced measures of the full pain sensitivity spectrum to identify and disentangle the genetic architecture of pain sensitivity [39, 40]. This study focused only on a narrow set of pain sensitivity measurements. However, we demonstrated that it is now possible to deploy and collect relevant data at a population scale for fairly sophisticated tests such as the CPT. Large sample sizes are commonly needed for uncovering the genetic architecture of complex traits and our results confirmed that pain sensitivity will not be an exception. A larger PSQ dataset should be particularly informative, not only for validating some of the results of this study, but also for evaluating the potential genetic basis of pain sensitivity differences between females and males, and for focusing on specific imaginary pain situations included in the PSQ.

Materials and Methods

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.

Pain sensitivity traits

For the assessment of the pain interpretation and perception, we used a pain sensitivity questionnaire (PSQ) and an at-home version of cold pressor test (CPT) on two subsets of 23andMe research participants who self-reported chronic pain conditions [2]. The PSQ is an English-language version of the Pain Sensitivity Questionnaire [3] [41], supplemented with additional questions about the participant's own memory of painful experiences. The PSQ contains 14 questions in which you should imagine yourself in certain situations. You should then grade how painful they would be, from 0 that stands for no pain to 10, the most severe pain that you can imagine or consider possible. The total PSQ score is the mean of the 14 responses. We also computed two 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, 4). For the CPT, participants were asked to prepare their own bath of ice water at home, and to keep their non-dominant hand submerged to the wrist for no more than 150 seconds. A separate consent for the CPT was used: participants reporting neurological or

temperature-triggered conditions (e.g. migraine, history of syncope, or Raynaud's phenomenon) or current injuries to their non-dominant hands at the time of recruitment were ineligible. For additional information on the PSQ and CPT, in particular the validity of these approaches for estimating pain sensitivity [18].

Genotyping and variant imputation

DNA extraction and genotyping were performed on saliva samples by LabCorp, Inc. Participants were genotyped on one of five Illumina genotyping platforms, containing between 550,000 to 950,000 variants, for a total of 1.6 million of genotyped variants. Samples that failed to reach 98.5% call rate were re-analyzed. Genotyping quality controls included discarding variants with a Hardy-Weinberg $P<10^{-20}$, a call rate of <90%, or batch effects. About 57.5M 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 five genotyping platforms. For more details on genotyping, imputation process, and variant quality controls, see [21].

GWAS 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-value were computed using a likelihood ratio test. The CPT duration was analyzed using a cox proportional hazards model, a survival model on the CPT time. We included covariates for age, gender, genotyping platform, and the top five 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 1.3M genotyped and 25.5M 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 over-dispersion, reducing to 13.7M variants available for follow-up analyses. A detailed description of the variant quality control and GWAS methods can be found in [42]. 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.

MC1R and hair color

We defined three categories of *MC1R* variant carriers by combining three variants, rs1805007, rs1805008, and rs1805009: Non-carrier (0 *MC1R* alleles), Carrier1 (1 allele), and Carrier2+ (>1 alleles) [17]. A self-reported hair color phenotype was available for 63% of the participants in the cohort [43]. It contained six 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. We also converted CPT duration in ranks, and tested associations using ANOVAs. The same set of covariables used in GWAS was included in these models.

Genetic correlation, gene-based and pathway analyses

Genetic correlations between PSQ and a broad list of diseases and traits, were estimated with LD Hub v1.9.1 (http://ldsc.broadinstitute.org/ldhub/), using the default analysis parameters. The gene-based analysis was performed on MAGMA (v1.07) [44]. 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 (DEG).

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References

1. 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: 2421–2436. doi:10.1097/j.pain.0000000000001401

2. Fillingim RB, Loeser JD, Baron R, Edwards RR. Assessment of Chronic Pain: Domains, Methods, and Mechanisms. J Pain Off J Am Pain Soc. 2016;17: T10-20. doi:10.1016/j.jpain.2015.08.010

3. 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. doi:10.1016/j.pain.2009.06.020

4. Young EE, Lariviere WR, Belfer I. Genetic basis of pain variability: recent advances. J Med Genet. 2012;49: 1–9. doi:10.1136/jmedgenet-2011-100386

5. Zorina-Lichtenwalter K, Meloto CB, Khoury S, Diatchenko L. Genetic predictors of human chronic pain conditions. Neuroscience. 2016;338: 36–62. doi:10.1016/j.neuroscience.2016.04.041

6. Tsepilov YA, Freidin MB, Shadrina AS, Sharapov SZ, Elgaeva EE, van Zundert J, et al. Analysis of genetically independent phenotypes identifies shared genetic factors associated with chronic musculoskeletal pain at different anatomic sites. Genomics; 2019 Oct. doi:10.1101/810283

7. The 23andMe Research Team, Meng W, Adams MJ, Palmer CNA, Shi J, Auton A, et al. Genome-wide association study of knee pain identifies associations with GDF5 and COL27A1 in UK Biobank. Commun Biol. 2019;2: 321. doi:10.1038/s42003-019-0568-2

8. Meng W, Chan BW, Harris C, Freidin MB, Hebert HL, Adams MJ, et al. A genome-wide association study finds genetic variants associated with neck or shoulder pain in UK Biobank. Hum Mol Genet. 2020; ddaa058. doi:10.1093/hmg/ddaa058

9. International Headache Genetics Consortium, Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, et al. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat Genet. 2016;48: 856–866. doi:10.1038/ng.3598

10. Siewert KM, Klarin D, Damrauer SM, Chang K-M, Tsao PS, Assimes TL, et al. Cross-trait analyses with migraine reveal widespread pleiotropy and suggest a vascular component to migraine headache. Int J Epidemiol. 2020;49: 1022–1031. doi:10.1093/ije/dyaa050

11. Williams FMK, Scollen S, Cao D, Memari Y, Hyde CL, Zhang B, et al. Genes Contributing to Pain Sensitivity in the Normal Population: An Exome Sequencing Study. Leal SM, editor. PLoS Genet. 2012;8: e1003095. doi:10.1371/journal.pgen.1003095

12. Randall CL, Wright CD, Chernus JM, McNeil DW, Feingold E, Crout RJ, et al. A Preliminary Genome-Wide Association Study of Pain-Related Fear: Implications for Orofacial Pain. Pain Res Manag. 2017;2017: 1–12. doi:10.1155/2017/7375468

13. Li W-Q, 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: 239–242. doi:10.1111/pcmr.12445

14. Chen X, Chen H, Cai W, Maguire M, Ya B, Zuo F, et al. The melanoma-linked “redhead” MC1R influences dopaminergic neuron survival. Ann Neurol. 2017;81: 395–406. doi:10.1002/ana.24852

15. Flegr J, Sýkorová K. Skin fairness is a better predictor for impaired physical and mental health than hair redness. Sci Rep. 2019;9: 18138. doi:10.1038/s41598-019-54662-5

16. 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: 938–948. doi:10.1097/j.pain.0000000000001780

17. 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: 2397–2402. doi:10.1093/hmg/10.21.2397

18. McIntyre M, 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. doi: 10.1371/journal.pone.023169

19. 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. doi:10.1371/journal.pone.0134000

20. Gershlick DC, Schindler C, Chen Y, Bonifacino JS. TSSC1 is novel component of the endosomal retrieval machinery. Mol Biol Cell. 2016;27: 2867–2878. doi:10.1091/mbc.E16-04-0209

21. Battle A, Mostafavi S, Zhu X, Potash JB, Weissman MM, McCormick C, et al. Characterizing the genetic basis of transcriptome diversity through RNA-sequencing of 922 individuals. Genome Res. 2014;24: 14–24. doi:10.1101/gr.155192.113

22. Meloto CB, Benavides R, Lichtenwalter RN, Wen X, Tugarinov N, Zorina-Lichtenwalter K, et al. Human pain genetics database: a resource dedicated to human pain genetics research. PAIN. 2018;159: 749–763. doi:10.1097/j.pain.0000000000001135

23. 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 Off J Am Pain Soc. 2012;13: 220–227. doi:10.1016/j.jpain.2011.10.014

24. Kuperman P, Granovsky Y, Bahouth H, Fadel S, Ben Lulu H, Bosak N, et al. 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. doi:10.1097/PR9.0000000000000821

25. Veinante P, Yalcin I, Barrot M. The amygdala between sensation and affect: a role in pain. J Mol Psychiatry. 2013;1: 9. doi:10.1186/2049-9256-1-9

26. Thompson JM, Neugebauer V. Amygdala Plasticity and Pain. Pain Res Manag. 2017;2017: 1–12. doi:10.1155/2017/8296501

27. Mano H, Seymour B. Pain: A Distributed Brain Information Network? PLoS Biol. 2015;13: e1002037. doi:10.1371/journal.pbio.1002037

28. Spisak T, Kincses B, Schlitt F, Zunhammer M, Schmidt-Wilcke T, Kincses ZT, et al. Pain-free resting-state functional brain connectivity predicts individual pain sensitivity. Nat Commun. 2020;11: 187. doi:10.1038/s41467-019-13785-z

29. Pereira V, Goudet C. Emerging Trends in Pain Modulation by Metabotropic Glutamate Receptors. Front Mol Neurosci. 2019;11: 464. doi:10.3389/fnmol.2018.00464

30. Calvo M, Zhu N, Tsantoulas C, Ma Z, Grist J, Loeb JA, et al. Neuregulin-ErbB Signaling Promotes Microglial Proliferation and Chemotaxis Contributing to Microgliosis and Pain after Peripheral Nerve Injury. J Neurosci. 2010;30: 5437–5450. doi:10.1523/JNEUROSCI.5169-09.2010

