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Genome-wide association analysis identifies susceptibility loci for migraine without aura

Tobias Freilinger[1,2,35], **Verneri Anttila**[3,4,35], **Boukje de Vries**[5,35], **Rainer Malik**[1], **Mikko Kallela**[6], **Gisela M Terwindt**[7], **Patricia Pozo-Rosich**[8,9], **Bendik Winsvold**[10,3], **Dale R Nyholt**[11], **Willebrordus P J van Oosterhout**[7], **Ville Artto**[6], **Unda Todt**[12], **Eija Hämäläinen**[3,4], **Jèssica Fernández-Morales**[3,9], **Mark A Louter**[7,13], **Mari A Kaunisto**[4,14], **Jean Schoenen**[15], **Olli Raitakari**[16], **Terho Lehtimäki**[17], **Marta Vila-Pueyo**[18], **Hartmut Göbel**[19], **Erich Wichmann**[20], **Cèlia Sintas**[21,22], **Andre G Uitterlinden**[23], **Albert Hofman**[24], **Fernando Rivadeneira**[23,24], **Axel Heinze**[19], **Erling Tronvik**[25], **Cornelia M. van Duijn**[24], **Jaakko Kaprio**[4,26,27], **Bru Cormand**[21,22,28], **Maija Wessman**[4,14], **Rune R Frants**[5], **Thomas Meitinger**[29,30], **Bertram Müller-Myhsok**[31], **John-Anker Zwart**[10], **Markus Färkkilä**[6], **Alfons Macaya**[18], **Michel D Ferrari**[7], **Christian Kubisch**[12], **Aarno Palotie**[3,4,32,33,34,36], **Martin Dichgans**[1,36], **Arn M J M van den Maagdenberg**[5,7,36], and **International Headache Genetics Consortium**[37]

[1]Institute for Stroke and Dementia Research, Klinikum der Universität München, Munich, Germany [2]Department of Neurology, Klinikum der Universität München, Munich, Germany [3]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK [4]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland [5]Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands [6]Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland [7]Department of Neurology, Leiden University Medical Centre, Leiden, The Netherlands [8]Department of Neurology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain [9]Headache Research Group, Vall d'Hebron Research Institute, Universitat Autonoma de Barcelona, Barcelona, Spain [10]Department of Neurology, Oslo University Hospital and University of Oslo, Oslo, Norway [11]Neurogenetics Laboratory, Queensland Institute of Medical Research, Brisbane, Australia [12]Institute of Human Genetics, University of Ulm, Ulm, Germany [13]Department of Psychiatry, Leiden University Medical Centre, Leiden [14]Folkhälsan Research Center, Helsinki, Finland [15]Headache Research Unit, Department of Neurology and Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA)-Neurosciences, Liège University, Liège, Belgium [16]Department of Clinical Physiology, University of Turku and Turku University Central Hospital, Turku, Finland [17]Department of Clinical Chemistry, Tampere University Hospital and University of

Correspondence: Prof. Dr. A. Palotie, Head of Medical Sequencing, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK; Tel: +440 122 349 6863; Fax: +440 122 349 6802; ap8@sanger.ac.uk, or, Prof. Dr. A.M.J.M. van den Maagdenberg, Departments of Human Genetics and Neurology; Leiden University Medical Centre, Einthovenweg 20; PO Box 9600, 2300 RC Leiden, The Netherlands; Tel: +31 71 5269460; Fax: +31 71 5268285; maagdenberg@lumc.nl.
[35]These authors contributed equally to this work.
[36]These authors jointly directed this work.
[37]A full list of members and affiliations is provided in the Supplementary Note.

Author contributions

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Tampere, Tampere, Finland [18]Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Barcelona, Spain [19]Kiel Pain and Headache Center, Kiel, Germany [20]Institute of Epidemiology, Helmholtz Center Munich, Neuherberg, Germany [21]Department of Genetics, University of Barcelona, Barcelona, Spain [22]Biomedical Network Research Centre on Rare Diseases (CIBERER), Barcelona, Spain [23]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands [24]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands [25]Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway [26]Department of Public Health, University of Helsinki, Helsinki, Finland [27]Department of Mental Health and Alcohol Research, National Institute for Health and Welfare, Helsinki, Finland [28]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain [29]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany [30]Institute of Human Genetics, Klinikum rechts der Isar, Technische Universität München, Munich, Germany [31]Max Planck Institute of Psychiatry, Munich, Germany [32]The Broad Institute of MIT and Harvard, Boston, Massachusetts, USA [33]Department of Medical Genetics, University of Helsinki, Helsinki, Finland [34]Department of Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland

