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Detangling red hair from pain: phenotype-specific contributions from different genetic variants in melanocortin-1 receptor

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1. Introduction

The gene *MC1R* encodes the melanocortin-1 receptor, MC1R, a G protein-coupled receptor (GPCR) responsible for skin and hair pigment biosynthesis [10]. Once endogenously activated by the melanocyte stimulating hormone, α-MSH, or the adrenocorticotropic hormone, ACTH, MC1R signals via cAMP upregulation for a switch from the default pheomelanin to eumelanin production [22, 39]. The ratio of eumelanin to phaeomelanin determines hair colour, and a functionally compromised MC1R leads to red hair colour [45].

While its connection to red hair has been known for decades, *MC1R*'s involvement in pain was first reported in the early 2000s, by Mogil and colleagues [31, 32]. They showed an increased response to opioid analgesics in individuals with at least two *MC1R* variants – previously reported as associated with red hair – undergoing experimental heat, ischemic, and electrical current sensitivity protocols. The later publication also showed a lower baseline sensitivity to electrical current pain in the two *MC1R*-variant allele group. By contrast, two contemporary studies by Liem and colleagues found an increased anaesthetic requirement in red-haired individuals [27, 28], with a higher baseline sensitivity to hot and cold stimuli [27].

Given that red hair is known to result from loss of function (LOF), measured by cellular responses to receptor stimulation, we set out to test four possible explanations for these apparently divergent findings: 1. LOF in *MC1R* leads to reduced sensitivity to pain (in agreement with Mogil's publications); 2. LOF in *MC1R* leads to increased sensitivity to pain (in agreement with Liem's publications); 3. there is a non-linear relationship between red hair variants in *MC1R* and pain sensitivity; 4. there is no relationship between red hair variants in *MC1R* and pain sensitivity (original association results were spurious).

The studies done by Mogil and Liem suggest that *MC1R* may affect the response to different noxious stimuli. Therefore, it is plausible that *MC1R*'s polymorphisms affect nociception at the point of noxious stimulus integration – the spinal dorsal horn – or higher in the ascending or descending pain signalling pathway. To capture this activity at the first hub of

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pain processing, we selected two measures from the heat pain wind-up paradigm: temporal summation and pain sensitivity. The heat protocol was chosen as the only shared modality affected by MC1R reported in Mogil's and Liem's studies.

To address the possibility that different genetic variants were responsible for hair colour and pain sensitivity, we included all common genotyped *MC1R* variants in the discovery cohort analysis [29]. For validation, we used the U.K. Biobank (UKBB) dataset. While no QSTs were tested in this cohort, a surrogate pain phenotype was available – a count of persistent clinical pain conditions. The mechanisms underlying nociceptive temporal summation and pain sensitivity have been shown to overlap with the mechanism responsible for central sensitisation [2, 30], the facilitation of both of which increases one's risk for developing chronic pain [8, 36]. The availability of hair colour data in this cohort was an additional benefit and allowed us to query directly the relationship between these two phenotypes as mediated by genotype.

2. Materials and Methods

2.1. Cohorts

The association analysis was first conducted in the Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA) cohort [29]. In this prospective study of the temporomandibular disorder (TMD), participants aged 18–44, recruited from 4 U.S. study sites – the University of Maryland at Baltimore, the University of Buffalo (New York), the University of North Carolina at Chapel Hill, and the University of Florida at Gainesville – were enrolled from 2006 to 2008 and diagnosed for the presence of TMD at recruitment and follow-up. Additionally, participants were characterised for a battery of quantitative sensory testing (QST) measures to determine sensitivity to heat, mechanical cutaneous, and pressure pain. Genotyping was done at 2.5 million single variant positions on the Illumina Infinium Omni2.5Exome-8 panel, which comprehensively covers the protein content of the genome. After quality control (sex mismatch, batch effects, relatedness, chromosomal anomalies, and sample and variant quality) and missing data filters, 464 and 412 Caucasian women and men, respectively, were included in the study, Table 1.

Validation was conducted in the U.K. Biobank (UKBB) cohort of 500,000 participants aged 40–69, who were recruited between 2006 and 2010 (application 20802). We used imputed genotypes for European Caucasians, as specified in the UKBB Data Field, hereafter DF, 22006. Quality control filters consisted of heterozygosity rate (DF22010), sex mismatch, final call rate, heterozygosity outliers, unintended duplicates, and ancestry principal component outliers (DF22051). Individuals who withdrew from the study were likewise removed. The final sample size was 402,000 individuals.

For the OPPERA cohort, the study was approved by the institutional review boards at all participating institutions. For the UKBB cohort, the study was approved by the North West Multicentre Research Ethics Committee (REC reference number: 06/MRE08/65), in accordance with the principles of the Declaration of Helsinki (information available at www.ukbiobank.ac.uk).

2.2. Heat QST

In the OPPERA cohort, the phenotypes analysed were measures of nociceptive summation and pain sensitivity using a heat pain wind-up protocol that preferentially stimulates C-fibres [6], as described in [21]. Specifically, we queried temporal summation, a measure of the central nervous system's ability to temporally integrate the perception of noxious stimuli; and pain sensitivity, reflected by the participant's perceptual response to the first stimulus and by the area under the wind-up curve [6]. Briefly, a thermode was applied to the participants' inner forearm in 3 series of 10 pulses, 1 second apart, starting at 38°C and ending at 46°C, 48°C, and 50°C, respectively. Ratings were collected from patients, on a scale from 0 to 100. The following derived measures were used as outcomes: slope of the first 3 ratings (beta, temporal summation), first and highest rating difference (delta, temporal summation), and the area under the curve (AUC, sensitivity) plotting ratings on an arbitrary scale, 0–100, as a function of pulse count, Supplementary Figure S1. The measures for 3 series amount to 12 tested phenotypes.

2.3. Count of reported clinical pain sites

In the UKBB, the pain phenotype used for analysis was derived from Data Field (DF) 6159, which tallied pain conditions/body sites with pain experienced at the time of assessment and for more than 3 months prior. The individual pain sites/phenotypes were: head (DF3571), face (DF4067), neck/shoulder (DF3404), abdomen (DF3741), back (DF3571), hip (DF3414), knee (DF3773), and general pain all over the body (DF2956). The phenotype tested for association was the total count of pain sites, ranging from "0" to "7", and "8" corresponding to DF2956.

2.4. Hair colour

Hair colour was collected only in UKBB. Using DF1747 we constructed a "red versus dark" phenotype, in which the "dark" category comprised dark brown and black hair colours. We excluded blonde and light brown to avoid overlap with strawberry blonde and auburn brown, which brought the effective sample size to 187,560. The more purely dichotomous phenotype was desirable for maximum sensitivity in testing the effects of variants expected to have a comparatively small effect.

2.5. Statistical analyses

In the OPPERA cohort, we analysed for association between all common genotyped (17) *MC1R* variants (passing the quality control filters and minimum minor allele frequency, MAF, 1% threshold), Table 2, and heat pain sensitivity and temporal summation phenotypes. The model of inheritance used was additive, a common default, because it is sufficiently sensitive to capture recessive and dominant effects and reduces the multiple testing penalty. Via David Nyholt's spectral decomposition method (https://sites.google.com/site/qutsgel/software) [35], the starting number of variants was reduced to 12 independent genotypes. This approach uses principal component analysis (PCA) of matrices of pairwise variant LD scores to assign an Eigenvalue to each variant (principal component, PC). The number of PCs with an Eigenvalue >= 1 is then used as the number of independent variants. For an analogous reduction in phenotype variables we referred to the PCA of 22 heat QST

measures, which output 2 independent phenotype constructs for temporal summation and pain sensitivity, described in previous publications [6, 21]. These constructs were: Component 1, with heavy loadings from temporal integration (AUC) measures for all 3 series – 46°C, 48°C, and 50°C – and Component 5, with heavy loadings from temporal summation – beta and delta derived measures for all 3 series [21]. The resulting correction was done for 24 tests, the product of 12 genotypes and 2 phenotypes. Association analysis was done using a data storage, retrieval, and analysis portal [26], which integrates PLINK 1.07 code. Linear regression with TMD status, age, sex, study site, and 3 ancestry PCs as covariates was run.