31. Tomita H, Cornejo F, Aranda-Pino B, Woodard CL, Rioseco CC, Neel BG, et al. The Protein Tyrosine Phosphatase Receptor Delta Regulates Developmental Neurogenesis. Cell Rep. 2020;30: 215-228.e5. doi:10.1016/j.celrep.2019.11.033

32. International Parkinson's Disease Genomics Consortium, 23andMe Research Team, Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat Genet. 2017;49: 1511–1516. doi:10.1038/ng.3955

33. BIOS consortium, Social Science Genetic Association Consortium, Baselmans BML, Jansen R, Ip HF, van Dongen J, et al. Multivariate genome-wide analyses of the well-being spectrum. Nat Genet. 2019;51: 445–451. doi:10.1038/s41588-018-0320-8

34. Allen JD, Bishop JR. A systematic review of genome-wide association studies of antipsychotic response. Pharmacogenomics. 2019;20: 291–306. doi:10.2217/pgs-2018-0163

35. Cao S, Fisher DW, Yu T, Dong H. The link between chronic pain and Alzheimer's disease. J Neuroinflammation. 2019;16: 204. doi:10.1186/s12974-019-1608-z

36. Stockton SD, Gomes I, Liu T, Moraje C, Hipólito L, Jones MR, et al. Morphine Regulated Synaptic Networks Revealed by Integrated Proteomics and Network Analysis. Mol Cell Proteomics. 2015;14: 2564–2576. doi:10.1074/mcp.M115.047977

37. 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 Apr 13;16:87. doi: 10.1186/s12891-015-0545-y. PMID: 25888381; PMCID: PMC4397667.

38. Demontis D, Walters RK., Martin J et al. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat Genet 51, 63–75. 2019

39. Rolke R, Baron R, Maier C, Tölle TR, Treede - D. R., Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values: Pain. 2006;123: 231–243. doi:10.1016/j.pain.2006.01.041

40. Garland EL. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim Care. 2012;39: 561–571. doi:10.1016/j.pop.2012.06.013

41. Ruscheweyh R, Verneuer B, Dany K, Marziniak M, Wolowski A, Colak-Ekici R, et al. Validation of the pain sensitivity questionnaire in chronic pain patients. Pain. 2012;153: 1210–1218. doi:10.1016/j.pain.2012.02.025

42. Tian C, Hromatka BS, Kiefer AK, Eriksson N, Noble SM, Tung JY, et al. Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nat Commun. 2017;8: 599. doi:10.1038/s41467-017-00257-5

43. Morgan MD, Pairo-Castineira E, Rawlik K, Canela-Xandri O, Rees J, Sims D, et al. Genome-wide study of hair colour in UK Biobank explains most of the SNP heritability. Nat Commun. 2018;9: 5271. doi:10.1038/s41467-018-07691-z

44. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput Biol. 2015;11: e1004219. doi:10.1371/journal.pcbi.1004219

Figures

Figure 1

Manhattan plots for PSQ and CPT GWAS.

Figure 2

Results of MC1R and hair color association with PSQ score.

Figure 3

Regional association plot for TSSC1 locus and QQ plot from the PSQ GWAS.

Supporting information

S1 Table. Top GWAS and MCR1 association results for PSQ and CPT traits.
S2 Table. Linear models on PSQ score analyzing MC1R carriers and hair colors.

S3 Table. Survival models on CPT duration analyzing MC1R carriers and hair colors.

S4 Table. ANOVA models on CPT duration (converted in ranks), analyzing MC1R carriers and hair colors.

S5 Table. Pathway enrichment results for PSQ.

S6 Table: Pathway enrichment results for CPT.

S7 Table: Gene-based (MAGMA) results for 21 nociception genes.

S1 Figure. PSQ and CPT distributions.

S2 Figure. Regional association plot of the top associated locus and QQ plot from the CPT GWAS.

S3 Figure. Results of MC1R and hair color models for CPT duration converted in ranks.

S4 Figure. Kaplan-Meier curves for MC1R and hair color survival models on CPT duration.

S5 Figure: Tissue expression enrichment analysis for PSQ, based on the 58 genes in Table 3 (GTEx v8).

S6 Figure: Enrichment analysis of cellular components for PSQ, based on the 58 genes in Table 3.

S7 Figure: Tissue expression enrichment analysis for CPT, based on the 50 genes in Table 3 (GTEx v8).

Tables

Table 1: Total number of participants included in the different analyses and mean (and SE) of PSQ score and CPT by sex and age classes.

	PSQ (score)		**CPT (in seconds)**	
	Females	Males	Females	Males
Full cohort (used in GWAS):				
# of participants	*18,060*	*7,261*	*4,343*	*2,510*
All	3.23 (0.01)	3.11 (0.01)	72.39 (0.75)	93.9 (1.01)
Per age classes:				
<40 years	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:				
Non-carrier	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 cohort:				
# of participants with hair color information	*10,928*	*4,239*	*3,375*	*1,868*
Proportion of redhead	*5.1%*	*3.2%*	*5%*	*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)

Table 2: Top GWAS variants and MCR1 association results for PSQ and CPT traits.

PSQ score were analyzed using a linear model. CPT duration (in seconds) were analyzed using a survival model.

						PSQ GWAS			**CPT GWAS**		
CHR	*POS*	*ID*	*Alleles*	*MAF*	*Gene context*	*Effect*	*SE*	*P-value*	*Effect*	*SE*	*P-value*
Top PSQ GWAS Variants:											
2	3280983	rs58194899	G/A	0.47	TSSC1	***-0.051***	***0.009***	***1.9e-08***	-0.01	0.018	5.8e-01
18	10008669	rs142738119	T/C	0.001	VAPA, APCDD1	0.696	0.138	4.7e-07	-0.035	0.275	9.0e-01
13	101893919	rs12583902	G/A	0.166	NALCN	0.059	0.012	7.6e-07	-0.025	0.023	2.9e-01
Top CPT GWAS Variant:											
17	65520139	rs141828201	G/A	0.027	PITPNC1	0.036	0.031	2.6e-01	0.356	0.066	2.4e-07
MC1R Variants:											
16	89986144	rs1805008	C/T	0.071	MC1R	0.031	0.017	6.8e-02	-0.032	0.034	3.5e-01
16	89986117	rs1805007	C/T	0.075	MC1R	0.047	0.017	5.1e-03	-0.039	0.033	2.4e-01
16	89986546	rs1805009	G/C	0.019	MC1R	0.041	0.032	2.0e-01	-0.089	0.063	1.5e-01

Table 3: Gene-based (MAGMA) and pathway analysis results for PSQ and CPT.

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

		PSQ		CPT		
Gene	**Position**	**# of variants**	**P-value**	**# of variants**	**P-value**	**Pathways**
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	
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
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***	

Table 4: Top genetic correlations between PSQ and LD Hub phenotypes

Trait	rG	rG se	z	P-value	h2	h2 se
Pneumothorax	0.8217	0.4166	-1.9722	0.0486	0.0031	0.0014
Fracture resulting from simple fall	0.7123	0.3442	-2.0692	0.0385	0.0518	0.0154
Neck/shoulder pain for 3+ months	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
Nasal polyps	0.6148	0.3046	-2.0187	0.0435	0.0053	0.0016
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
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 (Dentures)	0.3069	0.1363	2.2521	0.0243	0.048	0.0028
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
Overweight	0.221	0.1114	1.9843	0.0472	0.1093	0.0068
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
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

Figure 1: Manhattan plots for PSQ and CPT GWAS.

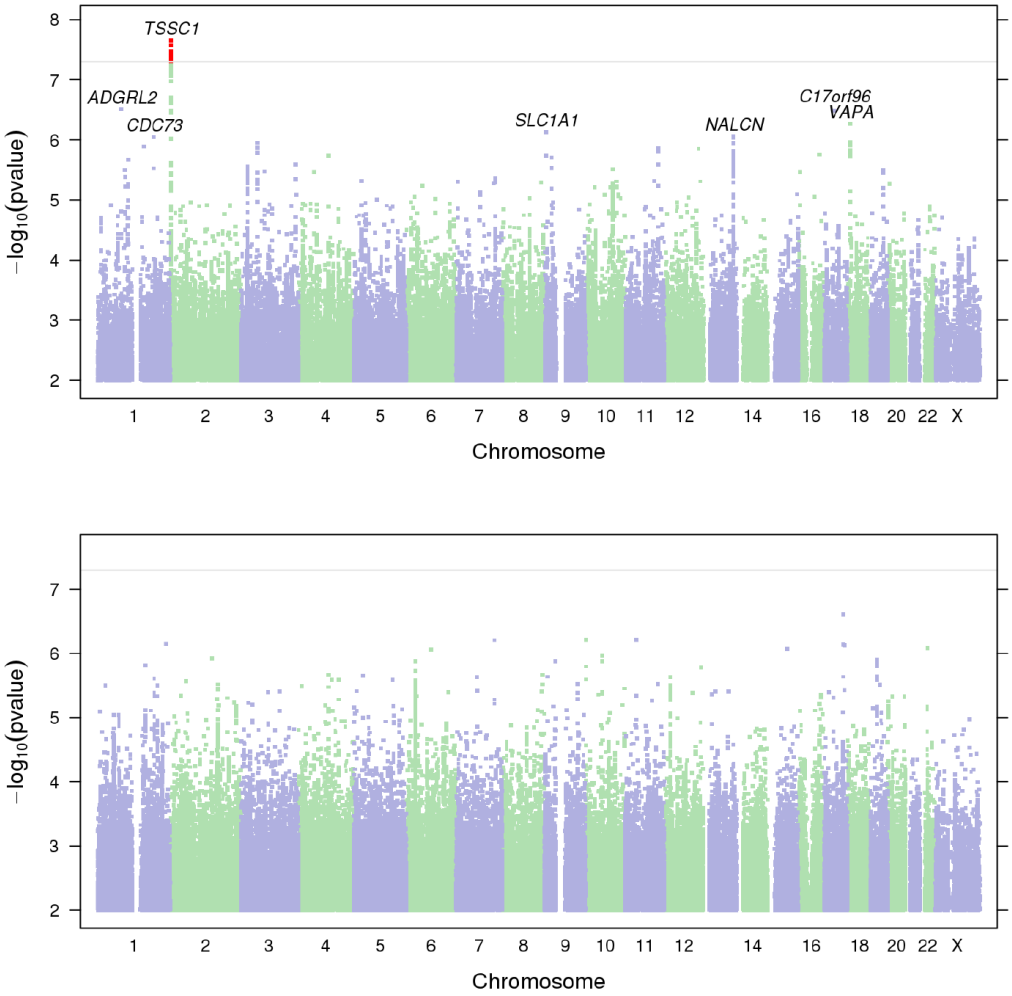


Figure 2: Regional association plot for TSSC1 locus and QQ plot from the PSQ GWAS

