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Common variants at 5q33.1 in Europeans predispose to migraine in African-American children

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Abstract

Background—Genome-wide association studies (GWAS) have identified multiple susceptibility loci for migraine in European adults. However, no large-scale genetic studies have been performed in children or African Americans with migraine.

Methods—We conducted a GWAS of 380 African-American children and 2,129 ancestry-matched controls to identify variants associated with migraine. We then attempted to replicate our primary analysis in an independent cohort of 233 AA patients and 4,038 non-migraine control subjects.

Results—The results of this study indicate that common variants at 5q33.1 associated with migraine risk in African-American children (rs72793414, $P = 1.94 \times 10^{-9}$). The association was validated in an independent study ($P = 3.87 \times 10^{-3}$) for an overall meta-analysis P -value of 3.81×10^{-10} . eQTL analysis of the GTEx data also show the genotypes of rs72793414 were strongly correlated with the mRNA expression levels of *NMUR2* at 5q33.1. *NMUR2* encodes a G protein-coupled receptor of NMU. NMU, a highly conserved neuropeptide, participates in diverse physiological processes of the central nervous system (CNS).

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Contributors

H.H. and X.C. designed the study and edited the manuscript; X.C. performed the statistical analysis and drafted the manuscript; P.R. and J.G. conducted the TaqMan genotyping assays; M.M. and J.S. contributed to DNA sample collection; F.M. assisted with organizing the phenotype data; C.H. performed the SNP genotyping and QC. Y.L., J.L. and P.S. assisted in discussing and revising the manuscript. All authors read and approved the final manuscript.

Competing interests

None declared.

Ethics approval

The Research Ethics Board of CHOP approved this project and provided written informed consent from all subjects by nursing and medical assistant staff under the direction of CHOP clinicians.

Conclusions—This study provides new insights into the genetic basis of childhood migraine and allow for precision therapeutic development strategies targeting migraine patients of African-American ancestry.

Introduction

Migraine is a common neurological disorder characterized by recurrent and intense headaches[1]. Although the mechanism of migraine is still unknown, migraines are believed to be caused by the interactions of multiple environmental and genetic factors [1]. Twin studies have revealed heritability estimates of migraine ranging from 34% to 51%[2]. Consistent with the high heritability estimates, GWA studies of European adults have successfully identified many migraine susceptibility genes involved in neuronal and vascular mechanism[3 4]. As the origins of migraine can be traced into childhood and adolescence for many adult sufferers, the early onset of migraine may reflect elevated biological predisposition or increased susceptibility to environmental risk factors. In addition, as the prevalence of migraines varies across ethnicities, the genetics risk factors may be different in African ancestries and European ancestries. To our knowledge, no large-scale GWAS has been performed in either children or African Americans with migraine.

Methods

Sample Collection

Case subjects were defined as subjects with a diagnosis of migraine (ICD9 code: 346.00–346.93) and registered through the EMR of the Children’s Hospital of Philadelphia (CHOP). All patients fulfilled the International Headache Society diagnostic criteria (ICHD-3b) [5] for migraine. The number of patients diagnosed with migraine with and without aura was provided in Table S1.

All blood samples were collected at the time of diagnosis, and most were annotated with clinical information comprising gender and age at diagnosis. Control subjects were recruited from the Philadelphia region through the CHOP Health Care Network, including four primary-care clinics and several group practices and outpatient practices that included well-child visits. Eligibility criteria for control subjects were (i) self-reporting as Caucasian or African American; (ii) availability of 1.5 µg of high-quality DNA from peripheral-blood mononuclear cells; and (iii) no serious underlying medical disorder, including mental disorders (ICD9 code: 290.0–319). The Research Ethics Board of CHOP provided written informed consent from all subjects by nursing and medical assistant staff under the direction of CHOP clinicians. SNP genotyping was performed using the Illumina Infinium II HumanHap550 and Human Quad610 BeadChips.