In UKBB, imputed data was converted from .BGEN to binary (.BED) format, using PLINK 2.0 [37]. Imputation quality was translated to hard genotype calls and coded as missing given greater than 10% uncertainty. Univariate logistic regression in PLINK 1.9 was used to test for association between *MC1R* genotypes and individual pain sites. Generalised linear regression (GLM) in R, version 3.5, was used to analyse for association between *MC1R* genotypes and the count of pain conditions and between *MC1R* genotypes and hair colour (red versus dark), as well as between the count of pain conditions and hair colour. Specifically, glm was run for individual variant and haplo.glm (as part of the haplo.stats package [42]) for haplotype analysis, assuming a Poisson distribution. All haplotypes above the frequency 1% were compared to the highest frequency, ancestral, haplotype as terms in the same multivariate model – omnibus test. For all analyses in UKBB, age, sex, recruitment site, and 10 ancestry PCs were used as covariates.

Linkage disequilibrium (LD) was visualised in Haploview software [3].

Expression quantitative trait locus (eQTL) effects were obtained from the genotype-tissue expression (GTEx) database (https://gtexportal.org/home/), supported by the Common Fund of the U.S. National Institutes of Health, NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. Summary statistics from linear regression on normalised mRNA expression levels as a function of the queried variant's minor allele count (genotype) are freely available with their GTEx eQTL Calculator tool (https://gtexportal.org/home/testyourown).

For all analyses, the direction of effect is shown with respect to the minor allele count of the tested variant.

3. Results

3.1. Temporal summation and pain sensitivity: discovery cohort

Single-variant association analysis was run on 17 *MC1R* variants with a minimum minor-allele frequency of 1%. Twelve correlated temporal summation sensitivity phenotypes at 3 temperatures were assessed (Supplementary Table S1). After correcting for multiple tests (α = 0.05/16 = 0.0031), 5 variants in the 5'-untranslated region (5'UTR) of the gene were associated with the phenotype 50°C AUC (sensitivity), (Table 3). The direction of effect was protective for all minor alleles. At the nominal significance level α = 0.05, these 5 5'-UTR variants were associated with a number of heat QST derived measures (Supplementary Table S1). LD visualisation showed all 5 variants to be members of one haploblock (Figure 1,

Block 1), tagged by rs3212357 and rs3212361 (Table 4). Given that rs3212361 was the variable with the best association statistics, we tested for a possible independent contribution of rs3212357 to 50°C AUC by running a multivariate regression model and found that it did not contribute above and beyond rs3212361 ($p = 0.64$). Therefore, for subsequent analyses, rs3212361 was retained as the sole representative of the 5' UTR variant set (Figure 1, Block 1), and 50°C AUC was used as the phenotype best capturing the sensitivity for association with *MC1R*, Figure 2A and 2C.

3.2. Association of missense variants with pain sensitivity

Given that previous reports connected missense *MC1R* variants to both red hair and pain sensitivity, next we restricted our analysis for association with 50°C AUC to common (minimum 1% MAF) variants known for their contribution to red hair: 6 high-penetrance, or R (rs1805006, rs11547464, rs1805007, rs1110400, rs1805008, and rs1805009) and 3 low-penetrance, or r (rs1805005, rs2228479, and rs885479) [21]. None of these variants passed the significance threshold adjusted for 9 genotypes ($\alpha = 0.05/9 = 0.0083$), but rs885479 was nominally significant ($p = 0.008$), Table 5, Figure 2B and 2C. An examination of all results from this analysis – including other pain sensitivity modalities from the complete QST panel [21] – at the nominal significance level $\alpha = 0.05$, showed that rs885479 was associated with a number of tested measures, and rs1110400 was associated with one, Supplementary Table S1.

3.3. Genetic correlation between 5'-UTR and missense variants

Next we examined the LD structure between the 5'-UTR variant rs3212361 and the 9 genotyped missense variants (rs1805005, rs1805006, rs2228479, rs11547464, rs1805007, rs1110400, rs1805008, rs885479, and rs1805009). Figure 1 (Block 2) and Table 6 show that 1) all nine missense variants are in high LD (D') with rs3212361; 2) each missense variant's minor allele appears as part of only one haplotype; 3) the minor alleles of missense variants do not co-occur; 4) only two of the missense variants, rs885479 and rs1805008, have their minor alleles (A and T, respectively) on the same haplotype with the minor allele of rs3212361, A.

3.4. Validation cohort: count of persistent pain conditions

In an attempt to validate our findings, we analysed rs3212361 for association with the count of persistent pain conditions, ranging from 0 (none) to 8 (general pain all over the body) in the UKBB, Table 7, Figure 3A. The minor allele of rs3212361 was indeed associated with a reduced count of pain conditions in the UKBB. We then also ran association analyses for common missense variants known for their contribution to red hair to determine if the larger sample size compared to the OPPERA cohort would identify an association. The minor allele of rs885479, but not the other tested variants, was associated with a reduced count of pain conditions in the UKBB (Table 7, Supplementary Table S2), reinforcing the original association pattern.

Next, we tested the variants rs3212361 and rs885479 for association with individual pain conditions, Figure 4. The 5'-UTR variant rs3212361 was significantly associated with neck and nominally associated with back/shoulder and hip pain, for all of which its minor allele

was protective. The missense variant rs885479 was significantly associated with back/shoulder pain, and nominally associated with hip, knee, and neck pain. Thus, we identified a stable pattern of association between these variants and musculoskeletal pain, and neither variant had consistently better association statistics than the other. Given the particular haplotypic structure of *MC1R*, which may lead to erroneous association effects in individual variant analysis [49], we investigated this further using a global haplotype association analysis on the entire set of variants in Table 5 with rs3212361. The count of persistent pain conditions was used as the test phenotype. The only significant association found was for haplotype 7 with minor alleles at rs3212361 and rs885479 ($p < 5.0 \times 10^{-3}$), Table 8. The next lowest p (5.1×10^{-2}) was for haplotype 6, carrying a minor allele at rs1805008 – the only other missense variant tagging the minor allele at rs3212361.

Interestingly, haplotype 3, carrying the minor allele at only rs3212361 was not significantly associated ($p = 2.57 \times 10^{-1}$), possibly because its weak effect was diluted when its minor allele was distributed among three separate model predictors (haplotypes 3, 6, and 7). We furthermore tested the additional effect of rs885479 in a global multivariate haplotype test, setting the haplotype with a minor allele only at rs3212361 (haplotype 3, Table 8) as the base. This analysis showed a significant association only for the rs885479 minor allele-carrying haplotype (haplotype 7 in Table 8, $p = 8.0 \times 10^{-3}$, $beta = -2.18 \times 10^{-2}$). It was not possible to run an analogous haplotype association analysis in the OPPERA cohort due to an insufficient sample size for a 10-variant block.

3.5. eQTL analysis

Given that one of the variants associated with pain sensitivity is located in the regulatory region of the gene and the other one does not result in a strong change in cellular function [4, 5, 38], we investigated both rs3212361 and rs885479 for possible effects on gene expression using the GTEx eQTL repository. The tissues we selected to investigate were the tibial nerve, representing the peripheral nervous system, and CNS structures with an established role in pain transmission or interpretation: spinal cord, amygdala, caudate, cerebellum, hippocampus, hypothalamus, putamen, anterior cingulate cortex, and nucleus accumbens. Our results show that after correcting for multiple tests (2 variants X 10 different tissues = 20, p threshold set at 0.05/20 = 0.0025), rs3212361 is significantly associated with reduced *MC1R* mRNA expression levels in caudate, nucleus accumbens, and tibial nerve and nominally associated with anterior cingulate cortex, cerebellum, putamen, and spinal cord, while rs885479 is only nominally associated with reduced *MC1R* expression in the tibial nerve, Table 9. Taking into account the strong LD between rs3212361 and rs885479, the association of rs885479 with *MC1R* expression in the tibial nerve is likely a reflection of the rs3212361 eQTL effect.

To determine if eQTL effects distinguished pain-associated *MC1R* variants from non-pain-associated *MC1R* variants, we also extracted eQTL summary statistics for all missense variants from Table 5. Supplementary Table S3 shows all results with $p < 0.05$. After correcting for 80 tests (8 variants X 10 tissues), rs2228479 is a significant eQTL in all but one brain tissue (hippocampus), however its minor allele is associated with increased levels of the *MC1R* transcript. Importantly, the minor allele of rs2228479 tags the major allele at

rs3212361 (Table 6, haplotype 4) and has the opposite direction of effect from the minor allele of rs3212361 (Table 7). However, it is not the only marker of this major allele among the analysed missense *MC1R* variants. The GTEx eQTL Calculator tool does not provide for haplotype association analysis. Therefore, we have not been able to test joint effects of rs3212361 and rs885479 on *MC1R* mRNA levels.