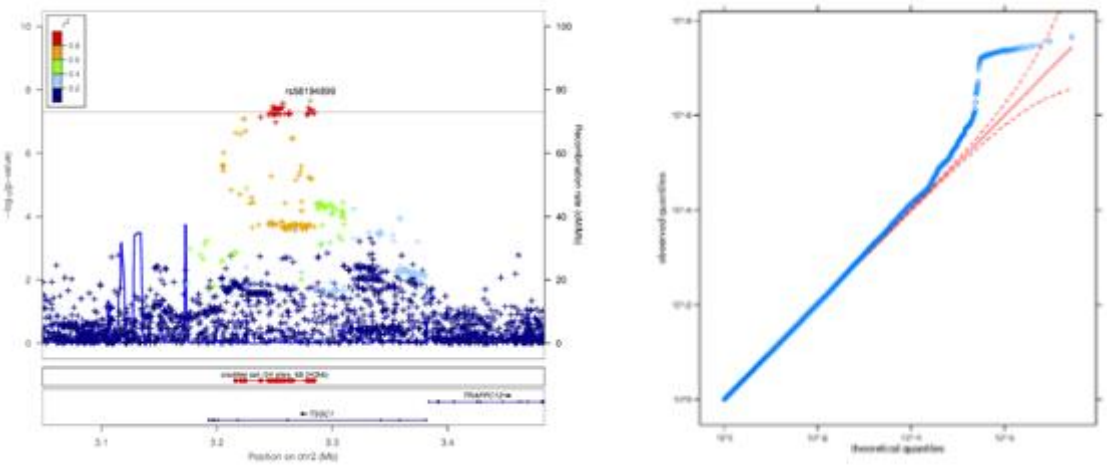
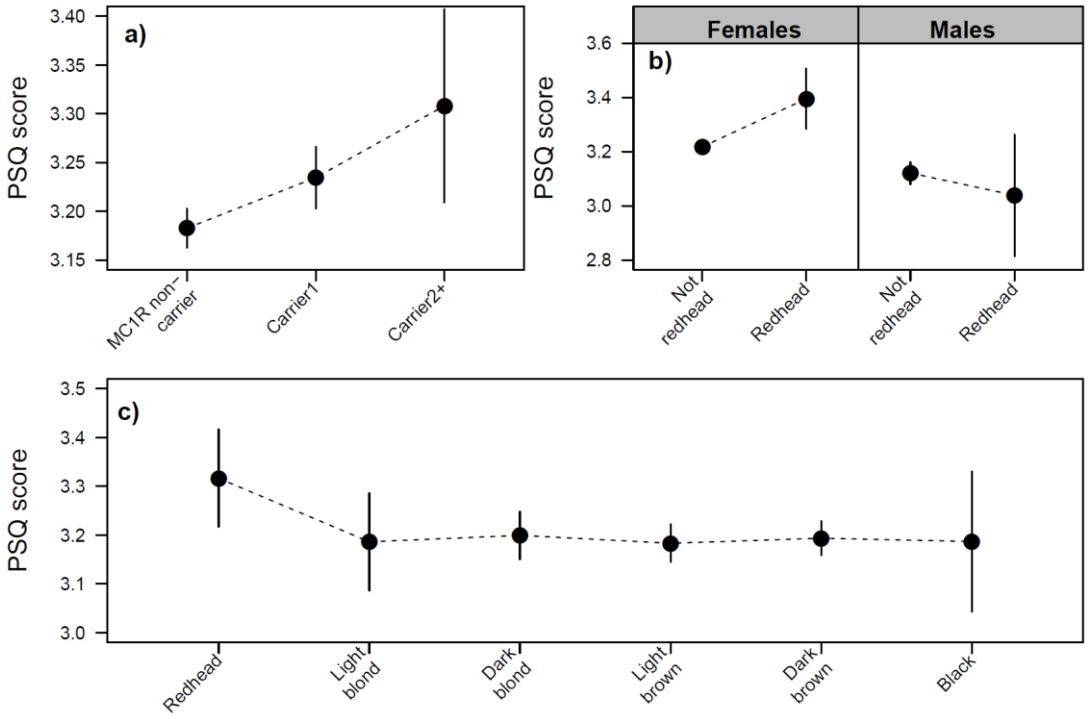


Figure 3: Results of MC1R and hair color association with PSQ score.



S1 table: Top GWAS and MCR1 association results for PSQ and CPT traits. *PSQ score were analyzed using a linear model. CPT duration (in seconds) were converted into three categories (δ50s; δ100s; and δ150s), and analyzed using a linear model.*

						PSQ GWAS			CPT (CAT.) GWAS		
CHR	*POS*	*ID*	*Alleles*	*MAF*	*Gene context*	*Effect*	*SE*	*P-value*	*Effect*	*SE*	*P-value*
Top PSQ GWAS Variants:											
2	3280983	rs58194899	G/A	0.47	TSSC1	***-0.051***	***0.009***	***1.9e-08***	0.005	0.015	7.2e-01
18	10008669	rs142738119	T/C	0.001	VAPA,APCDD1	0.696	0.138	4.7e-07	0.343	0.235	1.4e-01
13	101893919	rs12583902	G/A	0.166	NALCN	0.059	0.012	7.6e-07	0.012	0.02	5.3e-01
Top CPT GWAS Variants:											
17	65520139	rs141828201	G/A	0.027	PITPNC1	0.036	0.031	2.6e-01	-0.203	0.056	2.7e-04
MC1R Variants:											
16	89986144	rs1805008	C/T	0.071	MC1R	0.031	0.017	6.8e-02	0.011	0.029	7.0e-01
16	89986117	rs1805007	C/T	0.075	MC1R	0.047	0.017	5.1e-03	0.04	0.028	1.5e-01
16	89986546	rs1805009	G/C	0.019	MC1R	0.041	0.032	2.0e-01	0.061	0.052	2.4e-01

S2 table: Linear models on PSQ score analyzing MC1R carriers and hair colors.

	MC1R model				**Hair color model**				**Redhead x Sex model**			
	Beta	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*
Intercept	2.9708	0.0330	89.94	**0.00e+00**	2.9736	0.0415	71.72	**0.00e+00**	3.0909	0.0656	47.14	**0.00e+00**
Age	0.0025	0.0005	4.71	**2.51e-06**	0.0031	0.0007	4.59	**4.56e-06**	0.0031	0.0007	4.62	**3.80e-06**
Sex (Female)	0.1283	0.0188	6.84	**8.37e-12**	0.0966	0.0246	3.92	**8.77e-05**	0.1051	0.0244	4.30	**1.68e-05**
PC1	-2.2823	0.2112	-10.81	**3.70e-27**	-2.3501	0.2709	-8.68	**4.52e-18**	-2.3482	0.2747	-8.55	**1.37e-17**
PC2	-1.4966	0.4507	-3.32	**9.00e-04**	-2.0023	0.5706	-3.51	**4.51e-04**	-2.0155	0.5717	-3.53	**4.24e-04**
PC3	-1.3377	0.5047	-2.65	**8.05e-03**	-0.9214	0.6497	-1.42	1.56e-01	-0.9332	0.6540	-1.43	1.54e-01
PC4	1.4997	0.7604	1.97	**4.86e-02**	0.9815	0.9600	1.02	3.07e-01	0.9726	0.9647	1.01	3.13e-01
PC5	0.7175	0.9523	0.75	4.51e-01	0.4473	1.1739	0.38	7.03e-01	0.4317	1.1748	0.37	7.13e-01
MC1R carrier1	0.0517	0.0191	2.71	**6.81e-03**								
MC1R carrier2+	0.1253	0.0515	2.43	**1.51e-02**								
Redhead									-0.0823	0.1164	-0.71	4.80e-01
Light blond					-0.1303	0.0718	-1.81	6.97e-02				
Dark blond					-0.1170	0.0566	-2.07	**3.87e-02**				
Light brown					-0.1334	0.0546	-2.44	**1.45e-02**				
Dark brown					-0.1230	0.0540	-2.28	**2.29e-02**				
Black					-0.1297	0.0896	-1.45	1.48e-01				
Redhead x Sex									0.2597	0.1300	2.00	**4.58e-02**

S3 table: Survival models on CPT duration analyzing MC1R carriers and hair colors.