TaqMan SNPs and Genotyping

Selection of SNPs were conducted based on information from the dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Two polymorphisms rs1946225 and rs72793414 were included for the genotyping tests. Real-time PCR using the following calculations: 2.5uL Genotyping Master Mix, 0.25uL SNP Assay-probes, and 2.25uL DNA template (at 5ng/uL= 11.25ng total). Thermal cycling conditions were as follows: 60C 30secs Pre-read,

95 °C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60 °C for 1 min, then 60C 30secs Post-read. Genotyping of the polymorphisms were carried out using the 5' exonuclease TaqMan Allelic Discrimination assay, which was performed utilizing minor groove binder probes fluorescently labeled with VIC or FAM and the protocol recommended by the supplier (Applied Biosystems, Foster City, CA, USA). Analysis for interpretation was performed with Via7 software and Taqman Genotyper software calling.

Quality control

Individuals were genotyped on the HumanHap550 v1, HumanHap550 v3 and Human Quad610 arrays. SNPs were further filtered with genotype missing rate, minor allele frequency, and Hardy-Weinberg equilibrium *P* value. The analysis was implemented in PLINK[6]. After quality control, 506950 and 522471 SNPs were kept for the association analysis of the European-American and African-American cohort respectively. Here, we only considered samples with a genotype call rate no less than 95%. To remove cryptic relatedness between samples, we calculated the identity-by-descent (IBD) scores and remove one individual in the pairs of subjects with IBD great than 0.25. We also conducted principal component analysis among three cohorts separately using EIGENSTRAT to detect and correct for potential substructures and outliers[7].

Local ancestry estimation

We used MULTIMIX to infer the local ancestry of our African-American data [8]. The reference data of African (code: YRI) and European (code: CEU) ancestries were downloaded from the 1000 Genome Project.

Statistic analysis

The association analysis were carried out in PLINK using both allelic association test and logistic regression model. For the association test of logistic regression model, we used age, gender, and the first 10 PCs as covariates. The genomic inflation factor of migraine GWAS in African-American children was 1.01 for the logistic association. Meta-analysis was performed by GWAMA[9]. Fixed effects *P* values were reported.

Genotype imputation at the 3q25.32 locus was performed with IMPUTE2 using the reference panel 1000 Genome Phase I integrated variants set (Dec 2013 release)[10], which is provided as a single worldwide haplotype file per chromosome (rather than splitting the files by population or group). We used SHAPEIT recommended by Howie *et al* to infer the haplotypes before imputation[11]. In consideration of the uncertainty of imputation, association analysis of the imputed genotypes was calculated with the SNPTEST v2 package[12].

Results

Association analysis

To identify genes associated with susceptibility to childhood migraine, we genotyped 1,267 DNA samples isolated from blood obtained from children with history of migraine, who were receiving their care at The Children's Hospital of Philadelphia (CHOP. Blood-derived

DNAs from 9,693 children without migraine/headache diagnosis were genotyped as controls. All subjects were genotyped using the Illumina HumanHap550 or 610 SNP array. After completion of population stratification and quality control measures, our data set was comprised of 380 migraine patients and 2,129 controls for the African-American (AA) children, and 599 migraine patients and 7,327 controls for the European-American (EA) children (Fig S1 and Table S2). We tested associations between SNP markers and migraine.

As no genome-wide significant signal was identified in the EA children, we examined for associations at loci previously reported in European adults [3 4]. The 1p13.1 and 2q37.1 loci revealed a nominal significance in a meta-analysis of our pediatric cohorts (rs2078371, $P = 0.034$; rs7577262, $P = 0.029$, Table S3). The two loci also showed consistent direction of effects with similar strength between the adult and pediatric cohorts (Table S3). While the other loci did not replicate, our relatively small sample size of pediatric patients is likely the reason. In this regards, the locus at 1q23.1 was close to nominal significance (rs2274316, $P < 0.061$, Table S3), suggesting that larger pediatric sample size would likely yield a more robust P -value.