3.6. The effect of the 5'-UTR variant on red hair

Like the coding region variants associated with the red hair phenotype, which have been determined to result in a hypofunctional MC1R in cellular assays, the overall effect of the 5'-UTR region variant is also a reduction in the availability of functional MC1R. As such, we would expect it to correlate with the red hair phenotype as well. This is borne out by our analysis, which shows that the minor allele A of rs3212361 is positively correlated with red hair ($OR = 1.34$, $p = 2.0 \times 10^{-16}$), although the effect size is substantially smaller than for missense variants [49].

3.7. The effect of red hair on pain

Lastly, we ran a regression analysis to test for association between hair colour (red versus dark) and the count of persistent clinical pain conditions. The binary-coded phenotype, red hair = 0, dark hair = 1, showed a positive association with the count of pain conditions ($beta = 4.08 \times 10^{-2}$, $p = 5.39 \times 10^{-5}$), meaning that dark hair conferred a higher risk of – and by extent red hair was protective against – having more pain conditions, Figure 3B.

4. Discussion

Here, we tested *MC1R* genetic variants for association with pain. Unexpectedly, we found that regulatory but not missense red hair variants in this gene locus appear to affect pain sensitivity. Specifically, LOF variants rs3212361 and rs885479 are associated with temporal summation and pain sensitivity. Temporal summation is a manifestation of increased firing from spinal cord dorsal horn neurons upon frequent repetitive stimulation [14, 15], demonstrated to be mediated specifically by NMDA receptors [20] and mitigated by GABA agonists [1]. Pain sensitivity, measured by the AUC, also appears to be centrally mediated [30], although sensitisation of peripheral afferents to noxious stimuli may contribute as well. Both types of summary measures are modulated by central processing at the level of the dorsal horn [9, 19, 44, 48]. We were furthermore able to validate these findings in the UKBB, using the count of persistent clinical pain conditions as a proxy for nociceptive integration as well as the clinically relevant assessment of central sensitisation. Generally, both variants showed similar association statistics, and the only tested haplotype with significant association had minor alleles at rs3212361 and rs885479 ($p < 5.0 \times 10^{-3}$). Additionally, we found both variants to be associated with individual musculoskeletal conditions.

The motivation behind this study lay in reconciling previously published findings about *MC1R* variants and pain sensitivity. Our results show that in the *MC1R* locus, contribution to pain sensitivity may stem from the 5'-UTR, tagged by rs3212361, and from one low-penetrance red-hair variant, rs885479. The explanation for the divergent results reported

earlier [27, 28, 31, 32] may lie in the haplotypic structure of *MC1R*'s coding region variants, associated with red hair, and the 5'-UTR variant, associated with pain sensitivity. Given the distribution of red hair alleles on the pain-associated variant's background and allele frequencies in earlier studies, we estimate the odds of a redhaired individual to be less sensitive to pain as about 50/50, with a slight bias toward reduced sensitivity, borne out by our test of the effect of red hair on pain. Of the 3 studies that report genotypes Mogil's 8 of 14 (57%) and 20 of 29 (69%) carried either the pain-protective haplotype with minor alleles at both rs885479 (and by extension rs3212361) or just at rs3212361 as tagged by rs1805008 [31, 32]. In Liem's 2004 study, 4 of 10 (40%) had at least one minor allele at either rs1805008 or rs885749 [28]. We therefore extrapolate that participants were enriched for the pain-protective 5'-UTR genotype in the former and for the pain-sensitive genotype in the latter.

The difference in the functional roles of the two relevant regions in *MC1R* invites several possible explanations. First, previously characterised variants have been shown to result in red hair by affecting cAMP-mediated activity but not *MC1R* mRNA [4, 5, 41]. Our eQTL query suggests that *MC1R* transcript levels are reduced in all relevant tissues by the 5'-UTR variant. Thus, cAMP-dependent protein function and transcript levels, each appears to primarily affect hair colour and pain sensitivity, respectively. The first part of this claim is consistent with previous reports that have shown that eumelanin production is directly linked to cAMP activity and could be rescued in MC1R-deficient cells using forskolin [16]. As for pain sensitivity, it is possible that a change in *MC1R* expression levels affects nociception or its inhibition through a feedback mechanism. As has been previously suggested, such a feedback loop likely involves the opioid system [46]. Along with MC1R's main agonist, α-MSH, the latter's precursor peptide, pro-opiomelanocortin (POMC) processing also yields β-endorphin, an endogenous opioid agonist [11]. Thus, it is plausible that reduced levels of *MC1R* may signal for an upregulation of POMC, which increases the availability of the opioid agonist and its analgesic activity, Figure 5. Our discovery that rs3212361 is an eQTL in tibial nerve, cerebellum, putamen, and caudate provides additional evidence for a role for MC1R in ascending nociceptive transmission, implicating both the peripheral and central nervous systems [7, 33]. The tibial nerve association further suggests a possible involvement of sensitised C-fibres.

The missense variant, rs885479, is not an eQTL in the tested tissues, excepting a nominal association in the tibial nerve. However, its reported mild cellular functional effects on reduction in ligand-binding, cell surface expression, and cAMP production [4, 5] together with the effects of rs3212361 on mRNA expression would lead to a reduction in the activity of MC1R. It is unclear how this hypofunction is different from an analogous combination of LOF alleles on haplotype 6 (Table 8), which carries the minor alleles of rs3212361 and rs1805008. In particular, both variants are located within the fourth transmembrane domain. The arginine-to-glutamine change, R163Q, encoded by rs885479 causes a switch from a charged to polar amino acid, while the arginine to tryptophan change, R160W, is a more drastic switch from a charged to non-polar amino acid. Thus, while both rs885479 and rs1805008 are LOF variants, one would expect a stronger effect of rs1805008 rather than rs885479, as in red hair penetrance [49]. Furthermore, the observed effect could be similar to that triggered by a full gene knockout but not partial knockdown [40]. In our study the

more deleterious double-LOF combination of the *MC1R* transcript-reducing allele of rs3212361 and MC1R activity-compromising rs1805008 could trigger compensation, masking the effects of these variants on pain sensitivity, which a milder double-LOF rs3212361/rs885479 combination does not.

Another explanation for our results is that the effect on pain sensitivity is exerted not by the additive effects of the individual minor alleles of variants rs3212361 and rs885479 but by their joint effect. The minor alleles of each of the two contributing variants may interact to produce a novel functional effect, for example, altering the secondary structure of the *MC1R* transcript and leading to low translation efficiency [34]. Alternatively, rs3212361 and rs885479 may be markers for the true causal variant or region, yet to be found. Overall, the evidence available to-date, including our present findings, suggests that while MC1R does not directly participate in nociception, a reduction in its expression levels, especially when coupled with mild cellular functional impairment may modulate the nociceptive response or its inhibition.

Interestingly, while with one exception we did not find missense variants to contribute to pain sensitivity, the 5'-UTR variants, tagged by rs3212361, do contribute to red hair colour. This is consistent with the findings reported previously, which showed one 5'-UTR variant (rs76337330) in the parsimonious mRMR classifier-selected set of 10 best red hair colour predictors and 7 5'-UTR variants (rs148003355, rs3212359, rs3212361, rs3212371, rs3212379, rs577907985, and rs868197501) LASSO-selected set for a more complete genetic variant prediction model for red hair [49]. Furthermore, when we tested binary hair colour (red versus dark) with the count of persistent pain conditions, we found a significant association between these two phenotypes, an effect possibly mediated by 5'-UTR variants: redheads showed a tendency to report fewer persistent pain conditions.