	MC1R model				**Hair color model**				**Redhead model**			
	Beta	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*
Age	0.0	0.0	0.91	3.62e-01	0.0	0.0	0.58	5.62e-01	0.0	0.0	0.48	6.32e-01
Sex (Female)	0.5	0.0	15.79	**0.00e+00**	0.4	0.0	13.25	**0.00e+00**	0.4	0.0	13.21	**0.00e+00**
PC1	-0.7	0.4	-1.78	7.49e-02	-0.6	0.5	-1.21	2.27e-01	-0.6	0.5	-1.32	1.87e-01
PC2	1.2	0.7	1.58	1.14e-01	1.2	0.9	1.35	1.76e-01	1.2	0.9	1.41	1.58e-01
PC3	-2.8	0.9	-3.22	**1.28e-03**	-2.7	1.0	-2.68	**7.43e-03**	-2.8	1.0	-2.73	**6.34e-03**
PC4	2.1	1.3	1.64	1.00e-01	1.8	1.5	1.25	2.11e-01	1.9	1.5	1.26	2.07e-01
PC5	-0.1	1.6	-0.08	9.36e-01	-0.4	1.8	-0.19	8.46e-01	-0.3	1.9	-0.16	8.72e-01
Platform v2	0.2	0.3	0.76	4.46e-01	0.1	0.3	0.47	6.35e-01	0.2	0.3	0.52	6.04e-01
Platform v3	0.1	0.2	0.41	6.81e-01	0.1	0.2	0.91	3.65e-01	0.2	0.2	0.94	3.49e-01
Platform v4	0.1	0.1	0.62	5.37e-01	0.1	0.1	0.94	3.46e-01	0.1	0.1	0.99	3.22e-01
Platform v5	0.1	0.1	0.74	4.60e-01	0.1	0.1	0.99	3.24e-01	0.1	0.1	1.04	2.99e-01
MC1R carrier1	-0.1	0.0	-1.85	6.40e-02								
MC1R carrier2+	-0.1	0.1	-0.87	3.85e-01								
Redhead												
Light blond					0.2	0.1	1.51	1.31e-01				
Dark blond					0.0	0.1	0.42	6.78e-01				
Light brown					0.1	0.1	1.16	2.48e-01				
Dark brown					0.0	0.1	0.37	7.15e-01				
Black					0.2	0.1	1.20	2.32e-01	-0.1	0.1	-0.76	4.49e-01

S4 table: ANOVA models on CPT duration (converted in ranks), analyzing MC1R carriers and hair colors.

	MC1R model				**Hair color model**				**Redhead x Sex model**			
	Beta	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*	*Beta*	*SE beta*	*t value*	*P-value*
Intercept	4221.9	232.4	18.17	**4.72e-72**	4222.4	251.9	16.76	**1.79e-61**	4339.1	279.5	15.52	**3.62e-53**
Age	-3.1	1.6	-1.96	**4.99e-02**	-2.7	1.8	-1.50	1.33e-01	-2.6	1.8	-1.44	1.49e-01
Sex (Female)	-849.6	48.6	-17.47	**6.26e-67**	-806.5	57.4	-14.06	**4.26e-44**	-822.2	56.8	-14.48	**1.25e-46**
PC1	1512.0	698.4	2.16	**3.04e-02**	1305.1	801.0	1.63	1.03e-01	1355.4	806.1	1.68	9.27e-02
PC2	-1271.8	1218.3	-1.04	2.97e-01	-1701.4	1410.2	-1.21	2.28e-01	-1766.1	1412.8	-1.25	2.11e-01
PC3	4224.3	1534.3	2.75	**5.92e-03**	4050.8	1770.5	2.29	**2.22e-02**	4168.4	1780.1	2.34	**1.92e-02**
PC4	-3320.6	2232.4	-1.49	1.37e-01	-3377.7	2577.8	-1.31	1.90e-01	-3523.9	2597.5	-1.36	1.75e-01
PC5	-104.6	2695.7	-0.04	9.69e-01	953.3	3077.8	0.31	7.57e-01	854.8	3081.5	0.28	7.81e-01
Platform v2	-457.6	446.3	-1.03	3.05e-01	-315.9	488.1	-0.65	5.18e-01	-319.9	488.3	-0.66	5.12e-01
Platform v3	-165.1	255.9	-0.64	5.19e-01	-291.9	275.8	-1.06	2.90e-01	-290.8	275.8	-1.05	2.92e-01
Platform v4	-157.7	218.9	-0.72	4.71e-01	-204.7	235.6	-0.87	3.85e-01	-206.4	235.7	-0.88	3.81e-01
Platform v5	-156.1	219.6	-0.71	4.77e-01	-199.5	236.7	-0.84	3.99e-01	-201.9	236.8	-0.85	3.94e-01
MC1R carrier1	90.5	53.0	1.71	8.75e-02								
MC1R carrier2+	110.9	141.9	0.78	4.35e-01								
Redhead									324.1	235.4	1.38	1.69e-01
Light blond					-275.3	180.6	-1.52	1.27e-01				
Dark blond					-81.4	138.7	-0.59	5.57e-01				
Light brown					-149.0	135.4	-1.10	2.71e-01				
Dark brown					-68.2	133.3	-0.51	6.09e-01				
Black					-264.2	223.1	-1.18	2.36e-01				
Redhead x Sex									-305.7	280.7	-1.09	2.76e-01

S5 table: Pathway enrichment results for PSQ.

