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

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Number of pages: 6

Number of figures: 2

Number of tables: 4

Supplementary material: 5 figures, 4 tables

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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 of 5 adults. Pain sensitivity varies broadly between individuals, and it is notoriously difficult to measure in large population. Although pain sensitivity is known to be moderately heritable, only a limited numbers of genetics studies have been published, with limited success at identifying the genetic architecture of pain sensitivity. In this study, we deployed a pain sensitivity questionnaire (PSQ) and an at-home version of cold pressor test (CPT) in a large genotyped cohort. We performed genome-wide association study (GWAS) analysis on the PSQ scores and CPT duration, collected for 25,321 and 6,853 participants, respectively. Despite a reasonably large sample size, we identified only one genome-wide significant locus, located in the TSSC1 gene, associated with PSQ score. Genetic correlation analysis suggested that PSQ has several traits that are correlated with chronic pain states and lifestyle factors. We found that PSQ score are positively correlated to neck and shoulder pain that lasts more than 3 months and negatively correlated to acute pain sensations like fracture. Gene-based analysis followed by pathway analysis suggested that GWAS results are enriched in genes controlling Thyroid peroxidase and Melanin production. We showed that genetic variation in MC1R gene and redhead hair pigmentation is associated with an increase of pain sensitivity measured by the PSQ scores.

Introduction/Background

The assessment of psychometric and experimental pain sensitivity is increasingly important and may for instance be applied to predict postoperative pain and chronification at an early stage. In general, such assessments are time consuming within clinical settings. We are seeking to identify easily accessible genetic biomarkers that can be used to alert physicians in clinical practice and start early treatment to prevent the process of chronification in pain indications. The interindividual variability in pain sensitivity and perception is however large. Several genetic root causes for differences in pain signal transduction or in the interpretation of such signals in the insula and anterior cingulate cortex have been reported. So far it is still unclear what the quantitative effects of these identified mutations have in terms of magnitude. The assessment of pain sensitivity is in general difficult since there is no objective measurement of pain available and therefore subjective with a high interindividual variability. Consequently, the requirements for minimum sample sizes are high and can reach easily reach more than 100 subjects if a statistical power of 80% and p-values of 0.05 are essential.

In earlier studies, it could be shown that people with red hair need higher doses of anesthetics to reach a comparable level of anesthesia than other subgroups with a different hair color phenotype. The MC1R gene plays a pivotal role for the redhair phenotype and is highly polymorphic. In our study we have analyzed and quantified the contributions for the three major MC1R SNPs to the pain sensitivity as well as novel SNPs using GWAS by using a validated pain sensitivity questionnaire with imagined painful situations as well as an experimental pain test, namely the cold pressor test.

Materials and methods

Subjects

All participants included in the analyses were drawn from the customer base of 23andMe, Inc., a consumer genetics company. Participants provided informed consent and answered surveys online under a protocol approved by Ethical and Independent Review Services, an independent AAHRPP-accredited institutional review board (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. The PSQ is an English-language version of the Pain Sensitivity Questionnaire (Ruscheweyh 2009, 2012), 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, see McIntyre *et al.*, 2019.

Genotyping and variant imputation

DNA extraction and genotyping were performed on saliva samples by Laboratory Corporation of America. Samples 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 $P<10^{-20}$, or a call rate of <90%. Samples were then imputed in a single unified imputation reference panel, combining the May 2015 release of the 1000 Genomes Phase 3 haplotypes with the UK10K imputation reference panel. The imputation panel contains a total of 65 million variants. We flagged variants with $r^2<0.3$, as well as variants that had strong evidence of a batch effect. Principal components were computed using ~65,000 high quality genotyped variants present in all five genotyping platforms. For more details on genotyping and imputation process, see Tian *et al.*, 2017.

GWAS analysis

Imputed dosages and genotyped data were both tested for association with PSQ scores 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. 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.

MC1R and hair color

We defined three categories of MC1R carriers by combining three variants, rs1805007, rs1805008, and rs1805009: Non-carrier (0 MC1R allele), Carrier1 (1 allele), and Carrier2+ (>1 alleles). A self-reported hair color phenotype was available for 63% of the participants in the cohort. It contained six hair color categories: redhead, light blond, dark blond, light brown, dark brown, and black. We also built a binary redhead variable from this categorical hair color phenotype. Association of MC1R carrier, hair color, and redhead variables were tested against PSQ scores 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.