It is important to recognise limitations in this study. Arguably the biggest limitation is the lack of equivalent phenotypes in our discovery and validation cohorts. As time-consuming procedures requiring specialised equipment, QST protocols are rare to be performed in large population studies. Nevertheless, we have shown that *MC1R* variants rs3212361 and rs885479 affect both experimental measures of central sensitisation and the presence of clinical conditions, in line with the reported correlation of these two phenotypes [17]. Numerous studies in patients with fibromyalgia [12, 20, 43], chronic low back pain [18, 24], hip osteoarthritis [23], migraine [47], whiplash [13, 25], and TMD [30] have shown differences in temporal summation between patients and pain-free controls, with fibromyalgia, TMD, and whiplash patients showing greater pain intensity AUC. Furthermore, the larger UKBB sample size enabled us to test and verify the corresponding haplotype association with a clinically relevant outcome. However, our results should be replicated in other cohorts with heat temporal integration and summation measures and possibly other QSTs. Additionally, we were not able to test the joint effect of the two identified variants in the OPPERA cohort and in the eQTL dataset. Lastly, the effect sizes reported here for both OPPERA and UKBB analyses are not large, although their magnitude is in line with any common genetic variant's effect on a common phenotype. The presence of statistically significant associations is indicative of its contribution to pain processing and

therefore sheds additional light on the highly complex and intricate network involved in this clinically relevant phenomenon.

In conclusion, we report here that *MC1R* genetic variants relevant to pain sensitivity are distinct from red hair-conferring genetic variants. Our findings provide evidence for the different mechanisms that may be responsible for MC1R's role in these two phenotypes, build upon our understanding of the genetic architecture of human pain sensitivity, and have important potential implications for clinical practice. Specifically, rs3212361 and rs885479 could be a useful inclusion into a panel of genetic contributors in pain sensitivity profiling, to facilitate targeted therapy. Additionally, our results have broad implications for correspondence between genetic variants, cellular effects, and downstream clinical phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

5. Acknowledgements

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References

[1]. Arendt-Nielsen L, Frøkjær JB, Staahl C, Graven-Nielsen T, Huggins JP, Smart TS, and Drewes AM Effects of gabapentin on experimental somatic pain and temporal summation.

[2]. Arendt-Nielsen L, and Petersen-Felix S Wind-up and neuroplasticity: is there a correlation to clinical pain? European journal of anaesthesiology. Supplement 10 (1995), 1–7. [PubMed: 7535689]

[3]. Barrett JC, Fry B, Maller J, and Daly MJ Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 2 (2004), 263–265. [PubMed: 15297300]

[4]. Beaumont KA, Newton RA, Smit DJ, Leonard JH, Stow JL, and Sturm RA Altered cell surface expression of human MC1R variant receptor alleles associated with red hair and skin cancer risk. Human molecular genetics 14, 15 (2005), 2145–2154. [PubMed: 15972726]

[5]. Beaumont KA, Shekar SL, Newton RA, James MR, Stow JL, Duffy DL, and Sturm RA Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. Human molecular genetics 16, 18 (2007), 2249–2260. [PubMed: 17616515]

[6]. Bhalang K, Sigurdsson A, Slade GD, and Maixner W Associations among four modalities of experimental pain in women. The Journal of Pain 6, 9 (2005), 604–611. [PubMed: 16139779]

[7]. Bingel U, Quante M, Knab R, Bromm B, Weiller C, and Büchel C Subcortical structures involved in pain processing: evidence from single-trial fMRI. Pain 99, 1–2 (2002), 313–321. [PubMed: 12237210]

[8]. Brodin E, Malin E, and Olgart L Neurobiology: General considerations– from acute to chronic pain. Nor. Tannlegeforen. Tid 126 (2016), 28–33.

[9]. Budai D, and Larson AA Role of substance P in the modulation of C-fiber evoked responses of spinal dorsal horn neurons. Brain research 710, 1–2 (1996), 197–203. [PubMed: 8963659]

[10]. Chhajlani V, and Wikberg JE Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. FEBS letters 309, 3 (1992), 417–420. [PubMed: 1516719]

[11]. Cone RD Anatomy and regulation of the central melanocortin system. Nature neuroscience 8, 5 (2005), 571. [PubMed: 15856065]

[12]. Craggs JG, Staud R, Robinson ME, Perlstein WM, and Price DD Effective connectivity among brain regions associated with slow temporal summation of C-fiber-evoked pain in fibromyalgia patients and healthy controls. The Journal of Pain 13, 4 (2012), 390–400. [PubMed: 22480771]

[13]. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, and Radanov BP Central hypersensitivity in chronic pain after whiplash injury. The Clinical journal of pain 17, 4 (2001), 306–315. [PubMed: 11783810]

[14]. Davies SN, and Lodge D Evidence for involvement of N-methyl-aspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain research 424, 2 (1987), 402–406. [PubMed: 2823998]

[15]. Dickenson A, and Sullivan AF Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 26, 8 (1987), 1235–1238. [PubMed: 2821443]

[16]. D'Orazio JA, Nobuhisa T, Cui R, Arya M, Spry M, Wakamatsu K, Igras V, Kunisada T, Granter SR, Nishimura EK, et al. Topical drug rescue strategy and skin protection based on the role of MC1R in UV-induced tanning. Nature 443, 7109 (2006), 340. [PubMed: 16988713]

[17]. Edwards RR Individual differences in endogenous pain modulation as a risk factor for chronic pain. Neurology 65, 3 (2005), 437–443. [PubMed: 16087910]

[18]. George SZ, Wittmer VT, Fillingim RB, and Robinson ME Sex and pain-related psychological variables are associated with thermal pain sensitivity for patients with chronic low back pain. The Journal of Pain 8, 1 (2007), 2–10. [PubMed: 17207739]

[19]. Gozariu M, Bragard D, Willer J-C, and Le Bars D Temporal summation of C-fiber afferent inputs: competition between facilitatory and inhibitory effects on C-fiber reflex in the rat. Journal of neurophysiology 78, 6 (1997), 3165–3179. [PubMed: 9405536]

[20]. Graven-Nielsen T, Kendall SA, Henriksson KG, Bengtsson M, Sörensen J, Johnson A, Gerdle B, and Arendt-Nielsen L Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. Pain 85, 3 (2000), 483–491. [PubMed: 10781923]

[21]. Greenspan JD, Slade GD, Bair E, Dubner R, Fillingim RB, Ohrbach R, Knott C, Mulkey F, Rothwell R, and Maixner W Pain sensitivity risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case control study. The Journal of Pain 12, 11 (2011), T61–T74. [PubMed: 22074753]

[22]. Ito S, and Wakamatsu K Chemistry of mixed melanogenesis—pivotal roles of dopaquinone. Photochemistry and photobiology 84, 3 (2008), 582–592. [PubMed: 18435614]

[23]. Izumi M, Petersen KK, Laursen MB, Arendt-Nielsen L, and Graven-Nielsen T Facilitated temporal summation of pain correlates with clinical pain intensity after hip arthroplasty. Pain 158, 2 (2017), 323–332. [PubMed: 27870648]

[24]. Kleinböhl D, Görtelmeyer R, Bender H-J, and Hölzl R Amantadine sulfate reduces experimental sensitization and pain in chronic back pain patients. Anesthesia & Analgesia 102, 3 (2006), 840–847. [PubMed: 16492838]

[25]. Lemming D, Graven-Nielsen T, Sörensen J, Arendt-Nielsen L, and Gerdle B Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women. Journal of rehabilitation medicine 44, 8 (2012), 648–657. [PubMed: 22729792]

[26]. Lichtenwalter RN, Zorina-Lichtenwalter K, and Diatchenko L Genotypic data in relational databases: efficient storage and rapid retrieval. In European Conference on Advances in Databases and Information Systems (2017), Springer, pp. 408–421.

[27]. Liem EB, Joiner TV, Tsueda K, and Sessler DI Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. The Journal of the American Society of Anesthesiologists 102, 3 (2005), 509–514.

[28]. Liem EB, Lin C-M, Suleman M-I, Doufas AG, Gregg RG, Veauthier JM, Loyd G, and Sessler DI Anesthetic requirement is increased in redheads. Anesthesiology: The Journal of the American Society of Anesthesiologists 101, 2 (2004), 279–283.