Category	Gene set	# genes in gene set	Overlap	P-value	Adjusted P-value	Genes
GO bp	BIOLOGICAL ADHESION	1376	21	1.87e-10	1.38e-06	DAB1, PRKG1, CTNNA3, DLG2, NTM, OPCML, NRXN3, CDH13, PRKCE, PTPRT, CDH4, DSCAM, CNTN4, MAGI1, ROBO2, GRID2, PDZD2, TENM2, SDK1, CNTNAP2, PTPRD
GO bp	CELL CELL ADHESION VIA PLASMA MEMBRANE ADHESION MOLECULES	267	11	3.75e-10	1.38e-06	DAB1, CDH13, PTPRT, CDH4, DSCAM, CNTN4, ROBO2, GRID2, TENM2, SDK1, PTPRD
GO bp	NEURON RECOGNITION	47	6	5.55e-09	1.36e-05	NTM, OPCML, DSCAM, CNTN4, ROBO2, CNTNAP2
GO bp	CELL CELL ADHESION	806	15	1.02e-08	1.87e-05	DAB1, PRKG1, CTNNA3, DLG2, NRXN3, CDH13, PTPRT, CDH4, DSCAM, CNTN4, ROBO2, GRID2, TENM2, SDK1, PTPRD
GO bp	NEUROGENESIS	1572	20	1.36e-08	2.00e-05	DAB1, USH2A, PRKG1, NRG3, NTM, OPCML, SOX5, NRXN3, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, TENM2, PARK2, SDK1, CNTNAP2, NRG1, PTPRD
GO bp	NEURON DIFFERENTIATION	1328	18	3.47e-08	4.25e-05	DAB1, USH2A, PRKG1, NTM, OPCML, NRXN3, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, TENM2, PARK2, SDK1, CNTNAP2, NRG1, PTPRD
GO bp	NEURON DEVELOPMENT	1082	16	7.45e-08	7.82e-05	DAB1, PRKG1, NTM, OPCML, NRXN3, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, TENM2, PARK2, SDK1, CNTNAP2, PTPRD
GO bp	HOMOPHILIC CELL ADHESION VIA PLASMA MEMBRANE ADHESION MOLECULES	163	7	5.69e-07	5.22e-04	CDH13, PTPRT, CDH4, DSCAM, CNTN4, ROBO2, SDK1
GO bp	SYNAPSE ASSEMBLY	173	7	8.49e-07	6.94e-04	ASIC2, DSCAM, ROBO2, GRID2, SDK1, NRG1, PTPRD
GO bp	REGULATION OF NERVOUS SYSTEM DEVELOPMENT	890	13	1.82e-06	1.33e-03	DAB1, NRG3, ASIC2, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, PARK2, SDK1, NRG1, PTPRD
GO bp	CELL RECOGNITION	144	6	4.60e-06	3.08e-03	NTM, OPCML, DSCAM, CNTN4, ROBO2, CNTNAP2
GO bp	SYNAPTIC SIGNALING	704	11	6.78e-06	4.16e-03	NRG3, DLG2, ASIC2, PRKCE, CNTN4, GRID2, MCTP1, PARK2, NRG1, KCNQ3, PTPRD
GO bp	HETEROPHILIC CELL CELL ADHESION VIA PLASMA MEMBRANE CELL ADHESION MOLECULES	46	4	1.10e-05	6.20e-03	CDH4, GRID2, TENM2, PTPRD
GO bp	REGULATION OF NEURON PROJECTION DEVELOPMENT	478	9	1.19e-05	6.25e-03	DAB1, CDH4, DSCAM, ROBO2, GRID2, FSTL4, PARK2, SDK1, PTPRD
GO bp	REGULATION OF CELL DEVELOPMENT	904	12	1.28e-05	6.25e-03	DAB1, NRG3, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, PARK2, SDK1, NRG1, PTPRD
GO bp	REGULATION OF NEURON DIFFERENTIATION	633	10	1.71e-05	7.85e-03	DAB1, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, PARK2, SDK1, PTPRD
GO bp	CELLULAR COMPONENT MORPHOGENESIS	1107	13	1.93e-05	8.36e-03	DAB1, NRXN3, CDH13, CDH4, DSCAM, CNTN4, ROBO2, FSTL4, COL23A1, PARK2, CNTNAP2, NRG1, PTPRD
GO bp	REGULATION OF CELL PROJECTION ORGANIZATION	650	10	2.15e-05	8.76e-03	DAB1, CDH4, DSCAM, ROBO2, GRID2, FSTL4, TENM2, PARK2, SDK1, PTPRD
GO bp	CELL PROJECTION ORGANIZATION	1492	15	2.47e-05	9.12e-03	DAB1, PRKG1, NRXN3, CDH13, CDH4, DSCAM, CNTN4, ROBO2, GRID2, FSTL4, TENM2, PARK2, SDK1, CNTNAP2, PTPRD
GO bp	SYNAPSE ORGANIZATION	400	8	2.48e-05	9.12e-03	DLG2, ASIC2, DSCAM, ROBO2, GRID2, SDK1, NRG1, PTPRD
GO bp	CELL PART MORPHOGENESIS	671	10	2.82e-05	9.85e-03	DAB1, NRXN3, CDH4, DSCAM, CNTN4, ROBO2, FSTL4, PARK2, CNTNAP2, PTPRD
GO bp	DENDRITE DEVELOPMENT	219	6	4.97e-05	1.66e-02	DAB1, PRKG1, DSCAM, FSTL4, SDK1, PTPRD
GO bp	ADULT BEHAVIOR	143	5	7.00e-05	2.24e-02	DAB1, NRXN3, PARK2, SDK1, CNTNAP2
GO bp	STARTLE RESPONSE	28	3	8.03e-05	2.46e-02	GRID2, PARK2, CSMD1
GO bp	TAXIS	626	9	9.75e-05	2.87e-02	DAB1, NRG3, NRXN3, CDH13, CDH4, DSCAM, CNTN4, ROBO2, NRG1
GO bp	CENTRAL NERVOUS SYSTEM DEVELOPMENT	964	11	1.21e-04	3.42e-02	DAB1, PRKG1, NRG3, ASIC2, MACROD2, CNTN4, ROBO2, GRID2, PARK2, CNTNAP2, NRG1
GO bp	POSITIVE REGULATION OF NERVOUS SYSTEM DEVELOPMENT	517	8	1.50e-04	4.00e-02	DAB1, ASIC2, CDH4, DSCAM, ROBO2, GRID2, PARK2, PTPRD
GO bp	NEURON PROJECTION GUIDANCE	270	6	1.57e-04	4.00e-02	DAB1, NRXN3, CDH4, DSCAM, CNTN4, ROBO2
GO bp	POSTSYNAPTIC SPECIALIZATION ORGANIZATION	35	3	1.58e-04	4.00e-02	DLG2, GRID2, PTPRD
GO bp	REGULATION OF CALCIUM MEDIATED SIGNALING	96	4	1.99e-04	4.89e-02	CAMTA1, CDH13, NRG1, DMD
GO bp	REGULATION OF AXONOGENESIS	180	5	2.06e-04	4.89e-02	DAB1, CDH4, DSCAM, ROBO2, FSTL4
GO cc	SYNAPSE	1165	16	2.05e-07	2.05e-04	DAB1, USH2A, NRG3, DLG2, GPC6, CDH13, ASIC2, DSCAM, GRID2, MCTP1, TENM2, PARK2, SDK1, NRG1, PTPRD, DMD
GO cc	NEURON PROJECTION	1295	15	4.51e-06	2.16e-03	DAB1, USH2A, DLG2, CDH13, ASIC2, DSCAM, CNTN4, ROBO2, GRID2, TENM2, PARK2, CNTNAP2, NRG1, KCNQ3, DMD
GO cc	NEURON PART	1700	17	6.47e-06	2.16e-03	DAB1, USH2A, DLG2, CDH13, ASIC2, RBFOX3, DSCAM, CNTN4, ROBO2, GRID2, MCTP1, TENM2, PARK2, CNTNAP2, NRG1, KCNQ3, DMD
GO cc	CELL JUNCTION	1272	14	1.80e-05	4.50e-03	KAZN, CTNNA3, DLG2, CDH13, CDH4, DSCAM, MAGI1, GRID2, PDZD2, MCTP1, TENM2, SDK1, CNTNAP2, DMD
GO cc	MAIN AXON	68	4	5.21e-05	1.04e-02	DLG2, ROBO2, CNTNAP2, KCNQ3
GO cc	AXON	599	9	6.96e-05	1.16e-02	USH2A, DLG2, DSCAM, CNTN4, ROBO2, TENM2, CNTNAP2, NRG1, KCNQ3
GO cc	INTRINSIC COMPONENT OF PLASMA MEMBRANE	1678	15	9.57e-05	1.37e-02	NRG3, DLG2, GPC6, NRXN3, ASIC2, PTPRT, CDH4, DSCAM, KCNIP4, GRID2, TENM2, CNTNAP2, NRG1, KCNQ3, PTPRD
GO cc	AXON PART	375	7	1.28e-04	1.60e-02	USH2A, DLG2, DSCAM, ROBO2, TENM2, CNTNAP2, KCNQ3
GO cc	ANCHORED COMPONENT OF MEMBRANE	168	5	1.49e-04	1.66e-02	NTM, OPCML, GPC6, CDH13, CNTN4
GO cc	POTASSIUM CHANNEL COMPLEX	98	4	2.16e-04	2.16e-02	DLG2, KCNIP4, CNTNAP2, KCNQ3
GO cc	PLASMA MEMBRANE PROTEIN COMPLEX	567	8	2.80e-04	2.55e-02	DLG2, CDH13, CDH4, KCNIP4, GRID2, CNTNAP2, KCNQ3, DMD
GO cc	CELL CELL JUNCTION	447	7	3.73e-04	3.04e-02	KAZN, CTNNA3, CDH13, CDH4, MAGI1, PDZD2, CNTNAP2
GO cc	JUXTAPARANODE REGION OF AXON	10	2	3.94e-04	3.04e-02	DLG2, CNTNAP2
GO cc	GOLGI APPARATUS	1534	13	5.19e-04	3.71e-02	PRKG1, GPC6, PCSK6, RBFOX1, HS3ST4, WWOX, SLC39A11, TSSC1, PRKCE, LARGE, TENM2, PARK2, CNTNAP2
GO cc	GLUTAMATERGIC SYNAPSE	349	6	6.18e-04	4.12e-02	NRG3, DLG2, GPC6, GRID2, NRG1, PTPRD
GO cc	NEURON PROJECTION MEMBRANE	59	3	7.46e-04	4.67e-02	USH2A, ROBO2, CNTNAP2
BioCarta	BIOCARTA AGR PATHWAY	33	3	1.32e-04	3.82e-02	NRG3, NRG1, DMD
GO mf	RECEPTOR ACTIVATOR ACTIVITY	11	3	4.19e-06	6.90e-03	NRG3, PRKCE, NRG1
Curated gene sets	MIKKELSEN MCV6 HCP WITH H3K27ME3	425	9	4.63e-06	2.55e-02	NRG3, FAM155A, NPAS3, ASIC2, RBFOX3, PTPRT, DSCAM, FSTL4, KCNQ3
Curated gene sets	SILIGAN BOUND BY EWS FLT1 FUSION	47	4	1.19e-05	3.29e-02	CDH13, LRP1B, FHIT, PTPRD
Curated gene sets	SILIGAN TARGETS OF EWS FLI1 FUSION DN	18	3	2.04e-05	3.50e-02	DLG2, ASIC2, CNTNAP2
Curated gene sets	DING LUNG CANCER BY MUTATION RATE	20	3	2.84e-05	3.50e-02	LRP1B, ROBO2, PTPRD
Curated gene sets	BENPORATH SUZ12 TARGETS	1009	12	3.79e-05	3.50e-02	IHHAT, PRKG1, HS3ST4, CDH13, ASIC2, PRKCE, PTPRT, KCNIP4, PDZD2, CSMD1, NRG1, KCNQ3
Curated gene sets	REACTOME SHC1 EVENTS IN ERBB2 SIGNALING	22	3	3.82e-05	3.50e-02	NRG3, PRKCE, NRG1
Curated gene sets	MIKKELSEN MEF HCP WITH H3K27ME3	581	9	5.50e-05	4.33e-02	NRG3, NTM, RBFOX3, PTPRT, DSCAM, CNTN4, FSTL4, CSMD1, KCNQ3

S6 table: Pathway enrichment results for CPT.