Gene-based analysis and genetic correlation

We further processed the initial list of highly associated SNPs determined by cut-off at P<1e-05 using a gene-set analysis with Magma to detect associated genes. Correlation with other traits and multi-trait GWAS have been investigated to understand the extent of genetic overlap between PSQ and CPT. The genetic correlations were estimated for a broad set of medical diseases, disorders, and traits available at LD Hub from the Broad Institute, using a bivariate LD-score regression method. Significant correlations were found for several traits including schizophrenia (rG = 0.211, P = $1.03*10^{-5}$) and measures of cognitive ability, especially educational attainment (rG = 0.199, P = $2.56*10^{-9}$), thus indicating a substantial genetic overlap with these phenotypes (Figure 3).

Results and Discussion

A total of 25,321 and 6,853 participants were included in the PSQ and CPT analyses, respectively. The two cohorts are largely independent, only 1,534 participants completed the questionnaire and took the CPT (categorical values in supptable1). The sex-ratio is unbalanced in the two cohorts, with 71% and 63% of females, respectively (Table 1). Earlier genetic studies have shown that the three non-synonymous loss-of-function single-nucleotide variants rs1805009 (D294H), rs1805008 (R160W) and rs1805007 (R151C) in the G protein-coupled receptor MC1R are responsible for the red hair color phenotype. Additionally, our quantitative model shows an additivity between these three mutations with respect to contributions of shifts in pain sensitivity. The PSQ score within our model spans a range of 0.2 that is a small effect regarding difference in pain sensitivity but statistically significant. Further we have identified a new associated locus in the TSSC1/EIPR1 gene (lead SNP rs58194899, chr2:3277212 (GRCh38p12), MAF 0.351, OR=0.051, pvalue=$2.2*10^{-8}$) that reached GWAS significance. The SNP has not been reported in ClinVar or GWAS databases (see OpenTarget) so far but the corresponding protein TSSC1 is a component of the endosomal retrieval pathways and plays a critical co-localization 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. Two other SNPs (rs7581969 p= $5*10^{-10}$, rs12624302 p= $7*10^{-9}$) of the TSSC1/EIPR1 have been identified in one study of cognitive decline rate in late mild cognitive impairment (33, GWAS Catalog study: GCST003075). This may lead to the

conclusion that cognitive impairment can play a role in rating imagined pain situations. Further we have performed a LD score regression to compare with other GWAS summary-level results (34). The results are summarized in table 3. The most positive correlations are related to chronic pain states like neck and shoulder pain that lasted more than 3 months, self-reported rheumatoid arthritis, mononeuropathies as well as complex disease with heritability like ADHD and extreme BMI. Negative correlations identified are related to lower lipid levels, lipid metabolism, free fatty acids and acute pain experiences. For the CPT score, we have not seen any signals with GWAS statistical significance and therefore performed MAGMA analysis to see how the top-ranking SNPs are enriched in genes. Within the highest-ranking genes in our analysis, we have identified two genes that are Thyroid peroxidase (PERT/TPO) as well as PTPRD which are directly involved in the Melanin production and Tyrosine metabolism pathway. Further SORL1 are components of the lipid, cholesterol and steroid metabolic processes (low-density lipoprotein receptors). CNTNAP2/5 a neuronal cell adhesion protein, has been earlier reported to be relevant for changes in risk of social behavior like Autism Spectrum Disorder (ASD) and cognitive impairment where we have identified a new SNP in the TSSC1 gene.

CONCLUSION

The purpose of this study was to identify genetic factors contributing to the individual perception of pain. A sensory input like experimental pain or an imagined pain situation can lead to a different rating of pain by subjects. Most likely this is caused by individual differences in peripheral and central processing. Using a pain questionnaire in combination with GWAS and LD score regression analyses, we can show that in addition to the somatosensory elements of pain-processing the cognitive and life-style factors contribute a large part to the perception of pain. Positive correlations and higher pain sensitivity are found in the group of chronic pain patients like neck and should pain that last longer than 3 months, long working weeks and high BMI. Negative correlations depend on VLDL/HDL levels and acute pain sensations. Gene set enrichment revealed molecular pathway components that influence the pain perception. For instance, we have been able to show that subjects with red hair or a combination of three major mutations have indeed a higher pain sensitivity independent of age with reverse effect in males that can be described by an additive model of SNPs of the MC1R gene. Even more interesting, the same molecular pathways that are involved in the melanin production like Thyroid peroxidase, and subsequent secretion of endorphins also provide a reduced pain sensation that we found in our study by a quantitative model with 3 SNPs of the MC1R gene. Especially the PSQ questionnaire in combination with genetics revealed to be a more suitable tool to describe pain sensitivity on the molecular level than an experimental medicine method like the CPT.