[29]. Maixner W, Diatchenko L, Dubner R, Fillingim RB, Greenspan JD, Knott C, Ohrbach R, Weir B, and Slade GD Orofacial pain prospective evaluation and risk assessment study–the OPPERA study. The Journal of Pain 12, 11 (2011), T4–T11. [PubMed: 22074751]

[30]. Maixner W, Fillingim R, Sigurdsson A, Kincaid S, and Silva S Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain: evidence for altered temporal summation of pain. Pain 76, 1–2 (1998), 71–81. [PubMed: 9696460]

[31]. Mogil JS, Ritchie J, Smith SB, Strasburg K, Kaplan L, Wallace M, Romberg R, Bijl H, Sarton E, Fillingim R, et al. Melanocortin-1 receptor gene variants affect pain and μ-opioid analgesia in mice and humans. Journal of medical genetics 42, 7 (2005), 583–587. [PubMed: 15994880]

[32]. Mogil JS, Wilson SG, Chesler EJ, Rankin AL, Nemmani KV, Lariviere WR, Groce MK, Wallace MR, Kaplan L, Staud R, et al. The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. Proceedings of the National Academy of Sciences 100, 8 (2003), 4867–4872.

[33]. Moulton EA, Schmahmann JD, Becerra L, and Borsook D The cerebellum and pain: passive integrator or active participator? Brain research reviews 65, 1 (2010), 14–27. [PubMed: 20553761]

[34]. Nackley AG, Shabalina S, Tchivileva I, Satterfield K, Korchynskyi O, Makarov S, Maixner W, and Diatchenko L Human catecholo-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 314, 5807 (2006), 1930–1933. [PubMed: 17185601]

[35]. Nyholt DR A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. The American Journal of Human Genetics 74, 4 (2004), 765–769. [PubMed: 14997420]

[36]. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, and Vierck CJ Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. Pain 99, 1–2 (2002), 49–59. [PubMed: 12237183]

[37]. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, De Bakker PI, Daly MJ, et al. Plink: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81, 3 (2007), 559–575. [PubMed: 17701901]

[38]. Ringholm A, Klovins J, Rudzish R, Phillips S, Rees JL, and Schiöth HB Pharmacological characterization of loss of function mutations of the human melanocortin 1 receptor that are associated with red hair. Journal of Investigative Dermatology 123, 5 (2004), 917–923. [PubMed: 15482480]

[39]. Robbins LS, Nadeau JH, Johnson KR, Kelly MA, RoselliRehfuss L, Baack E, Mountjoy KG, and Cone RD Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72, 6 (1993), 827–834. [PubMed: 8458079]

[40]. Rossi A, Kontarakis Z, Gerri C, Nolte H, Hölper S, Krüger M, and Stainier DY Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature 524, 7564 (2015), 230. [PubMed: 26168398]

[41]. Scott MC, Wakamatsu K, Ito S, Kadekaro AL, Kobayashi N, Groden J, Kavanagh R, Takakuwa T, Virador V, Hearing VJ, et al. Human melanocortin 1 receptor variants, receptor function and melanocyte response to UV radiation. J Cell Sci 115, 11 (2002), 2349–2355. [PubMed: 12006619]

[42]. Sinnwell J, and Schaid D R package version 1.2. 2.

[43]. Sörensen J, Graven-Nielsen T, Henriksson K, Bengtsson M, and Arendt-Nielsen L Hyperexcitability in fibromyalgia. The Journal of rheumatology 25, 1 (1998), 152–155. [PubMed: 9458220]

[44]. Staud R The important role of cns facilitation and inhibition for chronic pain. International journal of clinical rheumatology 8, 6 (2013), 639. [PubMed: 24489609]

[45]. Valverde P, Healy E, Jackson I, Rees JL, and Thody AJ Variants of the melanocyte–stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nature genetics 11, 3 (1995), 328–330. [PubMed: 7581459]

[46]. Vrinten DH, Gispen WH, Kalkman CJ, and Adan RA Interaction between the spinal melanocortin and opioid systems in a rat model of neuropathic pain. Anesthesiology: The Journal of the American Society of Anesthesiologists 99, 2 (2003), 449–454.

[47]. Weissman-Fogel I, Sprecher E, Granovsky Y, and Yarnitsky D Repeated noxious stimulation of the skin enhances cutaneous pain perception of migraine patients in-between attacks: clinical evidence for continuous sub-threshold increase in membrane excitability of central trigeminovascular neurons. Pain 104, 3 (2003), 693–700. [PubMed: 12927642]

[48]. You H-J, Morch CD, Chen J, and Arendt-Nielsen L Simultaneous recordings of wind-up of paired spinal dorsal horn nociceptive neuron and nociceptive flexion reflex in rats. Brain research 960, 1–2 (2003), 235–245. [PubMed: 12505677]

[49]. Zorina-Lichtenwalter K, Lichtenwalter RN, Zaykin DD, Parisien M, Gravel S, Bortsov A, and Diatchenko L A study in scarlet: MC1R as the main predictor of red hair and exemplar of the flip-flop effect. Human molecular genetics (2019).

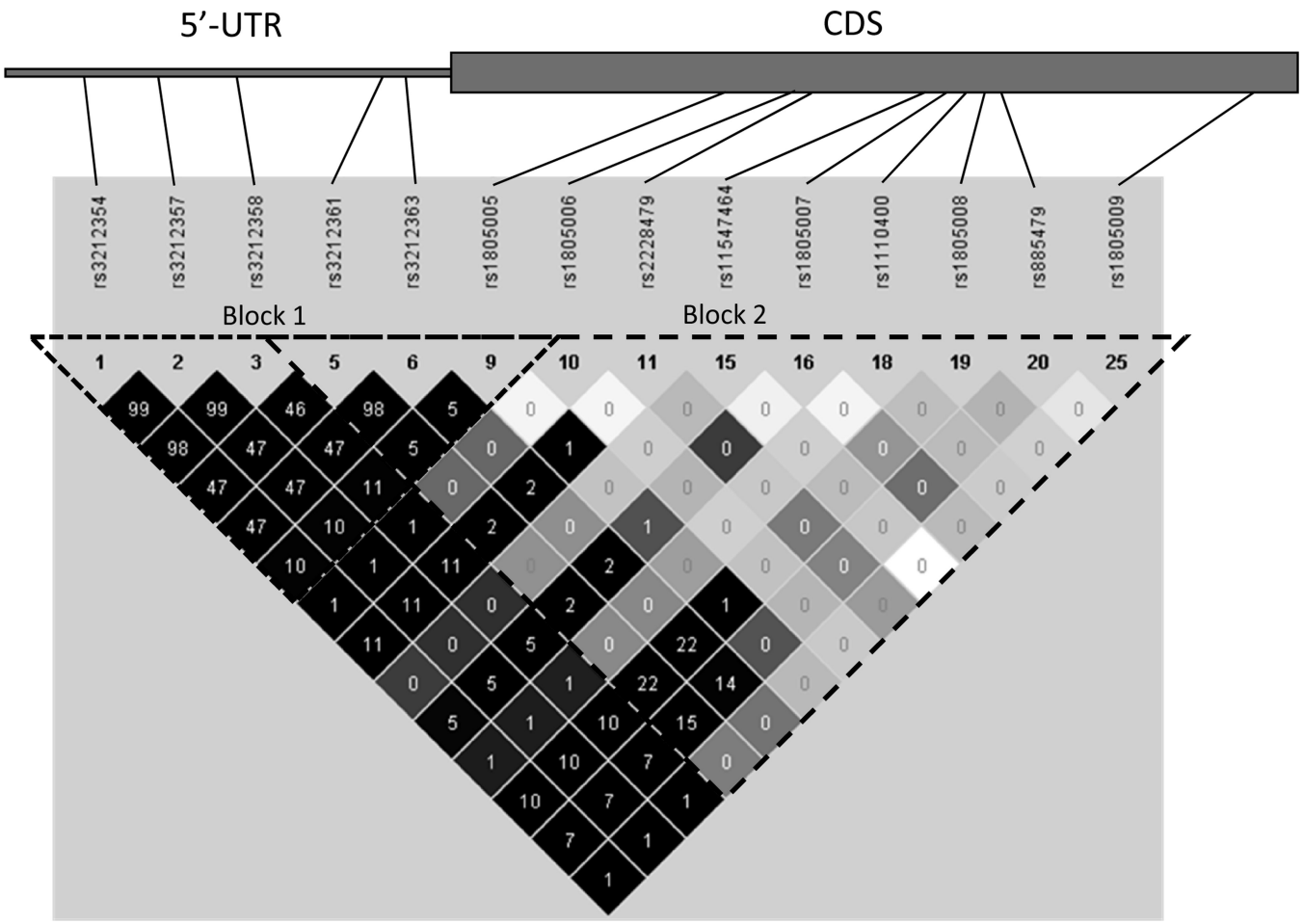

Figure 1:

Linkage disequilibrium (LD) plot for 5’-UTR and CDS (coding region) *MC1R* variants. Block 1 consists of variants rs3212354, rs3212357, rs3212358, rs3212361, and rs3212363. Block 2 consists of variants rs3212361, rs1805005, rs1805006, rs2228479, rs11547464, rs1805007, rs1110400, rs1805008, rs885479, and rs1805009. Metric coding: colour = D’ (white = 0; 1 < grey > 0; black = 1); values in the diamond: r-squared.