The pediatric AA migraine GWAS analysis uncovered a novel susceptibility locus at 5q33.1. The associated SNPs map to a linkage disequilibrium block harboring a gene *NMUR2* (Fig S2–4 and Table S4), which encodes a receptor for the neuromedin-U (NMU). The most significant genotyped SNP passed the genome-wide significant threshold by allelic association test (rs1946225, $P_{\text{allelic}} = 1.73 \times 10^{-8}$). The P -value calculated by logistic model was 8.28×10^{-8} after adjustment for age, gender and the first 10 PCs. The genomic inflation factor of logistic model is 1.01. We also examined the association of rs1946225 in the EA pediatric cohort, and found that this locus was not significant in the EA children with migraine ($P_{\text{logistic}} = 0.39$), suggesting that the 5q33.1 locus might be specifically associated with the migraine risk in African Americans.

Imputation of 5q33.1

To investigate the 5q33.1 region in greater detail and search for additional variants not assayed on the genotyping arrays directly, we imputed unobserved genotypes at 5q33.1 in the AA migraine cohort using the 1000 Genomes Project data as reference. In the AA children, imputation identified 83 additional SNPs surpassing genome-wide significance with the top imputed SNP, rs72793414, having $P = 1.94 \times 10^{-9}$ (Table S5). As shown, the genome-wide significant SNPs, both genotyped and imputed, were highly correlated in a strong linkage disequilibrium region (Fig 1A).

To explore additional GWAS signals at this region we conditioned the analysis on the top imputed SNP rs72793414 in the AA children. The association signal at 5q33.1 was abolished, including the top genotyped SNP rs1946225 ($P = 0.828$), after conditioning on rs72793414 suggesting no additional signal at this region (Fig S5).

Replication genotyping

To further confirm the association between 5q33.1 and migraine, we identified an independent AA pediatric cohort of 233 migraine patients and 4,038 non-migraine control subjects, all of African-American ancestry based on self-reported information within the

electronic medical records (EMRs) at CHOP. We next designed TaqMan genotyping assays for rs1946225 (top genotyped SNP) and rs72793414 (top imputed SNP) to determine the genotypes of the two SNPs in the migraine cases. The controls were already genotyped by the Illumina HumanHap550 or 610 SNP arrays.

We further assessed the SNP-trait associations of the two SNPs. The associations of both SNPs were successfully validated in the replication cohort (Table 1). The P values of both markers were significant after Bonferroni correction (rs1946225, $P_{corrected} = 0.03$; rs72793414, $P_{corrected} = 7.74 \times 10^{-3}$). In addition, the odds ratios of rs72793414 are comparable between the discovery and replication studies (Table 1). Meta-analysis on rs1946225 and rs72793414 after combining the discovery and replication cohorts revealed genome-wide significant P -values for both SNPs (rs1946225, $P_{combined} = 9.55 \times 10^{-9}$; rs72793414, $P_{combined} = 3.81 \times 10^{-10}$).

Admixture analysis

Given that African Americans (AA) in general have a proportion of admixture from European populations (~15–20%), we investigated the local ancestry of the participating AA individuals at 5q33.1. The estimation of the African ancestry at 5q33.1 from our study is consistent with previous reports [13–14]. Both cases and controls show African ancestry between 80% to 85% (Fig S6). The proportion of European and African ancestry at 5q33.1 was balanced between cases and controls (Wilcoxon rank sum test, $P = 0.367$), suggesting that the association detected at 5q33.1 is not driven by local ancestry differences between cases and controls. In consistent with this result, rs1946225 is still significantly associated with migraine, when both local and global ancestries are included as covariates (Table S6).