ACKNOWLEDGMENTS

This research was conducted by 23andMe and Grünenthal GmbH. All authors are employees of these companies. We thank the research participants of 23andMe for making this study possible.

Figures and tables

Table 1: Pain sensitivity questionnaire (PSQ) and Cold pressor test (CPT) summaries in 23andMe cohort.

	PSQ (score)		CPT (in seconds)	
	Females	Males	Females	Males
Full cohort:				
# 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:				
# 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 and MCR1 GWAS results for PSQ and CPT traits.
PSQ scores 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 Variants:											
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: MAGMA analysis results for PSQ p<1e-05. Genes from GWAS top ranking SNPs in green.

Gene	CHR	START	STOP	NSNPS	NPARAM	ZSTAT	P
TPO/PERT	2	1417233	1547445	3	2	5,7835	3,66E-09
CNTNAP2	7	145813453	148118090	4	2	5,6727	7,03E-09
SORL1	11	121322912	121504471	3	2	5,6542	7,83E-09
CNTNAP5	2	124782864	125672954	3	2	5,4114	3,13E-08
PTPRD	9	8314246	10612723	5	2	5,1594	1,24E-07
TSSC1/EIPR1	2	3192741	3381653	101	3	5,0828	1,86E-07
DCC	18	49866542	51062273	3	2	5,062	2,07E-07
EPOP	17	36827956	36831187	1	1	4,9991	2,88E-07
COL27A1	9	116918231	117072975	2	1	4,9396	3,91E-07
DNAH10	12	124246786	124420267	2	1	4,8475	6,25E-07
PLCB1	20	8112912	8865547	1	1	4,8425	6,41E-07
CDC73	1	193091088	193223945	1	1	4,8008	7,90E-07
NELL1	11	20691117	21597232	1	1	4,7993	7,96E-07
UNC13C	15	54270557	54920806	2	1	4,7631	9,53E-07
SCAPER	15	76640526	77197796	3	1	4,7528	1,00E-06
KAZN	1	14219646	15444544	2	1	4,7335	1,10E-06
WWOX	16	78133310	79246567	2	1	4,7321	1,11E-06
ATXN1	6	16299343	16761721	2	1	4,7278	1,13E-06
RORA	15	60780483	61521502	2	1	4,715	1,21E-06
GABRG1	4	46037786	46126082	1	1	4,7098	1,24E-06
FLNB	3	57994127	58157982	15	2	4,7008	1,30E-06
SLC1A1	9	4490427	4587469	1	1	4,655	1,62E-06
SLC9B1	4	103806205	103947552	1	1	4,6537	1,63E-06
NRG1	8	31496820	32622558	2	1	4,6394	1,75E-06
AKAP7	6	131456826	131604675	2	1	4,6219	1,90E-06
MAML2	11	95711440	96076344	5	2	4,5459	2,74E-06
EYS	6	64429876	66417118	3	1	4,5286	2,97E-06
BCL2L11	2	111878491	111926022	1	1	4,4813	3,71E-06
NALCN	13	101706130	102068813	51	2	4,4669	3,97E-06
UBQLN1	9	86274878	86323168	1	1	4,4472	4,35E-06
WIPI2	7	5229835	5273486	1	1	4,4385	4,53E-06
KCNQ3	8	133133105	133493004	1	1	4,4366	4,57E-06
ZFAND3	6	37787272	38161637	3	1	4,4298	4,72E-06
CASC1	12	25261223	25348096	1	1	4,4278	4,76E-06
COL11A1	1	103342023	103574052	2	1	4,4212	4,91E-06
PGLYRP3	1	153270338	153283194	1	1	4,4202	4,93E-06
BBX	3	107241783	107530176	4	1	4,3967	5,50E-06
NRG3	10	83635070	84746935	1	1	4,3949	5,54E-06
DNAJB6	7	157129692	157210133	1	1	4,3805	5,92E-06
CACNG4	17	64960980	65029518	1	1	4,3615	6,46E-06
PACRG	6	163148164	163736524	2	1	4,3485	6,85E-06
SUPT16H	14	21819631	21852425	1	1	4,3334	7,34E-06
TP63	3	189348942	189615068	1	1	4,3296	7,47E-06
CSNK2A2	16	58191811	58231782	1	1	4,3142	8,01E-06
PPFIA2	12	81652045	82153109	1	1	4,3042	8,38E-06
DAB2IP	9	124329162	124547809	2	1	4,2734	9,63E-06