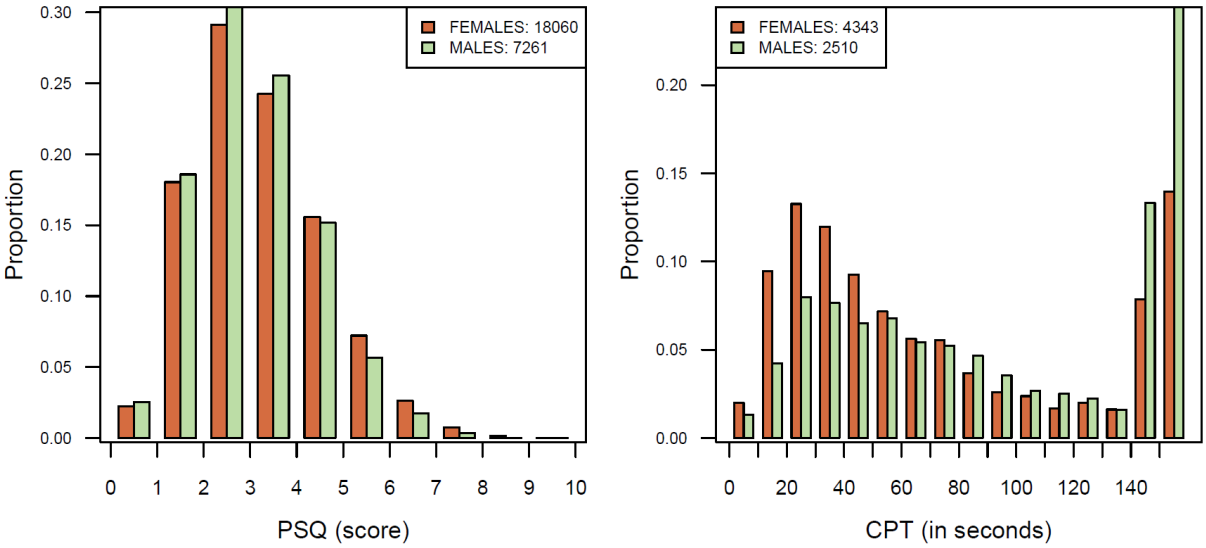
Category	Gene set	# genes in gene set	Overlap	P-value	Adjusted P-value	Genes
GO bp	CELL CELL ADHESION VIA PLASMA MEMBRANE ADHESION MOLECULES	267	9	1.72e-08	1.72e-08	CDH23, TENM4, KIRREL3, CDH13, CDH4, CNTN4, ROBO2, TENM3, PTPRD
GO bp	HOMOPHILIC CELL ADHESION VIA PLASMA MEMBRANE ADHESION MOLECULES	163	7	1.49e-07	1.49e-07	CDH23, KIRREL3, CDH13, CDH4, CNTN4, ROBO2, TENM3
GO bp	CENTRAL NERVOUS SYSTEM DEVELOPMENT	964	13	4.26e-07	4.26e-07	PRKG1, NRG3, NAV2, TENM4, KIRREL3, ASIC2, PLCB1, MACROD2, CNTN4, PTPRG, ROBO2, EGFR, CNTNAP2
GO bp	CELL CELL ADHESION	806	12	4.57e-07	4.57e-07	PRKG1, CDH23, TENM4, DLG2, KIRREL3, CDH13, CDH4, CNTN4, ROBO2, TENM3, EGFR, PTPRD
GO bp	SYNAPTIC SIGNALING	704	11	9.39e-07	9.39e-07	NRG3, DLG2, ASIC2, DLGAP1, PLCB1, CNTN4, CADPS, EGFR, PTPRN2, DLGAP2, PTPRD
GO bp	NEURON DEVELOPMENT	1082	13	1.57e-06	1.57e-06	PRKG1, CDH23, TENM4, KIRREL3, CDH4, CNTN4, PTPRG, ROBO2, TENM3, EGFR, MAGI2, CNTNAP2, PTPRD
GO bp	NEUROGENESIS	1572	15	3.63e-06	3.63e-06	PRKG1, CDH23, NRG3, NAV2, TENM4, KIRREL3, CDH4, CNTN4, PTPRG, ROBO2, TENM3, EGFR, MAGI2, CNTNAP2, PTPRD
GO bp	BIOLOGICAL ADHESION	1376	14	4.02e-06	4.02e-06	PRKG1, CDH23, TENM4, DLG2, CNTN5, KIRREL3, CDH13, CDH4, CNTN4, ROBO2, TENM3, EGFR, CNTNAP2, PTPRD
GO bp	SYNAPSE ASSEMBLY	173	6	4.30e-06	4.30e-06	CNTN5, KIRREL3, ASIC2, ROBO2, MAGI2, PTPRD
GO bp	HETEROPHILIC CELL CELL ADHESION VIA PLASMA MEMBRANE CELL ADHESION MOLECULES	46	4	5.11e-06	5.11e-06	TENM4, CDH4, TENM3, PTPRD
GO bp	HEAD DEVELOPMENT	760	10	1.44e-05	1.44e-05	PRKG1, NRG3, KIRREL3, PLCB1, MACROD2, CNTN4, PTPRG, ROBO2, EGFR, CNTNAP2
GO bp	NEURON DIFFERENTIATION	1328	13	1.47e-05	1.47e-05	PRKG1, CDH23, TENM4, KIRREL3, CDH4, CNTN4, PTPRG, ROBO2, TENM3, EGFR, MAGI2, CNTNAP2, PTPRD
GO bp	MUSCLE CELL CELLULAR HOMEOSTASIS	21	3	1.87e-05	1.87e-05	LARGE, SGCZ, DMD
GO bp	CELL CELL SIGNALING	1619	14	2.58e-05	2.58e-05	NRG3, DLG2, WWOX, ASIC2, DLGAP1, PLCB1, CNTN4, CADPS, EGFR, MAGI2, PTPRN2, DLGAP2, PTPRD, PCSK5
GO bp	TELENCEPHALON DEVELOPMENT	246	6	3.18e-05	3.18e-05	NRG3, KIRREL3, PLCB1, ROBO2, EGFR, CNTNAP2
GO bp	FOREBRAIN DEVELOPMENT	376	7	3.76e-05	3.76e-05	PRKG1, NRG3, KIRREL3, PLCB1, ROBO2, EGFR, CNTNAP2
GO bp	CIRCULATORY SYSTEM PROCESS	525	8	4.15e-05	4.15e-05	PRKG1, NAV2, ASIC2, CACNA2D3, EGFR, SGCZ, PCSK5, DMD
GO bp	CELL PROJECTION ORGANIZATION	1492	13	5.03e-05	5.03e-05	PRKG1, CDH23, KIRREL3, CDH13, CDH4, CNTN4, PTPRG, ROBO2, TENM3, EGFR, MAGI2, CNTNAP2, PTPRD
GO bp	REGULATION OF NERVOUS SYSTEM DEVELOPMENT	890	10	5.53e-05	5.53e-05	NRG3, TENM4, ASIC2, CDH4, CNTN4, PTPRG, ROBO2, TENM3, MAGI2, PTPRD
GO bp	SYNAPSE ORGANIZATION	400	7	5.56e-05	5.56e-05	DLG2, CNTN5, KIRREL3, ASIC2, ROBO2, MAGI2, PTPRD
GO bp	NEGATIVE REGULATION OF LOCOMOTION	312	6	1.19e-04	1.19e-04	PRKG1, NRG3, PLCB1, PTPRG, ROBO2, MAGI2
GO bp	DEPHOSPHORYLATION	465	7	1.42e-04	1.42e-04	DLG2, TMEM132D, PTPRG, PPP2R2C, MAGI2, PTPRN2, PTPRD
GO cc	NEURON PART	1700	17	3.26e-07	3.26e-07	CDH23, TENM4, DLG2, CNTN5, KIRREL3, CDH13, ASIC2, DLGAP1, CNTN4, CADPS, ROBO2, TENM3, MAGI2, CNTNAP2, PTPRN2, DLGAP2, DMD
GO cc	SYNAPSE	1165	14	5.57e-07	5.57e-07	NRG3, DLG2, CNTN5, CDH13, ASIC2, DLGAP1, PLCB1, CADPS, EGFR, MAGI2, PTPRN2, DLGAP2, PTPRD, DMD
GO cc	CELL JUNCTION	1272	13	9.27e-06	9.27e-06	KAZN, FRMD4A, DLG2, CDH13, DLGAP1, CDH4, CADPS, EGFR, MAGI2, CNTNAP2, PTPRN2, DLGAP2, DMD
GO cc	NEURON PROJECTION	1295	13	1.12e-05	1.12e-05	CDH23, TENM4, DLG2, KIRREL3, CDH13, ASIC2, CNTN4, ROBO2, TENM3, MAGI2, CNTNAP2, PTPRN2, DMD
GO cc	PLASMA MEMBRANE PROTEIN COMPLEX	567	8	7.13e-05	7.13e-05	DLG2, CDH13, CDH4, CACNA2D3, EGFR, CNTNAP2, SGCZ, DMD
GO cc	SYNAPSE PART	928	10	7.85e-05	7.85e-05	DLG2, CNTN5, ASIC2, DLGAP1, PLCB1, CADPS, MAGI2, PTPRN2, DLGAP2, DMD
GO cc	JUXTAPARANODE REGION OF AXON	10	2	2.70e-04	2.70e-04	DLG2, CNTNAP2
BioCarta	BIOCARTA AGR PATHWAY	33	3	7.50e-05	7.50e-05	NRG3, EGFR, DMD
GO mf	TRANSMEMBRANE RECEPTOR PROTEIN PHOSPHATASE ACTIVITY	17	3	9.62e-06	9.62e-06	PTPRG, PTPRN2, PTPRD
Curated gene sets	DING LUNG CANCER BY MUTATION RATE	20	4	1.59e-07	1.59e-07	LRP1B, PTPRG, ROBO2, PTPRD
Curated gene sets	ACEVEDO METHYLATED IN LIVER CANCER DN	792	11	2.95e-06	2.95e-06	KAZN, ADARB2, NAV2, TMEM132D, RBFOX1, CDH13, CDH4, PPP2R2C, PTPRN2, DLGAP2, DMD
Curated gene sets	SILIGAN BOUND BY EWS FLT1 FUSION	47	4	5.58e-06	5.58e-06	CDH13, LRP1B, FHIT, PTPRD
Curated gene sets	BENPORATH EED TARGETS	1035	12	6.22e-06	6.22e-06	ADARB2, PRKG1, CDH23, GALNT18, NAV2, NELL1, KIRREL3, PLCB1, SORCS2, EGFR, MAGI2, CSMD1
Curated gene sets	SILIGAN TARGETS OF EWS FLI1 FUSION DN	18	3	1.15e-05	1.15e-05	DLG2, ASIC2, CNTNAP2
Curated gene sets	BENPORATH SUZ12 TARGETS	1009	11	2.90e-05	2.90e-05	ADARB2, PRKG1, CDH23, GALNT18, NAV2, NELL1, KIRREL3, CDH13, ASIC2, PLCB1, CSMD1
Curated gene sets	DING LUNG CANCER MUTATED SIGNIFICANTLY	25	3	3.21e-05	3.21e-05	LRP1B, EGFR, PTPRD
Curated gene sets	BLALOCK ALZHEIMERS DISEASE DN	1236	12	3.68e-05	3.68e-05	NELL1, DLG2, ASIC2, DLGAP1, LRP1B, PLCB1, LARGE, FHIT, CNTNAP2, PTPRN2, DLGAP2, DMD
Curated gene sets	TAKADA GASTRIC CANCER COPY NUMBER DN	30	3	5.61e-05	5.61e-05	RBFOX1, WWOX, FHIT
Curated gene sets	SCHLESINGER METHYLATED DE NOVO IN CANCER	87	4	6.47e-05	6.47e-05	PRKG1, CDH13, ASIC2, CDH4
Curated gene sets	REACTOME PROTEIN PROTEIN INTERACTIONS AT SYNAPSES	87	4	6.47e-05	6.47e-05	DLG2, DLGAP1, DLGAP2, PTPRD
Curated gene sets	BIOCARTA AGR PATHWAY	33	3	7.50e-05	7.50e-05	NRG3, EGFR, DMD
Curated gene sets	TAVAZOIE METASTASIS	101	4	1.16e-04	1.16e-04	DLG2, PLCB1, CNTNAP2, PTPRN2

S7 table: Gene-based (MAGMA) results for 21 nociception genes.