eQTL analysis

We next sought to determine if the genotypes of rs1946225 and rs72793414 were correlated with the mRNA expression levels of *NMUR2*. The eQTL data from GTEx[15] demonstrated that the expression of *NMUR2* was significantly down-regulated in the frontal cortex (rs1946225, $P = 1.50 \times 10^{-9}$; rs72793414, $P = 8.50 \times 10^{-10}$, Fig 1B and Table 2) and thyroid tissue (rs1946225, $P = 5.40 \times 10^{-14}$; rs72793414, $P = 6.40 \times 10^{-14}$, Table 2) of individuals carrying the minor alleles of rs1946225 and rs72793414 (Fig S7). The minor alleles of rs1946225 and rs72793414 represented the risk variants for migraine in the African-American children. Interestingly, besides *NMUR2*, the minor alleles of rs1946225 and rs72793414 were also negatively associated with the expression levels of *GLRA1* in the cerebellar hemisphere (rs1946225, $P = 1.00 \times 10^{-9}$; rs72793414, $P = 7.70 \times 10^{-10}$; Table 2). *GLRA1* encodes glycine receptor subunit alpha-1, which is located at about 500kb upstream from the linkage disequilibrium (LD) block of migraine associated SNPs in AA children. We further confirmed that both *NMUR2* and *GLRA1* are highly expressed in a variety of brain tissues of human according to the GTEx data (Fig S8), suggesting a functional role for *NMUR2* and *GLRA1* in the central nervous system (CNS). Taken together, the eQTL data indicated that either rs1946225 and rs72793414 or variants in strong linkage disequilibrium with them may be responsible for the regulation of *NMUR2* and *GLRA1* expression in Brain tissues, and that low expression levels of *NMUR2* and *GLRA1* are likely linked to the pathophysiology of migraine.

In silico mining of putative causal variants at 5q33.1

Since all the genome-wide significant SNPs are non-coding (Table S5), we further explored the bioinformatic annotations of top imputed SNP rs72793414 and variants in the same LD block with rs72793414 ($r^2 > 0.8$) using HaploReg v4.1[16]. HaploReg annotation results indicate a number of SNPs in the LD block with rs72793414 are located in the regulatory region of genome. For example, rs72793414 may influence nine regulatory motifs as predicted by the position weight matrix (PWM) method suggesting rs72793414 could involve in the regulation process of its nearby genes. In addition, the annotation of top genotyped SNP rs1946225 ($r^2 = 1$ and $D' = 1$ with rs72793414) based on the full table of epigenomic information from Roadmap Epigenomics indicates[17] there is a cluster of enhancer activity in brain, and that it is classified as a enhancer (7_Enh) by the 15-state core model and weak enhancer (17_EnhW2) by the 25-state model. H3K4me1 contribute to the chromatin state assignment at this locus. Black cells of the table indicate that DNase was not assayed by Roadmap in these tissues. Notably, SNP rs4958306 ($r^2 = 1$ and $D' = 1$ with rs72793414) in the LD block overlaps with an HMM-predicted promoter and an HMM-predicted enhancer in multiple tissue types including the brain tissue. Moreover, ChIP-Seq data indicates there is a binding region of CTCF. PWM also consistently predicts rs4958306 may lead to CTCF motif disruption.

Discussion

In this study, we have identified a genomic locus at 5q33.1 that is associated with migraine in AA children. The locus was not associated with migraine in EA children implying that the causal variants are either not present in EA or are not tagged by the AA migraine associated SNPs. Analysis of eQTL data demonstrates that associated SNPs may regulate expression levels of nearby genes *NMUR2* and *GLRA1*. This discovery contributes to our understanding of the genetic etiology of migraine in African Americans and provides a novel gene target for therapeutic development.