Abstract

Migraine without aura is the most common form of migraine, characterized by recurrent disabling headache and associated autonomic symptoms. To identify common genetic variants for this migraine type, we analyzed genome-wide association data of 2,326 clinic-based German and Dutch patients and 4,580 population-matched controls. We selected SNPs from 12 loci with two or more SNPs with *P*-values $< 1 \times 10^{-5}$ for follow-up in 2,508 patients and 2,652 controls. Two loci, i.e. 1q22 (*MEF2D*) and 3p24 (near *TGFBR2*) replicated convincingly ($P = 4.9 \times 10^{-4}$, $P = 1.0 \times 10^{-4}$, respectively). Meta-analysis of the discovery and replication data yielded two additional genome-wide significant ($P < 5 \times 10^{-8}$) loci in *PHACTR1* and *ASTN2*. In addition, SNPs in two previously reported migraine loci in or near *TRPM8* and *LRP1* significantly replicated. This study reveals the first susceptibility loci for migraine without aura, thereby expanding our knowledge of this debilitating neurological disorder.

Main text

Migraine is a disabling episodic neurovascular brain disorder affecting 12% of the general population[1-4]. Migraine attacks are typically characterized by severe throbbing unilateral headache and nausea, vomiting and photo- and phonophobia (migraine without aura; MO). In up to one third of patients attacks may be associated with neurological aura symptoms (migraine with aura; MA). Previous genome-wide association studies (GWAS) identified a migraine susceptibility locus on chromosome 8q22, close to *MTDH*, in the clinic-based International Headache Genetics Consortium (IHGC) MA study[5] and three other loci in or near *PRDM16, LRP1*, and *TRPM8* in the population-based migraine Women's Genome Health Study (WGHS)[6]. For *TRPM8* there was suggestive association ($P < 1 \times 10^{-5}$) also in the clinic-based IHGC MA GWAS[5]. Here we report the first GWAS in MO, the most common form of migraine. We analyzed two large samples from headache centres in Germany and the Netherlands including 2,326 MO patients and 4,580 population-matched controls (Supplementary Note and Supplementary Fig. 1). A quantile-quantile plot of the joint analysis (Supplementary Fig. 2) and an overall inflation factor ($\lambda 1000$) of 1.03 were used as final quality control measures. The discovery dataset identified one genome-wide significant ($P < 5 \times 10^{-8}$) locus on chromosome 1q22 as well as eleven additional loci containing multiple SNPs with suggestive association ($P < 1 \times 10^{-5}$) (Supplementary Table 1). Eighteen SNPs from these 12 loci were taken forward to the replication stage in four

independent clinic-based European MO samples (2,508 cases and 2,652 controls) (Supplementary Fig. 1 and Supplementary Table 1). Eight SNPs in six loci showed *P*-values < 0.05 in the replication study, and five of these SNPs also showed *P*-values < 5×10^{-8} in the meta-analysis combining the discovery and replication cohorts (Table 1, Fig. 1 and Supplementary Fig. 3). Four loci (1q22, 3p24, 6p24, 9q33) replicated, although replication was less convincing for loci on 6p24 and 9q33 with replication *P*-values of 0.012 and 0.018, respectively, although *P*-values were < 5×10^{-8} in the overall meta-analysis. In addition, we tested top SNPs of the four previously identified migraine loci (1p36, 2q37, 8q22, 12q13)[5,6] in the replication stage (Fig. 2 and Supplementary Table 1), of which 2q37 and 12q13 convincingly replicated. Because migraine is more prevalent in women, we performed a gender interaction analysis for the reported SNPs (Supplementary Table 2). No significant interactions were observed and all SNPs had relatively similar odds ratios in both genders. To further extend our analyses, we searched for expression quantitative trait loci (eQTLs) in available data sets of tissues and cell lines[7,8], but did not observe consistently significant eQTLs for any of the SNPs of Table 1.