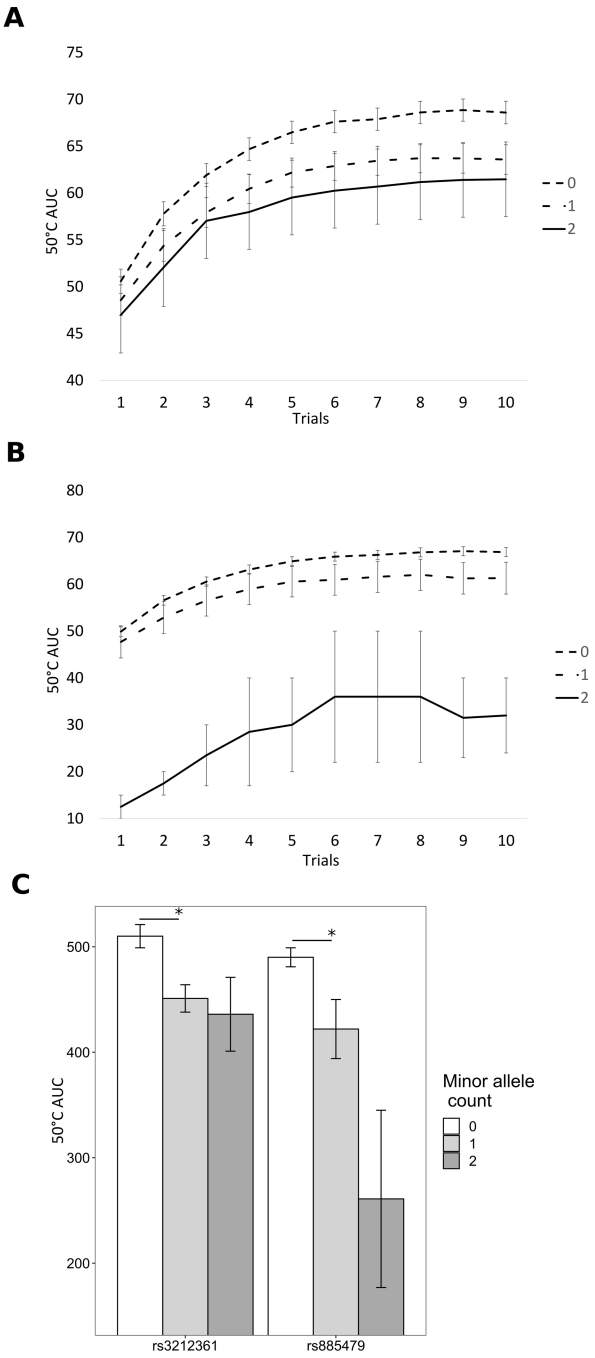

Figure 2:

Effects of variants rs3212361 and rs885479 on responses to temporal summation of repeated heat stimuli at 50°C in the OPPERA cohort. Mean visual analog scale (VAS) responses to 10 thermal stimuli (trials) were plotted for each genotype A, B: Responses categorised by genotype carrier for rs3212361 (A) and rs885479 (B). C: Distribution of the AUC phenotype for each genotype carrier for rs3212361 and rs885479. *$p < 0.05$; Student's unpaired t test.

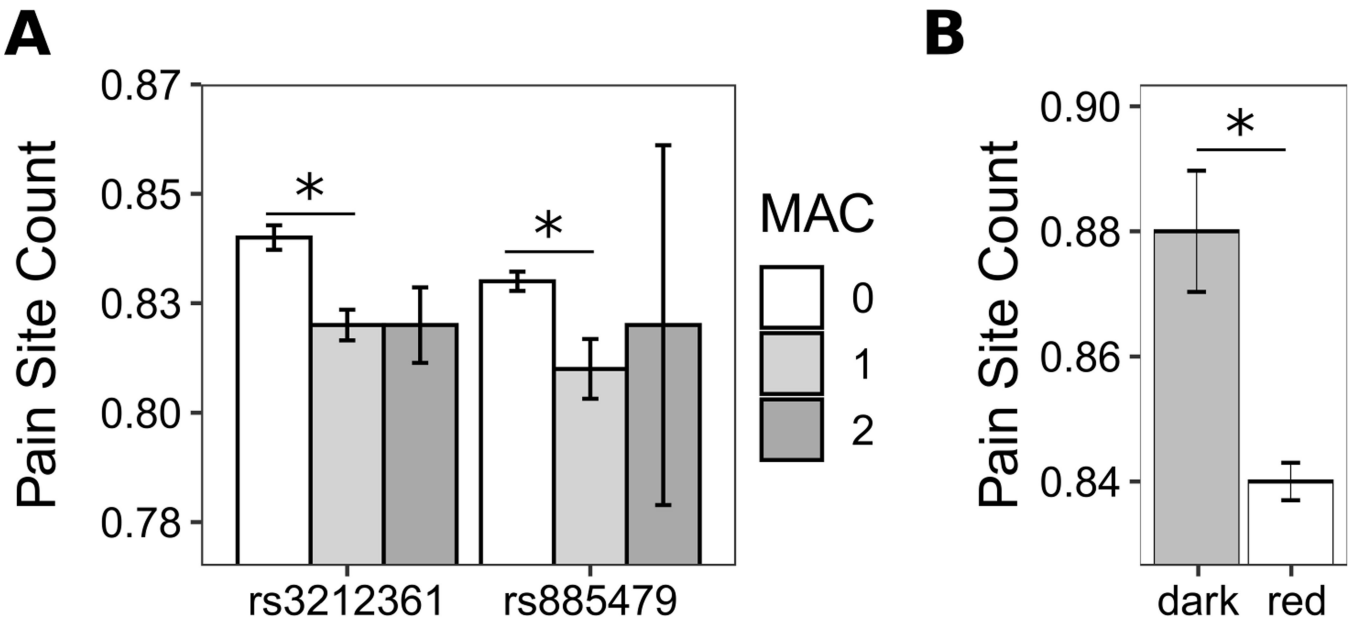

Figure 3:

Effects of *MC1R* genotypes in the UKBB cohort. A: Distribution of reported pain site counts grouped by genotype for rs3212361 and rs885479. B: pain site count in individuals grouped by hair colour, dark and red. *$p < 0.05$; Student's unpaired t test. MAC, minor allele count.

Condition	rs3212361 OR(L95−U95)	rs885479 OR(L95−U95)
Back	0.979(0.965−0.993)	0.955(0.927−0.983)
Facial	0.997(0.944−1.054)	1.057(0.950−1.175)
Hdche	1.001(0.982−1.020)	0.978(0.942−1.016)
Hip	0.974(0.956−0.993)	0.961(0.926−0.999)
Knee	0.986(0.971−1.000)	0.966(0.938−0.994)
Neck	0.976(0.961−0.991)	0.969(0.941−0.999)
Stmch	0.976(0.952−1.001)	0.969(0.922−1.019)

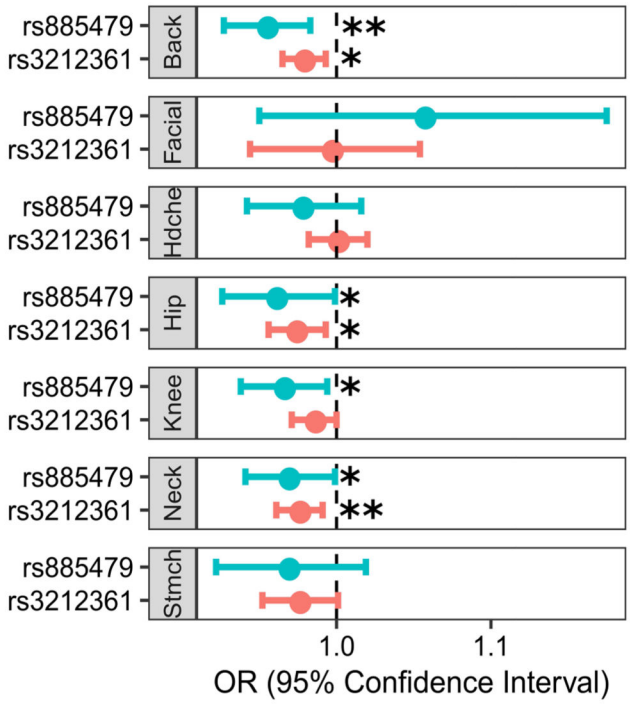

Figure 4:

Forest plot showing odds ratios (ORs), and 95% confidence intervals (L95 and U95, lower and upper boundaries) for variants rs3212361 and rs885479 in association with persistent clinical pain in the listed body sites in the UKBB. *$p < 0.05$, **$p < 0.0036$, as determined by the logistic regression analysis for association.