Table 4: Genetic correlations of the PSQ GWAS summary results

Trait	rG	SE_rG	z	P_rG	H2_obs	H2_obs_se	H2_int	H2_int_se
Neck/shoulder pain for 3+ months	0,7118	0,3416	2,0835	0,0372	0,0214	0,0064	1,0081	0,0072
Rheumatoid arthritis (self-reported)	0,6804	0,3244	2,0974	0,036	0,0044	0,0014	1,0073	0,0068
Attention deficit hyperactivity disorder	0,6738	0,3204	2,1031	0,0355	0,2428	0,0988	1,0057	0,0071
Length of working week for main job	0,6473	0,2681	2,4141	0,0158	0,0191	0,0029	1,0103	0,0068
Cholelithiasis/gall stones (self-reported)	0,5583	0,2568	2,1743	0,0297	0,0083	0,0015	1,0211	0,0082
Mononeuropathies of upper limb (ICD10:G56)	0,5271	0,2086	2,5264	0,0115	0,0131	0,0018	1,0138	0,0076
Frequency of other exercises in last 4 weeks	0,5203	0,2216	2,348	0,0189	0,0213	0,0033	0,9974	0,0069
Extreme bmi	0,2348	0,1271	1,8468	0,0648	0,6852	0,0576	1,0306	0,0115
Phospholipids in medium VLDL	-0,526	0,2938	-1,7904	0,0734	0,1053	0,033	0,9993	0,0071
Ever stopped smoking for 6+ months	-0,5407	0,3135	-1,7249	0,0845	0,0191	0,0059	1,0028	0,0076
Triglycerides in medium VLDL	-0,5529	0,2981	-1,8549	0,0636	0,0946	0,0301	1	0,007
Glycoprotein acetyls mainly a1-acid glycoprotein	-0,5544	0,2747	-2,0182	0,0436	0,0989	0,0284	0,9862	0,007
Celiac disease	-0,5604	0,2962	-1,8918	0,0585	0,2959	0,0502	1,0687	0,0107
Acetate	-0,5861	0,3195	-1,8344	0,0666	0,0556	0,0192	1,0022	0,007
Nasal polyps (self-reported)	-0,6148	0,3046	-2,0187	0,0435	0,0053	0,0016	1,005	0,007
Total lipids in small HDL	-0,6741	0,3715	-1,8146	0,0696	0,0532	0,0265	1,0023	0,0076
Fracture resulting from simple fall	-0,7123	0,3442	-2,0692	0,0385	0,0518	0,0154	0,9927	0,007
Pneumothorax (self-reported)	-0,8217	0,4166	-1,9722	0,0486	0,0031	0,0014	0,9868	0,007

Figure 1: Manhattan plots for PSQ and CPT GWAS.