The eQTL results further indicate that detected risk variants can lead to significant expression changes of *NMUR2* and *GLRA1* expression in brain tissues. *NMUR2* is a G protein-coupled receptor of NMU, a highly conserved neuropeptide, participates in diverse physiological processes such as cardiovascular effects, smooth-muscle contraction, energy homeostasis, nociception and stress response [18–22]. *NMUR2* is predominantly expressed in the CNS [23]. Accumulated evidence from the animal models suggests that NMU and *NMUR2* in the CNS mediate the regulation of food intake, energy balance, stress response, and nociception. For example, NMU knockout mice exhibit increased body weight and adiposity[21]. *NMUR2* knockdown rats display significantly greater consumption of high-fat diet leading to increased body weight[24]. Central administration of NMU elevates adrenocorticotropin and corticosterone release[19], and stimulates stress-related behaviors in rodents[22], whereas NMU knockout mice show blunted behavioral response to environmental stress[20]. In addition, both NMU and *NMUR2* knockout mice display reduced nociceptive responses[18 20 23]. Although no functional link between *NMUR2* and migraine has been established to this point, hormone imbalance, stress, obesity and nociceptor activation are believed to be triggers or risk factors for migraine[25–27]. The

protein encoded by *GLRA1* is a neurotransmitter-gated transmembrane ion channel, which mediates postsynaptic inhibition in the CNS. Defects of *GLRA1* can cause a rare neurological disorder, hyperekplexia[28]. It is well known that disruptions of three ion channel genes *CACNA1A*, *ATP1A2* and *SCN1A* are responsible for most familial hemiplegic migraine[29]. Previous GWA studies of migraine have implicated genes involved in neurotransmitter release pathways[4 29], suggesting that *GLRA1* could influence the risk of migraine through mediating neurotransmission processes. In addition, eQTL results suggest that expression levels of *NMUR2* are also significantly correlated with rs72793414 and rs1946225 genotypes in thyroid. In consistent with this, a few studies suggest that hypothyroidism and migraine could be linked[30–32], suggesting that altered expression levels of *NMUR2* in thyroid gland could also play a role in migraine. We further examined if potential interactants of *NMUR2* or *GLRA1* may be associated with migraine. No significant association of variants from *NMU* or *GLRB* was found.

In consistent with eQTL results, bioinformatic annotations of variants detected provided multiple lines of evidence that SNPs from this GWAS may affect the regulatory mechanisms at 5q33.1. For example, ChIP-seq and motif data suggest that CTCF binding affinity to the alleles of rs4958306 could be different, and further influence the expression of nearby genes such as *NMUR2* and *GLRA1*.

In summary, we performed the first large-scale GWAS of migraine in children, including both EA and AA ancestry, and identified a novel migraine risk locus at 5q33.1 in the AA children. The eQTL analysis implicated two genes *NMUR2* and *GLRA1* both involved in signaling of neural pathways in the CNS. Follow-up studies of *NMUR2* and *GLRA1* may provide new insights into the genetic basis of childhood migraine and allow for precision therapeutic development strategies targeting migraine patients of AA ancestry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. de Vries B, Frants RR, Ferrari MD, van den Maagdenberg AM. Molecular genetics of migraine. *Human genetics* 2009;126(1):115–32 doi: 10.1007/s00439-009-0684-z. [PubMed: 19455354]
2. Piane M, Lulli P, Farinelli I, Simeoni S, De Filippis S, Patacchioli FR, Martelletti P. Genetics of migraine and pharmacogenomics: some considerations. *The journal of headache and pain* 2007;8(6):334–9 doi: 10.1007/s10194-007-0427-2. [PubMed: 18058067]

3. Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers T, Farh K, Cunningham-Rundles C, Muona M, Furlotte N, Kurth T, I A, McMahon G. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nature genetics* 2016;48(8):856–66 [PubMed: 27322543]
4. Anttila V, Winsvold BS, Gormley P, Kurth T, Bettella F, McMahon G, Kallela M, Malik R, de Vries B, Terwindt G, Medland SE, Todt U, McArdle WL, Quaye L, Koiranen M, Ikram MA, Lehtimäki T, Stam AH, Ligthart L, Wedenoja J, Dunham I, Neale BM, Palta P, Hamalainen E, Schurks M, Rose LM, Buring JE, Ridker PM, Steinberg S, Stefansson H, Jakobsson F, Lawlor DA, Evans DM, Ring SM, Farkkila M, Artto V, Kaunisto MA, Freilinger T, Schoonen J, Frants RR, Pelzer N, Weller CM, Zielman R, Heath AC, Madden PA, Montgomery GW, Martin NG, Borck G, Gobel H, Heinze A, Heinze-Kuhn K, Williams FM, Hartikainen AL, Pouta A, van den Ende J, Uitterlinden AG, Hofman A, Amin N, Hottenga JJ, Vink JM, Heikkilä K, Alexander M, Müller-Myhsok B, Schreiber S, Meitinger T, Wichmann HE, Aromaa A, Eriksson JG, Traynor BJ, Trabzuni D, Rossin E, Lage K, Jacobs SB, Gibbs JR, Birney E, Kaprio J, Penninx BW, Boomsma DI, van Duijn C, Raitakari O, Jarvelin MR, Zwart JA, Cherkas L, Strachan DP, Kubisch C, Ferrari MD, van den Maagdenberg AM, Dichgans M, Wessman M, Smith GD, Stefansson K, Daly MJ, Nyholt DR, Chasman DI, Palotie A, North American Brain Expression C, Consortium UKBE, International Headache Genetics C. Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nature genetics* 2013;45(8):912–7 doi: 10.1038/ng.2676. [PubMed: 23793025]
5. Headache Classification Committee of the International Headache S. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013;33(9):629–808 doi: 10.1177/0333102413485658. [PubMed: 23771276]
6. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics* 2007;81(3):559–75 doi: 10.1086/519795. [PubMed: 17701901]
7. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics* 2006;38(8):904–9 doi: 10.1038/ng1847. [PubMed: 16862161]
8. Churchhouse C, Marchini J. Multiway admixture deconvolution using phased or unphased ancestral panels. *Genet Epidemiol* 2013;37(1):1–12 doi: 10.1002/gepi.21692. [PubMed: 23136122]
9. Magi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. *BMC bioinformatics* 2010;11:288 doi: 10.1186/1471-2105-11-288. [PubMed: 20509871]
10. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS genetics* 2009;5(6):e1000529 doi: 10.1371/journal.pgen.1000529. [PubMed: 19543373]
11. Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. *Nature methods* 2013;10(1):5–6 doi: 10.1038/nmeth.2307. [PubMed: 23269371]
12. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nature genetics* 2007;39(7):906–13 doi: 10.1038/ng2088. [PubMed: 17572673]
13. Murray T, Beaty TH, Mathias RA, Rafaels N, Grant AV, Faruque MU, Watson HR, Ruczinski I, Dunston GM, Barnes KC. African and non-African admixture components in African Americans and an African Caribbean population. *Genetic epidemiology* 2010;34(6):561–8 doi: 10.1002/gepi.20512. [PubMed: 20717976]
14. Bryc K, Durand EY, Macpherson JM, Reich D, Mountain JL. The genetic ancestry of African Americans, Latinos, and European Americans across the United States. *American journal of human genetics* 2015;96(1):37–53 doi: 10.1016/j.ajhg.2014.11.010. [PubMed: 25529636]
15. The GTEx Consortium. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015;348(6235):648–60 doi: 10.1126/science.1262110. [PubMed: 25954001]
16. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic acids research* 2016;44(D1):D877–81 doi: 10.1093/nar/gkv1340. [PubMed: 26657631]