The 1q22 locus contained six SNPs with genome-wide significant association ($P < 5 \times 10^{-8}$) already in the discovery stage of the analysis and that were all in close LD ($r^2 > 0.98$). SNPs rs1050316 and rs3790455 were taken forward to the replication stage and successfully replicated (overall meta-analysis *P*-values: 3.21×10^{-10} (OR = 1.19) and 7.06×10^{-11} (OR = 1.20), respectively) (Table 1, Fig. 1 and Supplementary Fig. 3). All associated SNPs are located within the *MEF2D* (myocyte enhancer factor 2D) gene (i.e. intronic and 3′-UTR) that encodes a transcription factor highly expressed in brain. MEF2D regulates neuronal differentiation by supporting survival of newly formed neurons[9]. Perhaps even more relevant to migraine, neuronal activity-dependent activation of MEF2D restricts the number of excitatory synapses[10]. As the migraine brain is hyperexcitable[11], it is tempting to speculate that MEF2D dysregulation may affect neuronal excitatory neurotransmission in MO patients. There is some evidence for increased glutamate (the main brain excitatory neurotransmitter) levels in migraine patients[12,13] and increased glutamatergic neurotransmission was reported in a transgenic knock-in mouse model with a pathogenic human hemiplegic migraine gene mutation[14]. A role for MEF2D dysregulation in migraine is also plausible given that several MEF2 target genes have been associated with other neurological disorders, such as epilepsy[15-17]. Notably, pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38), which is encoded by one of the activity-regulated MEF2D target genes and modulates excitatory synaptic transmission, may trigger migraine-like attacks in patients with MO[18]. In mice, PACAP-38 is involved in nitroglycerol-induced trigeminovascular activation[19], the presumed origin of the migraine headache[20].

The 3p24 locus contained top SNP rs7640543 (initial $P = 2.72 \times 10^{-6}$, OR = 1.20). This SNP showed strong replication ($P = 1.02 \times 10^{-4}$) and reached genome-wide significance in the meta-analysis of the discovery and replication samples ($P = 1.17 \times 10^{-9}$, OR = 1.19) (Table 1, Fig. 1 and Supplementary Fig. 3). Rs7640543 is located ~200 kb upstream of *TGFBR2* (transforming growth factor beta receptor 2), which encodes a serine-threonine kinase involved in the regulation of cell proliferation and differentiation as well as extracellular matrix production[21]. *TGFBR2* is an attractive candidate gene for migraine as the p.Arg460His missense mutation in *TGFBR2* not only causes seemingly monogenic, familial aortic dissection, but also migrainous headaches in 11 of 14 mutation carriers in a large multigenerational family[22]. This may fit with the observation that migraineurs seemed to have a two-fold increased risk for cervical artery dissection[23].

For the 6p24 locus, SNP rs9349379 reached genome-wide significance when combining the data of the discovery and replication samples ($P = 3.20 \times 10^{-8}$, OR = 0.86) (Table 1, Fig. 1 and Supplementary Fig. 3). Five SNPs ($P < 10^{-5}$) were taken forward to the replication stage

(see Supplementary Table 1). All five SNPs are located in *PHACTR1* (phosphastase and actin regulator 1), a member of the PHACTR/scapinin family, which controls synaptic activity and synapse morphology through regulation of protein phosphatase 1 and actin binding[24,25]. PHACTR1 has further been implicated in endothelial cell functioning[26] and susceptibility to early-onset myocardial infarction[27]. Thus, the link between *PHACTR1* and migraine could be neuronal through aberrant synaptic transmission or vascular since endothelial dysfunction, cardiovascular disease and myocardial infarction all seemed to be linked with migraine[28]. Like TGFBR2, PHACTR1 may also be involved in systemic vascular disease through a TGFβ signalling pathway.

The 9p33 locus with top SNP rs6478241 reached genome-wide significance in the meta-analysis of the discovery and replication samples, although there is heterogeneity ($I^2 = 0.57$) in the replication samples ($P = 3.86 \times 10^{-8}$, OR = 1.16) (Table 1, Fig. 1 and Supplementary Fig. 3). In one of the four replication cohorts the effect direction was opposite to the discovery sample and the other replication samples. This locus should be considered tentative and further studies are needed to confirm its relevance to migraine. Rs6478241 is located in *ASTN2*, a member of the astrotactin gene family, which plays a role in glial-guided migration that seems important for development of the laminar architecture of cortical regions in the brain[29]. Although structural abnormalities in migraineurs have been reported in the somatosensory cortex[30] and the cerebellum[31], they more likely reflect degenerative processes related to severe migraine attacks than developmental problems. Therefore, it remains at present unclear how ASTN2 could play a role in migraine pathophysiology.