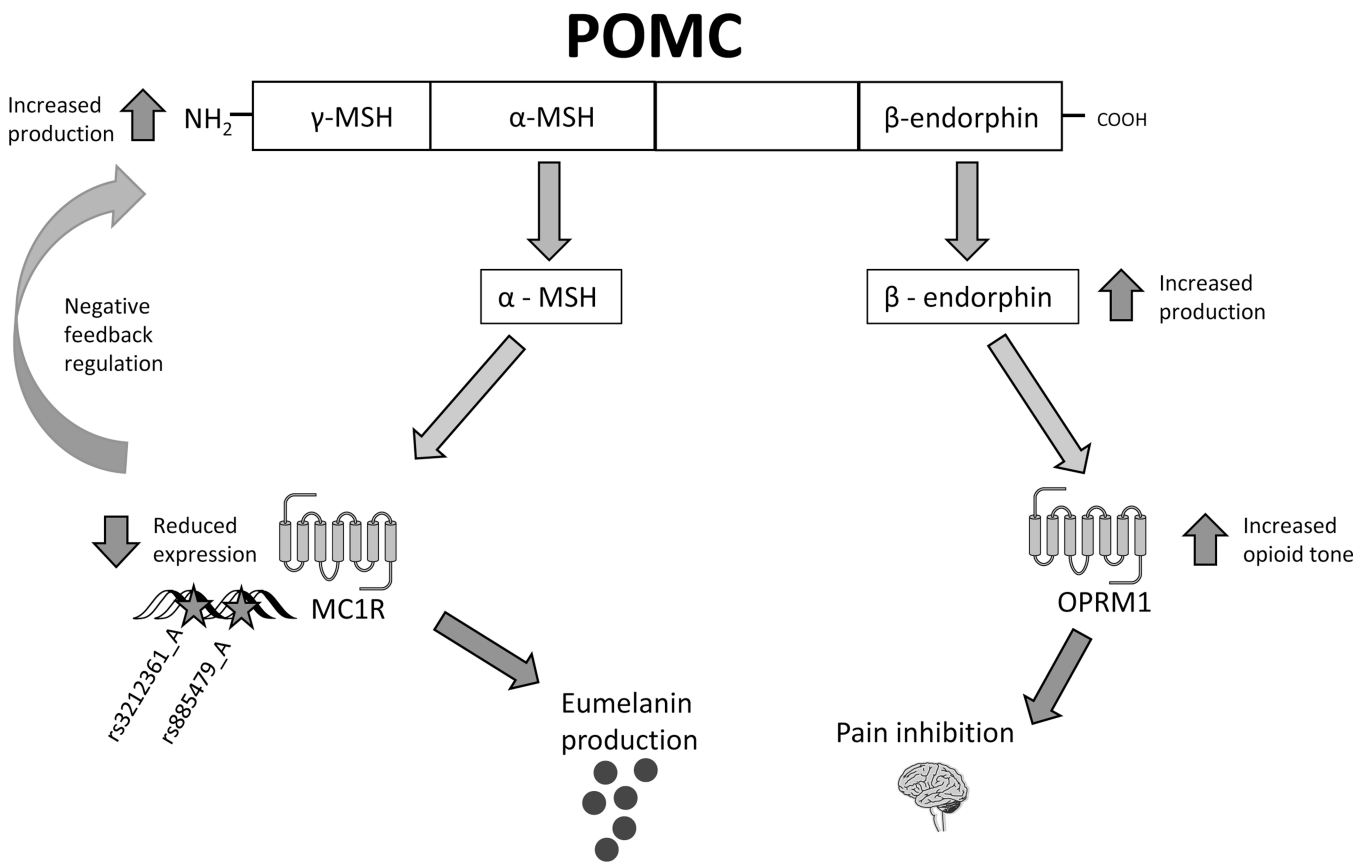

Figure 5:

Heuristic model of *MC1R* variant effects on pain processing. The reduced levels of MC1R expression resulting from allelic variants at rs3212361 and rs885479 may trigger negative feed-back cellular responses, leading to an upregulation of POMC, pro-opiomelanocortin, a hybrid precursor peptide for α-MSH (melanocyte stimulating hormone) and β-endorphin, which in turn could lead to an increased activity of the μ-opioid receptor (ORPM1). NH_2 and COOH, amino and carboxyl termini of the protein peptide.

Table 1:

Demographics

Metric	OPPERA (f)	OPPERA (m)	UKBB (f)	UKBB (m)
n	464	412	214,926	182,756
Age, avg. yrs.	27	25	57	57
TMD status (n)	195	74	NA	NA
Pain phenotype avg. (SE)	516 (12)	445 (12)	0.91 (0.003)	0.73 (0.003)

OPPERA, Orofacial Pain: Prospective Evaluation and Risk Assessment study; UKBB, U.K. Biobank; f, female; m, male; n, number (sample size); avg., average; yrs., years; TMD, temporomandibular disorder; SE, standard error. Pain phenotype avg.: 50°C AUC in OPPERA and pain site count in UKBB.

Table 2:

Descriptive statistics for genotypes

Variant	Min/Maj	MAF_O/MAF_U	Geno_OPPERA	Geno_UKBB	OH_O/OH_U	Position	L/FC
rs3212354	C/T	0.42/0.44	297/751/547	95529/231122/157343	0.47/0.48	89984370	5'-UTR
rs3212357	C/T	0.42/0.44	305/761/554	95773/230917/157153	0.47/0.48	89984604	5'-UTR
rs3212358	G/A	0.43/0.44	308/761/552	95979/231144/156666	0.47/0.48	89984739	5'-UTR
rs3212379	A/G	0.01/0.01	0/23/1598	22/5485/479433	0.01/0.01	89985131	5'-UTR
rs3212361	A/G	0.26/0.26	114/604/903	32869/180693/269559	0.37/0.37	89985222	5'-UTR
rs3212363	T/A	0.26/0.26	116/605/897	34986/181859/266225	0.37/0.38	89985441	5'-UTR
rs1805005	T/G	0.13/0.12	33/361/1227	7420/101779/378210	0.22/0.21	89985844	missense
rs1805006	A/C	0.01/0.01	0/31/1590	73/12045/475291	0.02/0.02	89985918	missense
rs2228479	A/G	0.08/0.10	11/227/1382	4682/83571/399156	0.14/0.17	89985940	missense
rs11547464	A/G	0.01/0.01	0/26/1595	33/6752/480624	0.02/0.01	89986091	missense
rs1805007	T/C	0.07/0.10	3/226/1388	5238/84855/397316	0.14/0.17	89986117	missense
rs1110400	C/T	0.01/0.01	0/25/1595	90/11219/474702	0.02/0.02	89986130	missense
rs1805008	T/C	0.07/0.08	10/214/1395	3634/74240/409535	0.13/0.15	89986144	missense
rs885479	A/G	0.05/0.05	5/152/1461	2324/45170/439915	0.09/0.09	89986154	missense
rs1805009	C/G	0.02/0.02	0/55/1542	144/20072/461197	0.03/0.04	89986546	missense
rs3212369	G/A	0.16/0.17	45/437/1138	15314/137687/331667	0.27/0.28	89986760	3'-UTR
rs3212371	G/A	0.10/0.12	15/295/1284	7658/103160/374645	0.19/0.21	89987201	3'-UTR

Min, minor allele; Maj, major allele; MAF_O/MAF_U, minor allele frequency in OPPERA and UKBB, respectively; Min, minor allele count; Geno_OPPERA/UKBB, counts of genotype carriers – MinMin/MinMaj/MajMaj – in OPPERA and UKBB, respectively; OH_O/OH_U, observed heterozygosity in OPPERA and UKBB, respectively; L/FC, location/functional consequence; UTR, untranslated region. Chromosomal position comes from the GRCh 37.