17. Roadmap Epigenomics C, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu YC, Pfennig AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shores N, Epstein CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuah E, Tam A, Canfield TK, Hansen RS, Kaul R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh KH, Feizi S, Karlic R, Kim AR, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Neph SJ, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthal KT, Sinnott-Armstrong NA, Stevens M, Thurman RE, Wu J, Zhang B, Zhou X, Beaudet AE, Boyer LA, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones SJ, Li W, Marra MA, McManus MT, Sunyaev S, Thomson JA, Tlsty TD, Tsai LH, Wang W, Waterland RA, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos JA, Wang T, Kellis M. Integrative analysis of 111 reference human epigenomes. *Nature* 2015;518(7539):317–30 doi: 10.1038/nature14248. [PubMed: 25693563]
18. Zeng H, Gragerov A, Hohmann JG, Pavlova MN, Schimpf BA, Xu H, Wu LJ, Toyoda H, Zhao MG, Rohde AD, Gragerova G, Onrust R, Bergmann JE, Zhuo M, Gaitanaris GA. Neuromedin U receptor 2-deficient mice display differential responses in sensory perception, stress, and feeding. *Molecular and cellular biology* 2006;26(24):9352–63 doi: 10.1128/MCB.01148-06. [PubMed: 17030627]
19. Thompson EL, Murphy KG, Todd JF, Martin NM, Small CJ, Ghatei MA, Bloom SR. Chronic administration of NMU into the paraventricular nucleus stimulates the HPA axis but does not influence food intake or body weight. *Biochemical and biophysical research communications* 2004;323(1):65–71 doi: 10.1016/j.bbrc.2004.08.058. [PubMed: 15351702]
20. Nakahara K, Kojima M, Hanada R, Egi Y, Ida T, Miyazato M, Kangawa K, Murakami N. Neuromedin U is involved in nociceptive reflexes and adaptation to environmental stimuli in mice. *Biochemical and biophysical research communications* 2004;323(2):615–20 doi: 10.1016/j.bbrc.2004.08.136. [PubMed: 15369794]
21. Hanada R, Teranishi H, Pearson JT, Kurokawa M, Hosoda H, Fukushima N, Fukue Y, Serino R, Fujihara H, Ueta Y, Ikawa M, Okabe M, Murakami N, Shirai M, Yoshimatsu H, Kangawa K, Kojima M. Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. *Nature medicine* 2004;10(10):1067–73 doi: 10.1038/nm1106.
22. Hanada R, Nakazato M, Murakami N, Sakihara S, Yoshimatsu H, Toshinai K, Hanada T, Suda T, Kangawa K, Matsukura S, Sakata T. A role for neuromedin U in stress response. *Biochemical and biophysical research communications* 2001;289(1):225–8 doi: 10.1006/bbrc.2001.5945. [PubMed: 11708803]
23. Torres R, Croll SD, Vercollone J, Reinhardt J, Griffiths J, Zabski S, Anderson KD, Adams NC, Gowen L, Sleeman MW, Valenzuela DM, Wiegand SJ, Yancopoulos GD, Murphy AJ. Mice genetically deficient in neuromedin U receptor 2, but not neuromedin U receptor 1, have impaired nociceptive responses. *Pain* 2007;130(3):267–78 doi: 10.1016/j.pain.2007.01.036. [PubMed: 17379411]
24. Benzon CR, Johnson SB, McCue DL, Li D, Green TA, Hommel JD. Neuromedin U receptor 2 knockdown in the paraventricular nucleus modifies behavioral responses to obesogenic high-fat food and leads to increased body weight. *Neuroscience* 2014;258:270–9 doi: 10.1016/j.neuroscience.2013.11.023. [PubMed: 24269937]
25. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. *Annual review of physiology* 2013;75:365–91 doi: 10.1146/annurev-physiol-030212-183717.
26. Ravid S, Shahar E, Schiff A, Gordon S. Obesity in children with headaches: association with headache type, frequency, and disability. *Headache* 2013;53(6):954–61 doi: 10.1111/head.12088. [PubMed: 23574609]
27. Schramm SH, Moebus S, Lehmann N, Galli U, Obermann M, Bock E, Yoon MS, Diener HC, Katsarava Z. The association between stress and headache: A longitudinal population-based study. *Cephalalgia : an international journal of headache* 2015;35(10):853–63 doi: 10.1177/0333102414563087. [PubMed: 25480807]
28. Chung SK, Vanbellinghen JF, Mullins JG, Robinson A, Hantke J, Hammond CL, Gilbert DF, Freilinger M, Ryan M, Kruer MC, Masri A, Gurses C, Ferrie C, Harvey K, Shiang R,