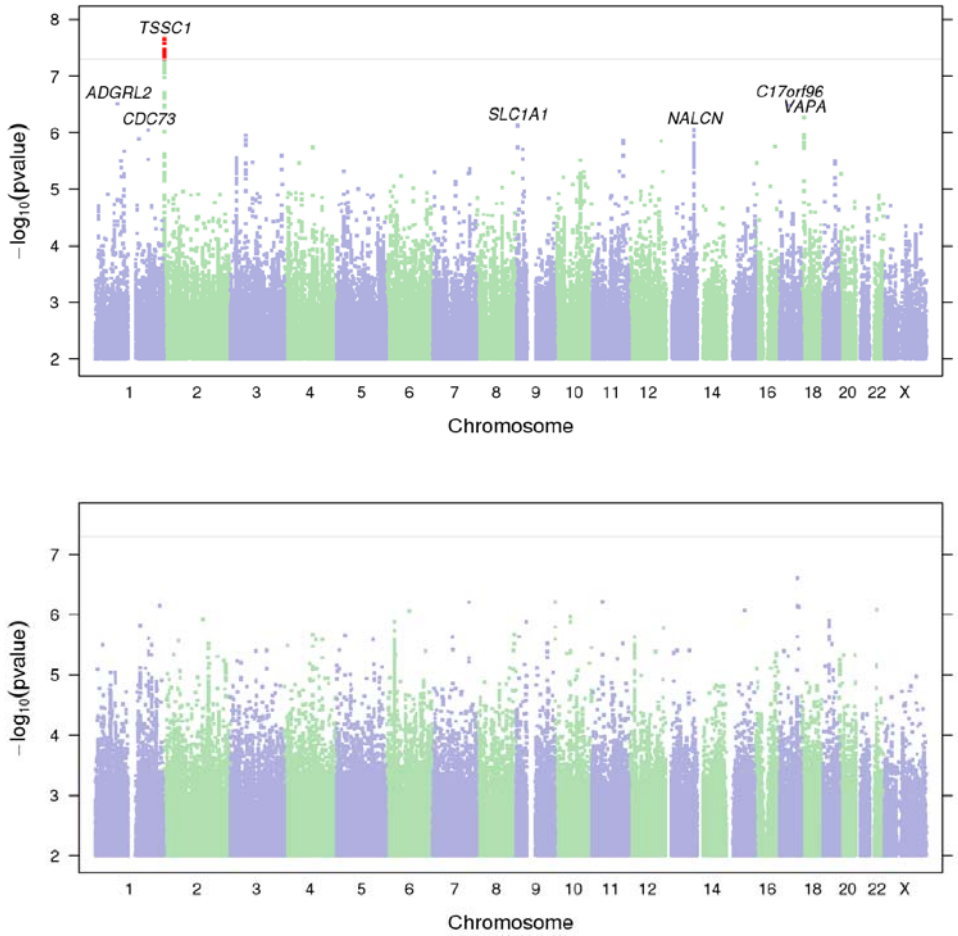
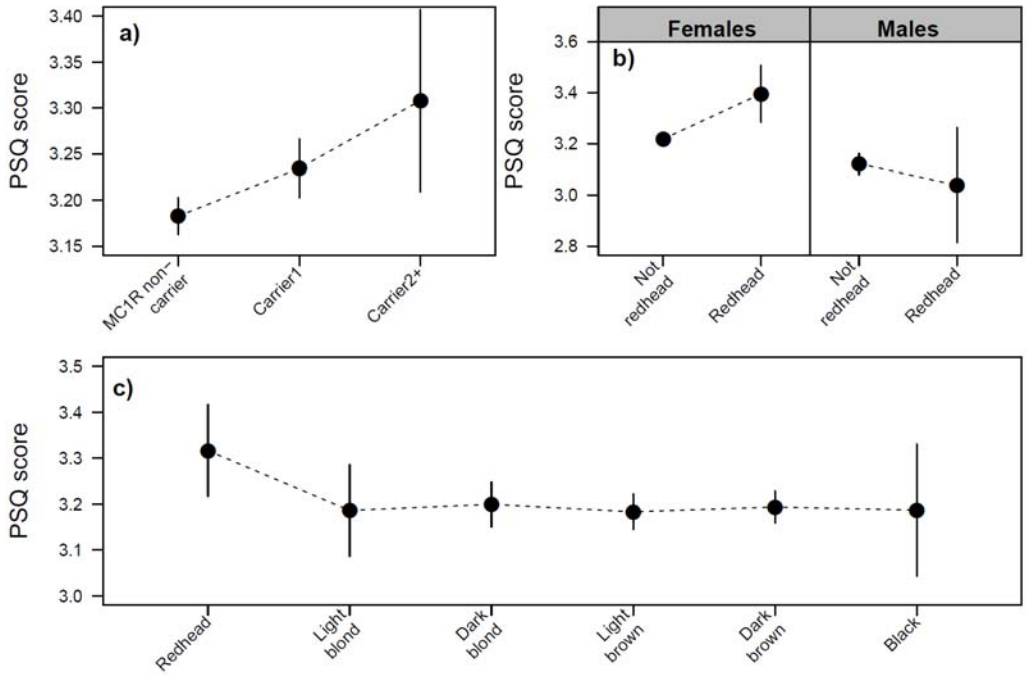


Figure 2: Results of MC1R and hair color models for PSQ scores.



Contributors Conception and design: PF and AK. Provision of study materials or patients: 23andMe and Grünenthal. Collection and assembly of data: 23andMe. Data analysis and interpretation: PF and AK. Manuscript writing: PF and AK. Final approval of manuscript: all authors

Patient consent Obtained

Ethics approval IRB approved study. OHRP/FDA registration number IRB00007807, study number 10044-11.

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