Table 3:

MC1R variant association analysis with 50°C AUC

Variant	Minor allele	MAF	Effect (*B*)	SE	*p*
rs3212361	A	0.26	−51.2	12.7	6.0×10^{-5} **
rs3212363	T	0.26	−49.8	12.7	1.0×10^{-4} **
rs3212357	C	0.42	−35.3	11.2	1.7×10^{-3} **
rs3212358	G	0.42	−34.8	11.2	2.0×10^{-3} **
rs3212354	C	0.42	−33.9	11.4	3.0×10^{-3} **

MAF, minor allele frequency; SE, standard error;

** statistically significant association ($p < 3.1 \times 10^{-3}$).

Table 4:

Haplotypes of *MC1R* 5'-UTR variants (Block 1) above HF 0.01

Haplotype	rs3212354	rs312357	rs3212358	rs3212361	rs3212363	HF	Carriers (het/hom)
1	T	T	A	G	A	0.57	1313/551
2	***C***	***C***	***G***	***A***	***T***	0.25	717/114
3	***C***	***C***	***G***	G	A	0.17	48¾6

In each haplotype the minor allele is highlighted in ***boldface Italicised*** font. The top haplotype is wild-type. HF, haplotype frequency; het., heterozygous; hom., homozygous.

Table 5:

MC1R missense variant association analysis with 50°C AUC

Variant	Minor allele	MAF	Penetrance	Effect (*B*)	SE	*p*
rs885479	A	0.05	r	−70.7	26.3	7.0×10^{-3} *
rs11547464	A	0.01	R	155.2	79.4	5.0×10^{-2}
rs1110400	C	0.01	R	65.1	51.9	2.1×10^{-1}
rs1805005	T	0.13	r	14.3	16.2	3.8×10^{-1}
rs1805009	C	0.02	R	35.1	40.1	3.8×10^{-1}
rs1805008	T	0.07	R	−16.5	22.6	4.7×10^{-1}
rs1805006	A	0.01	R	14.6	54.4	7.9×10^{-1}
rs2228479	A	0.08	r	5.8	21.9	7.9×10^{-1}
rs1805007	T	0.07	R	−0.2	22.3	9.9×10^{-1}

MAF, minor allele frequency; SE, standard error. Penetrance refers to previously reported strength of correlation with red hair, R = strong and r = weak.

* nominally significant association ($p < 0.05$)

Table 6:

Haplotypes of missense *MC1R* variants with 5'-UTR tag variant rs3212361 (Block 2), above HF 0.01, in the OPPERA cohort.

Haplotype	rs3212361	rs1805005	rs1805006	rs2228479	rs11547464	rs1805007	rs1110400	rs1805008	rs885479	rs1805009	HF	Carriers #
1	G	G	C	G	G	C	T	C	G	G	0.42	1133/297
2	G	***T***	C	G	G	C	T	C	G	G	0.14	33/394
3	***A***	G	C	G	G	C	T	C	G	G	0.14	34/476
4	G	G	C	***A***	G	C	T	C	G	G	0.07	11/240
5	G	G	C	G	G	***T***	T	C	G	G	0.07	3/231
6	***A***	G	C	G	G	C	T	***T***	G	G	0.07	9/260
7	***A***	G	C	G	G	C	T	C	***A***	G	0.05	5/181
8	G	G	C	G	G	C	T	C	G	***C***	0.02	0/89
9	G	G	C	G	G	C	***C***	C	G	G	0.01	0/40
10	G	G	***A***	G	G	C	T	C	G	G	0.01	0/32
11	G	G	C	G	***A***	C	T	C	G	G	0.01	0/27

In each haplotype the minor allele is highlighted in ***boldface Italicised*** font. The top haplotype is wildtype. Every other haplotype carries only one variant's minor allele.

UTR, untranslated region; HF, haplotype frequency.

Table 7:

MC1R variant association analysis with the count of persistent pain conditions in UKBB

Variant	Minor allele	MAF	Effect (*B*)	SE	*p*
rs3212361	A	0.26	-9.0×10^{-3}	3.5×10^{-3}	1.2×10^{-2} **
rs885479	A	0.05	-2.2×10^{-2}	7.0×10^{-3}	2.0×10^{-3} **

MAF, minor allele frequency; SE, standard error.

** statistically significant association ($p < 0.025$)

Table 8:

Haplotype association for Block 2 variants with the count of persistent pain conditions in UKBB

Haplotype	rs3212361	rs1805005	rs1805006	rs2228479	rs11547464	rs1805007	rs1110400	rs1805008	rs885479	rs1805009	HF	Effect (*B*)	SE	*p*
1	G	G	C	G	G	C	T	C	G	G	0.37	--	--	--
2	G	***T***	C	G	G	C	T	C	G	G	0.12	4.8×10^{-4}	4.8×10^{-3}	9.2×10^{-1}
3	***A***	G	C	G	G	C	T	C	G	G	0.11	-5.4×10^{-3}	4.8×10^{-3}	2.6×10^{-1}
4	G	G	C	G	G	***T***	T	C	G	G	0.10	-6.6×10^{-3}	5.1×10^{-3}	2.0×10^{-1}
5	G	G	C	***A***	G	C	T	C	G	G	0.10	-6.3×10^{-3}	5.2×10^{-3}	2.3×10^{-1}
6	***A***	G	C	G	G	C	T	***T***	G	G	0.09	-1.1×10^{-2}	5.5×10^{-3}	5.1×10^{-2}
7	***A***	G	C	G	G	C	T	C	***A***	G	0.05	-2.8×10^{-2}	6.9×10^{-3}	$<5.0 \times 10^{-3}$ **
8	G	G	C	G	G	C	T	C	G	***C***	0.02	7.6×10^{-3}	1.0×10^{-2}	4.6×10^{-1}
9	G	G	***A***	G	G	C	T	C	G	G	0.01	-1.6×10^{-3}	1.3×10^{-2}	9.0×10^{-1}
10	G	G	C	G	G	C	***C***	C	G	G	0.01	-2.1×10^{-2}	1.4×10^{-2}	1.2×10^{-1}
11	G	G	C	G	***A***	C	T	C	G	G	0.01	-1.0×10^{-2}	1.7×10^{-2}	5.5×10^{-1}

In each haplotype the minor allele is highlighted in ***boldface Italicised*** font. HF, haplotype frequency; SE, standard error. The highest-frequency haplotype (1) is used as the reference for the above analysis. The association statistics effect size and *p* are provided for analysis with the count of persistent pain conditions in UKBB.

** statistically significant association ($p < 5.0 \times 10^{-3}$). The effect size (***β***) refers to the difference in the number of pain conditions per haplotype.

Table 9:

eQTL analysis: rs3212361 effects on *MC1R* transcript levels in peripheral and central nervous systems

Tissue	rs3212361 Effect (β)	p	rs885479 Effect (β)	p
Amygdala	−0.25	7.2×10^{-2}	−0.31	2.6×10^{-1}
Anterior cingulate cortex	−0.25	3.9×10^{-2} *	−0.27	3.1×10^{-1}
Caudate	−0.26	3.6×10^{-4} **	−0.27	1.2×10^{-1}
Cerebellum	−0.21	1.8×10^{-3} **	0.03	8.8×10^{-1}
Hippocampus	−0.15	2.5×10^{-1}	−0.04	8.7×10^{-1}
Hypothalamus	−0.13	2.3×10^{-1}	−0.11	6.9×10^{-1}
Nucleus accumbens	−0.25	3.9×10^{-4} **	−0.35	5.3×10^{-2}
Putamen	−0.24	7.2×10^{-3} *	−0.24	2.7×10^{-1}
Spinal cord	−0.36	9.3×10^{-3} *	−0.09	7.5×10^{-1}
Tibial nerve	−0.17	1.2×10^{-5} **	−0.21	1.6×10^{-2} *

The effect size is computed in normalised space and magnitude has no direct biological interpretation (https://gtexportal.org/home/documentationPage).

** statistically significant association ($p < 5.0 \times 10^{-3}$);

* nominally significant association ($p < 5.0 \times 10^{-2}$).