- Christodoulou J, Andermann F, Andermann E, Thomas RH, Harvey RJ, Lynch JW, Rees MI. Pathophysiological mechanisms of dominant and recessive GLRA1 mutations in hyperekplexia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2010;30(28):9612–20 doi: 10.1523/JNEUROSCI.1763-10.2010. [PubMed: 20631190]
29. Ferrari MD, Klever RR, Terwindt GM, Ayata C, van den Maagdenberg AM. Migraine pathophysiology: lessons from mouse models and human genetics. *The Lancet Neurology* 2015;14(1):65–80 doi: 10.1016/S1474-4422(14)70220-0. [PubMed: 25496898]
30. Fallah R, Mirouliaei M, Bashardoost N, Partovee M. Frequency of subclinical hypothyroidism in 5- to 15-year-old children with migraine headache. *J Pediatr Endocrinol Metab* 2012;25(9–10):859–62 doi: 10.1515/jpem-2012-0121. [PubMed: 23426813]
31. Mirouliaei M, Fallah R, Bashardoost N, Partovee M, Ordooei M. Efficacy of levothyroxine in migraine headaches in children with subclinical hypothyroidism. *Iran J Child Neurol* 2012;6(4):23–6 [PubMed: 24665276]
32. Martin AT, Pinney SM, Xie C, Herrick RL, Bai Y, Buckholz J, Martin VT. Headache Disorders May Be a Risk Factor for the Development of New Onset Hypothyroidism. *Headache* 2017;57(1):21–30 doi: 10.1111/head.12943. [PubMed: 27676320]

Figure 1.

(a) Regional plot of the 5q33.1 locus, plotted are the significance of association ($-\log_{10}$ -transformed P values) and the recombination rate. SNPs are colored to reflect pairwise LD (r^2) with the most significantly associated imputed SNP. The most significant SNPs are marked in purple. (b) Box plot of *NMUR2* mRNA expression levels in the frontal cortex of individuals with rs72793414 genotype GG (65 individuals), GA (23 individuals), AA (4 individuals).

Table 1.

Association results for the lead genotyped and imputed SNPs at 5q33.1.

SNP	Study	A1/A2	Freq1	Freq2	OR	95% CI	$P_{logistic}$
rs1946225	Discovery	G/T	0.064	0.026	2.649	1.855–3.783	8.28×10^{-08}
	Replication	G/T	0.045	0.024	1.791	1.122–2.860	0.015
	Combined				2.294	1.728–3.046	9.55×10^{-09}
rs72793414	Discovery	A/G	0.066	0.025	2.695	1.910–3.803	1.94×10^{-09}
	Replication	A/G	0.047	0.023	2.020	1.254–3.255	3.87×10^{-03}
	Combined				2.441	1.847–3.227	3.81×10^{-10}

A1/A2: risk allele/protective allele.

Freq1: allele frequency in cases.

Freq2: allele frequency in controls.

Table 2.

eQTLs of rs72793414 and rs1946225 identified from the GTExPortal.

SNP	Gene	P-Value	Effect Size	Tissue
rs72793414	<i>NMUR2</i>	6.40E-14	−0.75	Thyroid
	<i>GLRA1</i>	7.70E-10	−0.87	Brain - Cerebellar Hemisphere
	<i>NMUR2</i>	8.50E-10	−1.2	Brain - Frontal Cortex (BA9)
	<i>NMUR2</i>	1.10E-08	0.47	Nerve - Tibial
rs1946225	<i>NMUR2</i>	5.40E-14	−0.75	Thyroid
	<i>GLRA1</i>	1.00E-09	−0.88	Brain - Cerebellar Hemisphere
	<i>NMUR2</i>	1.50E-09	−1.1	Brain - Frontal Cortex (BA9)
	<i>NMUR2</i>	1.30E-08	0.46	Nerve - Tibial