In addition, two of the four previously reported migraine loci[5,6] showed significant association in the current clinic-based MO GWAS. Two top SNPs ($r^2 = 0.52$) in the 2q37 migraine locus reached genome-wide significance in the overall MO meta-analysis (rs10166942, $P = 9.83 \times 10^{-13}$, OR = 0.78; rs17862920, $P = 5.97 \times 10^{-9}$, OR = 0.77) (Table 1, Fig. 2 and Supplementary Fig. 3). SNP rs10166942 is located about 1 kb upstream of the predicted transcription start of *TRPM8* (transient receptor potential melastatin 8), whereas rs17862920 is located in the first intron of *TRPM8. TRPM8* encodes a cold- and menthol-activated ion channel that is expressed in sensory neurons. The gene was identified as a migraine susceptibility gene both in the population-based WGHS migraine GWAS[6] and the clinic-based IHGC MA GWAS[5]. The effect direction of these SNPs is the same in all three studies, and the effect size estimates are similar (OR = 0.78 in the clinic-based IHGC MA study for rs17862920 versus 0.77 in the present study, and OR = 0.85 in the population-based WGHS migraine study for rs10166942 versus 0.78 in the present study). TRPM8 could be involved in cutaneous allodynia[32-34], which is defined as pain due to thermal or mechanical stimuli that normally do not provoke pain that is present in the majority of migraine patients.

The top SNP rs11172113 of the previously reported[6] 12q13 migraine locus reached genome-wide significance in the overall MO meta-analysis (rs11172113, overall $P = 2.97 \times 10^{-8}$, OR = 0.86) (Table 1, Fig. 2 and Supplementary Fig. 3). SNP rs11172113 is located within the first intron of *LRP1* that codes for the low density lipoprotein receptor-related protein 1 which is expressed in multiple tissues including neurons and the vasculature. LRP1 is a cell surface receptor that acts as a sensor of the extracellular environment: it is involved in the proliferation of vascular smooth muscle cells, and modulates synaptic transmission[35,36]. A possible role for LRP1 in migraine can be envisaged because of its neuronal and/or vascular function.

Addressing the question whether MA and MO represent different disease entities[37,38], we tested the top SNPs from the six loci from the current MO study *in silico* in our previous

IHGC MA GWAS data set[5] (Supplementary Table 3). Except for the 3p24 locus, all loci showed *P*-values below 0.05 in the MA data set and ORs going in the same direction in both studies. The *TRPM8* locus showed the most significant *P*-value (rs1786920: 2.19×10^{-5}, OR = 0.78 and rs10166942: 1.32×10^{-5}, OR = 0.82) in the MA data set. Interestingly, rs10166942 also showed association in the WGHS migraine study ($P = 2.30 \times 10^{-7}$; OR = 0.86 in the initial scan)[6]. This suggests that *TRPM8* may play a role in various forms of migraine. In contrast, the SNP rs1835740 in the 8q22 locus in the IHGC MA GWAS[5] pointing at *MTDH* as the putative migraine susceptibility gene[5], did not show association in the present GWAS ($P = 0.70$) nor the population-based WGHS migraine GWAS[6] ($P = 0.22$). This may suggest that *MTDH* confers more susceptibility to aura than to headache.

In conclusion, we present the first GWAS in MO, the most common migraine type, and identified several loci. Two loci (*MEF2D* and *TGFBR2*) showed convincing replication signals, whereas replication for the *PHACTR1* and *ASTN2* loci was weaker. Future studies will need to confirm their role as migraine susceptibility loci. In addition, two of the four previously identified migraine loci (i.e. *TRPM8* and *LRP1*) replicated in this clinic-based MO study. Functional studies are necessary to dissect the exact underlying molecular pathways to identify putative treatment targets for this common debilitating brain disorder.

ONLINE METHODS

Overall study design

The discovery stage of the study was based on an analysis of *de novo* genotyping in two large MO sample sets from headache clinics in Germany (Munich/Kiel) and the Netherlands (Leiden) (Supplementary Fig. 1). Population-matched controls were recruited from studies with existing genotyping data (for details on study cohorts and controls cf. Supplementary Note). For both sample sets, raw data were imputed to approximately 1.4M SNPs using HapMap3 release 2[39] as reference panel. As an initial step, genome-wide logistic regression analysis was performed independently in both samples, followed by meta-analysis of the two datasets. Subsequently, the top SNPs of the meta-analysis were tested for replication in four smaller clinic-based MO samples from Finland (Helsinki), Spain (Barcelona), Norway (Trondheim) and the Netherlands (Leiden) (Fig. 1 and Supplementary Fig. 1).

Ethical aspects

Written informed consent was obtained from all participants, and the study was approved by the respective local research ethics committees of the Klinikum Großhadern, Ludwig-Maximilians-University in Munich (Germany), the University of Leiden Medical Centre (The Netherlands), the Helsinki University Central Hospital (Finland), the Vall d'Hebron Research Institute in Barcelona (Spain), and the Regional Committee for Medical and Health Research Ethics in Trondheim (Norway).