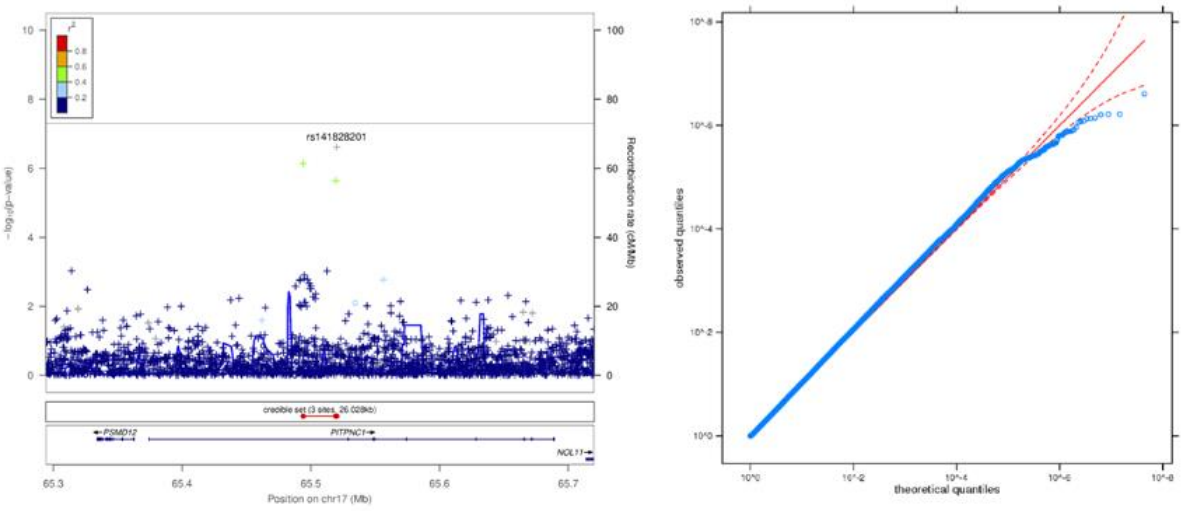
The list of nociception genes was obtained from https://humanpaingenetics.org/hpgdb/

		PSQ			CPT		
Gene	**Position**	**# of variants**	**P-value**	**Rank**	**# of variants**	**P-value**	**Rank**
ABCB1	7:87133179-87342639	22	4.43e-03	3649	53	2.55e-03	3101
ADRB1	10:115803806-115806667	1	2.58e-02	8264		NA	
ADRB2	5:148206156-148254628	4	8.08e-03	4863	22	1.23e-03	2212
ARRB2	17:4613789-4624795	4	1.80e-02	7074		NA	
ARVCF	22:19954126-20004325	14	1.30e-03	2094	8	2.25e-02	8062
BDNF	11:27676440-27743605	4	1.70e-03	2362	30	1.77e-03	2585
COMT	22:19929263-19957498	15	3.44e-03	3232	3	1.84e-02	7376
DRD3	3:113847499-113918254	45	1.13e-02	5714	31	9.15e-03	5469
EDNRA	4:148402069-148466106	26	1.53e-02	6566	4	3.14e-03	3376
FAAH	1:46859939-46879520	14	1.14e-02	5723	3	5.66e-02	11647
IL1A	2:113531492-113542971	1	8.80e-02	12944	1	5.97e-02	11851
MPDZ	9:13105700-13279660	33	5.80e-04	1508	14	2.72e-03	3192
OPRD1	1:29138654-29190208	34	4.26e-02	10209	20	5.32e-02	11389
OPRK1	8:54138276-54164257	2	3.87e-02	9835	26	1.01e-03	2046
OPRM1	6:154331631-154568001	32	1.76e-03	2400	51	2.04e-02	7708
SCN10A	3:38738837-38835501	23	5.14e-03	3894	34	5.01e-02	11140
SCN11A	3:38887260-38995136	34	4.22e-02	10165	106	1.12e-02	5961
SCN9A	2:167051695-167232497	8	4.86e-03	3799	20	6.61e-04	1721
TACR1	2:75273590-75426645	47	3.49e-03	3260	74	3.08e-04	1234
TRPA1	8:72933486-72987819	19	6.23e-03	4296	4	8.66e-04	1913
TRPV1	17:3468740-3512705	44	1.22e-04	796	64	8.46e-05	774

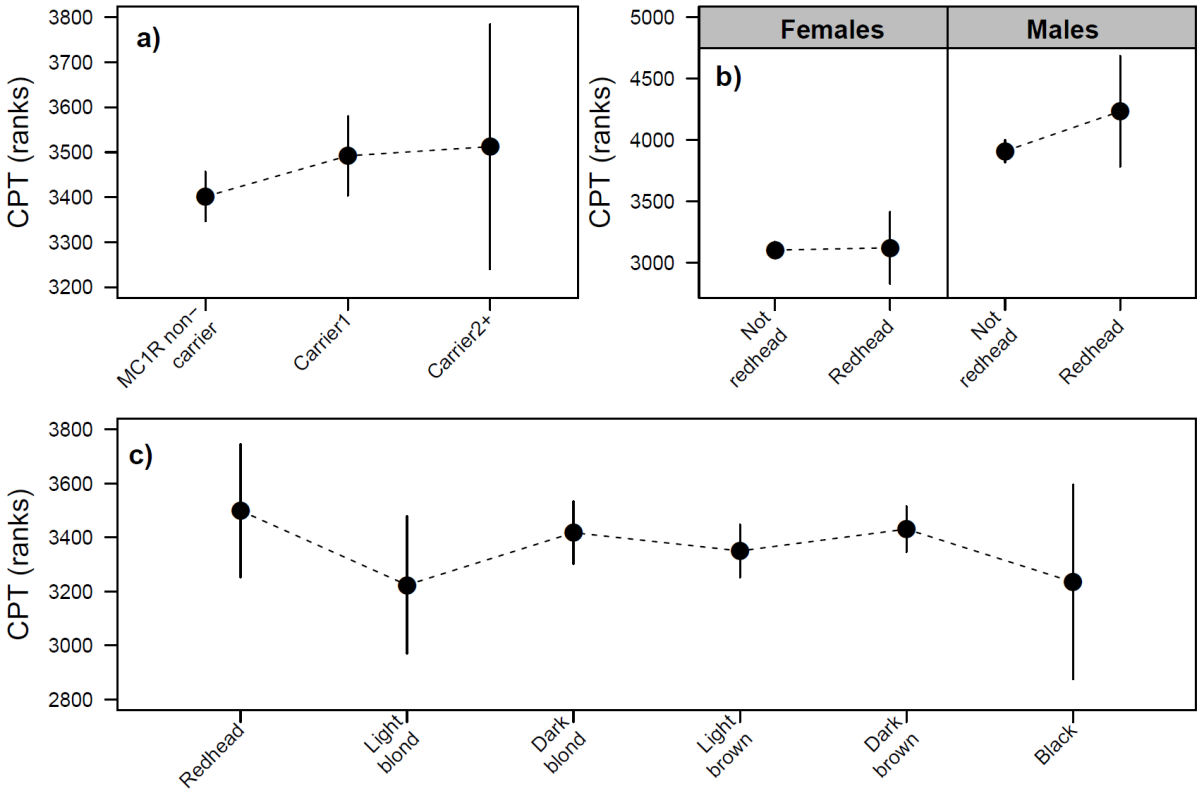
S1 figure: PSQ and CPT distributions.



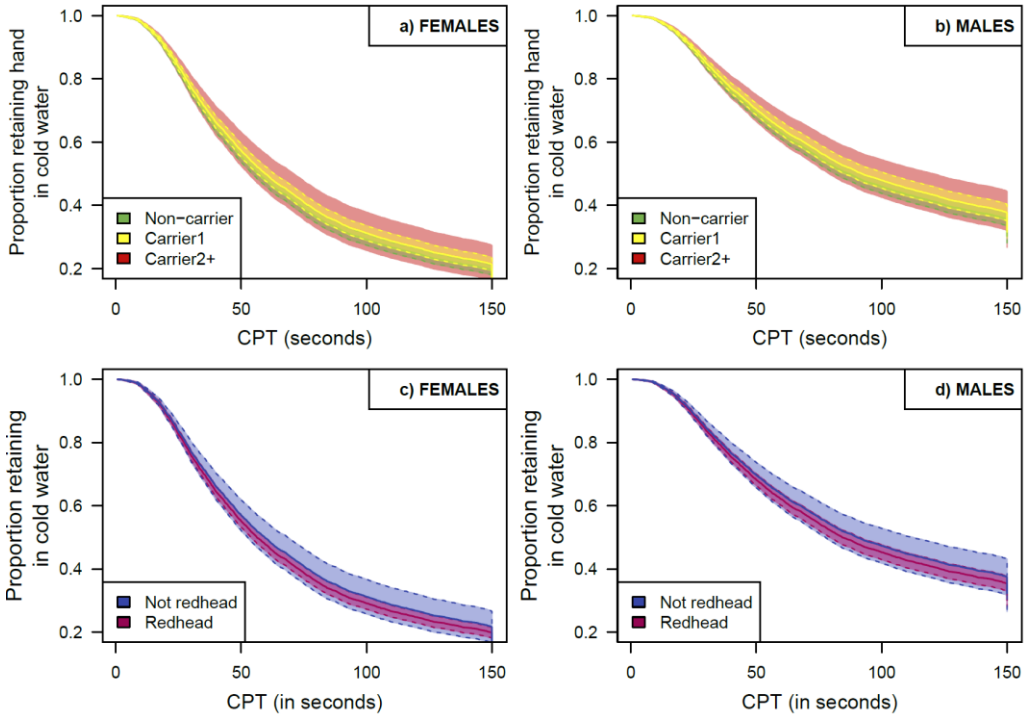
S2 figure: Regional association plot of the top associated loci and QQ plot from the CPT GWAS.



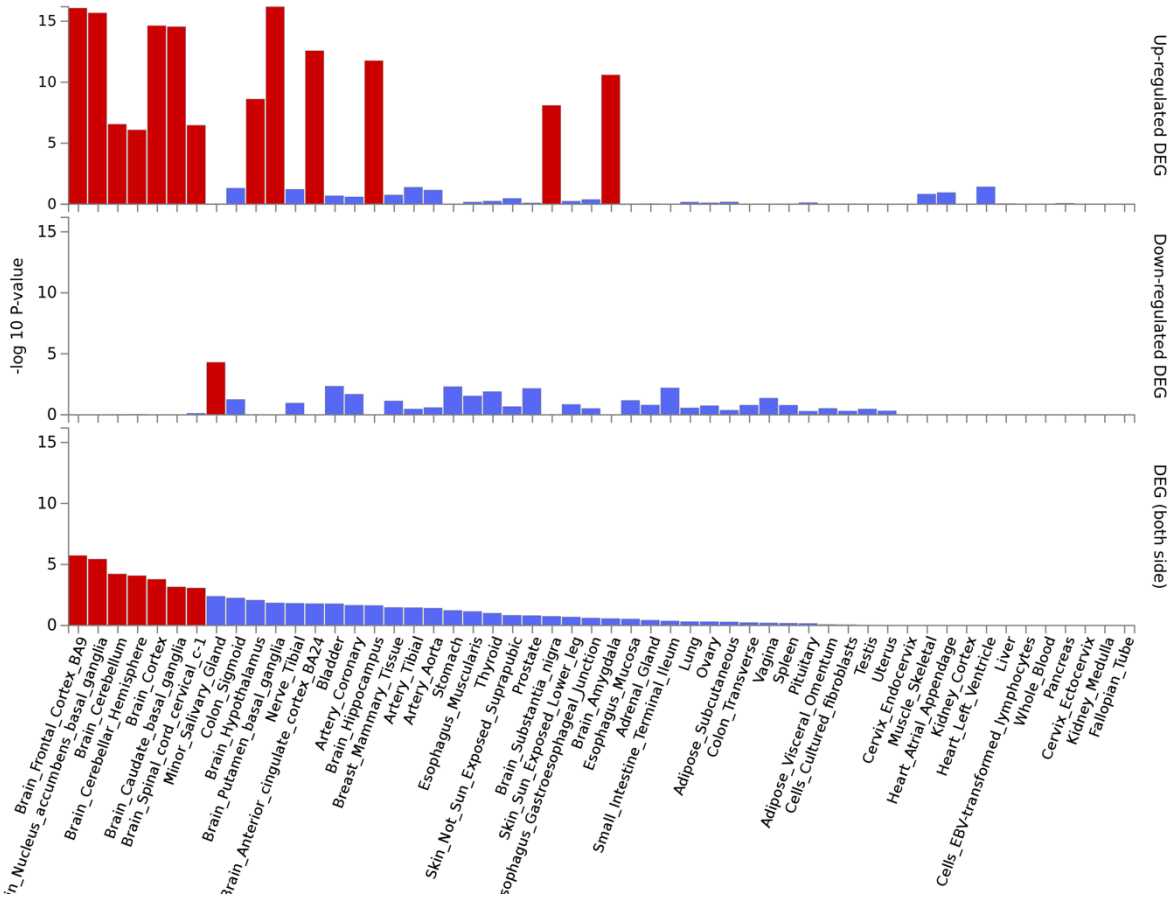
S3 figure: Results of MC1R and hair color models for CPT duration converted in ranks.



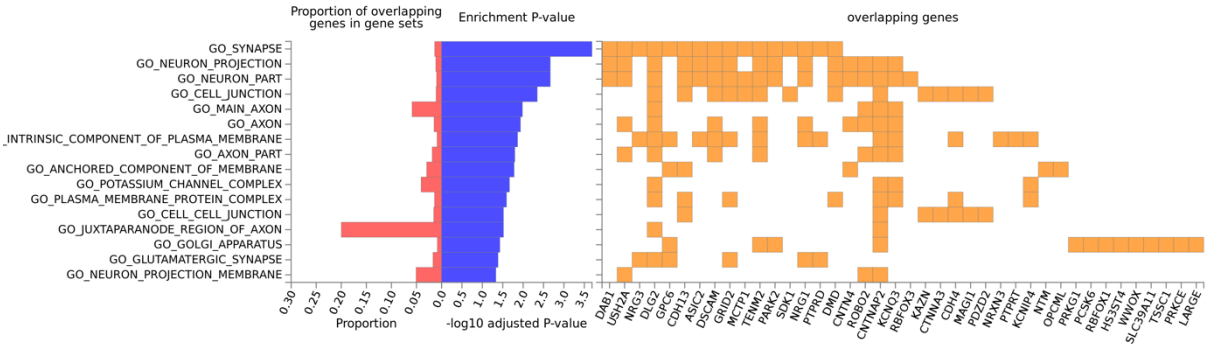
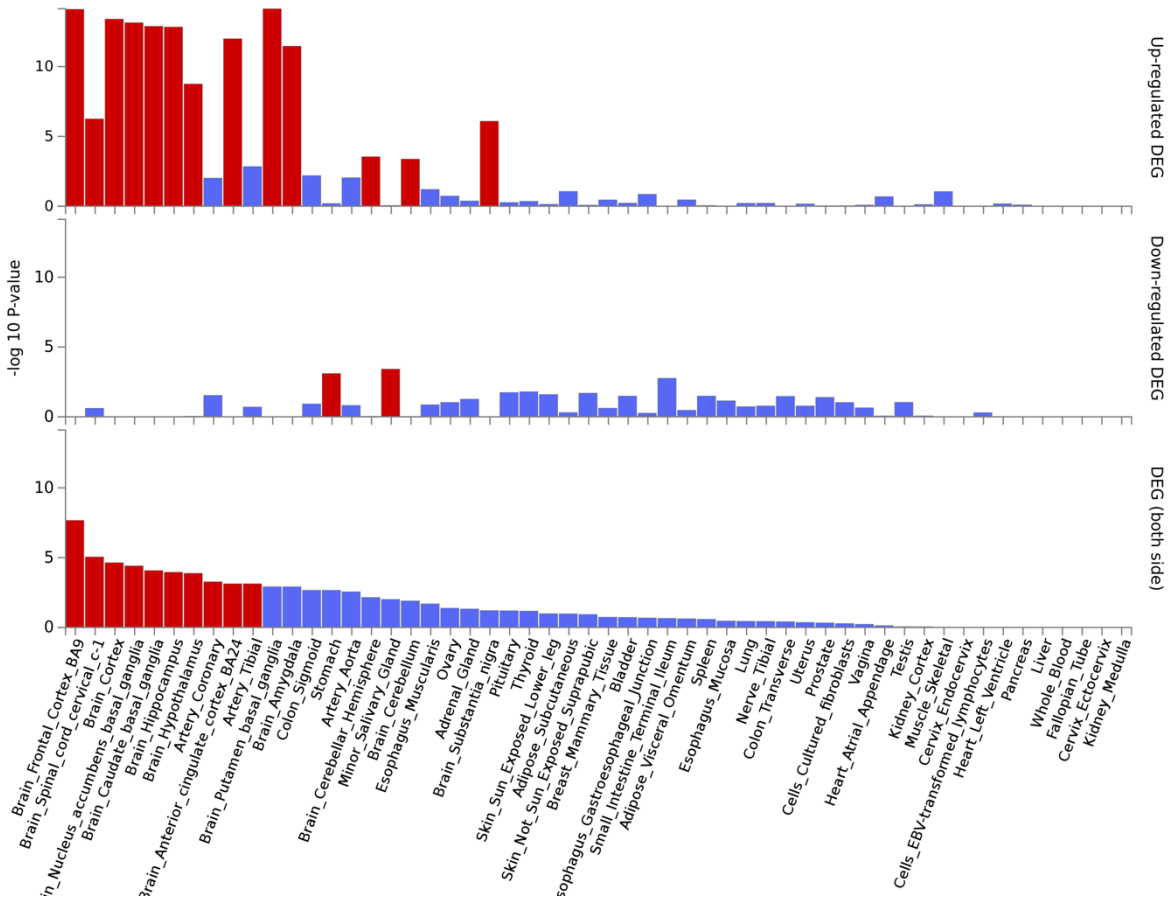
S4 figure: Kaplan-Meier curves for MC1R and hair color survival models on CPT duration.



S5 figure: Tissue expression enrichment analysis for PSQ, based on the 58 genes in Table 3 (GTEx v8).



S6 figure: Enrichment analysis of cellular components for PSQ, based on the 58 genes in Table 3.



S7 figure: Tissue expression enrichment analysis for CPT, based on the 50 genes in Table 3 (GTEx v8).