Discovery stage genotyping

Genomic DNA was extracted from peripheral blood samples according to standard protocols. Genotyping of the German GWAS sample was performed at Genome Analysis Center, Helmholtz Zentrum München, Germany using the Illumina Human 610-Quad v1 (n = 838) or Illumina Human 660W-Quad v1 SNP microarrays according to the Infinium II protocol from the manufacturer (Illumina Inc., San Diego, CA, USA) (n = 391). Genotype calling was performed using the Illumina Gencall data analyses software. Genotyping of the complete Dutch GWAS sample was performed at the Wellcome Trust Sanger Institute using the Illumina 660W technology. Genotype calling was performed using the Illuminus software.

Replication stage genotyping

For the replication study, all cases and controls were genotyped at the Wellcome Trust Sanger Institute using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAY™ methodology (Sequenom Inc, San Diego, CA, USA). Amplification reactions and parameters were based on the manufacturer's instructions. Each 384-wells plate contained positive (CEPH DNA) and negative controls, to check for assay performance and contaminations, respectively. Spectrocaller software supplied by the manufacturer was used to automatically call the genotypes. Clusters were checked manually and all doubtful calls were evaluated.

In the replication stage, we selected all loci with at least two SNPs with $P < 1 \times 10^{-5}$ for follow-up in the discovery stage (see Supplementary Table 1). Two SNPs each were selected from loci deemed to be most convincing (*MEF2D, PHACTR1, near TGFBR2, FHL5)*, and one SNP each from the remaining loci (see Supplementary Table 1). At the *PHACTR1* locus, as we observed both effect directions for minor alleles in the discovery stage, we chose three additional SNPs (for a total of five) for follow-up at this locus to robustly cover possible heterogeneity at this locus. In addition, the top reported SNPs from the four previously reported migraine loci (see Supplementary Table 1) were included in the replication stage.

Quality control

To ensure high data quality, the datasets from the primary study were subjected to per-SNP and per-sample quality control (QC) before and after imputation. In both cases, cut-offs of 1% for minor allele frequency and 1×10^{-6} for HWE were used for cases and controls independently (as the cases and controls were genotyped independently in both populations), and the latter again for the combined set of cases and controls. Further, SNPs with call rates < 97% were excluded. Subjects with a genotyping rate <97% and subjects which were closely related to each other (π_hat > 0.15) were removed, as were those with cryptic relatedness and those deemed to be heterozygosity outliers. In addition, population outliers were excluded using a manual selection from a multidimensional scaling plot of the genome-wide IBS pair-wise distance matrix in PLINK.

Before imputation, 452,154 SNPs for 3,772 individuals (1,208 cases and 2,564 controls) were in the German dataset, and 494,760 SNPs for 3,134 individuals (1,118 cases and 2,016 controls) were in the Dutch dataset.

Imputation

Imputation of the German and Dutch discovery samples was performed using IMPUTE 2 (v2.1.0 for the German samples, v2.1.2 for the Dutch samples)[40]. For the phased haploid reference panel, we used HapMap3 release 2 data for the 120 unrelated CEU trios[41]. We used the recommended parameters for the imputation, with the exception of a different number of copying states (κ = 60 for Germans, κ = 80 for Dutch) and a larger buffer size for the Dutch (500 kb instead of 250 kb). After imputation, we used individual call posterior probability > 0.9 and info measure I(A) > 0.6 as cut-offs to ensure high imputation data quality. From 1,411,821 SNPs after imputation, 165,433 SNPs were dropped due to QC reasons.

Statistical analysis

For the GWA data from the two initial study samples, we analyzed the imputed allele dosage data with the SNPTEST software (version 2.2.0; see Web Resources) to generate population-specific summary statistics. We used the presence of migraine as a binary phenotype, and assumed an additive model. Consistent with our previous clinic-based MA

GWA study[5] the association analysis was not corrected for age and gender. The primary reason for not using age as a covariate was that we lack age information for some of the control cohorts. However the majority of the individuals in the cohorts were of working age, similar to our case samples. The missing data score likelihood option was used for handling missing data. For the replication studies, genotyped markers were analyzed using the same model as the discovery samples for the population-specific results.

A fixed-effect meta-analysis of the summary statistics was conducted using GWAMA[42] (see Web Resources) first on the two discovery samples for the primary results. In the discovery sample meta-analysis, only SNPs that were present in both datasets were retained and filtered for the heterogeneity measure $I^2 < 0.5$. This moderately high threshold for I^2 was chosen to reflect the expectation of some differences in association signals of common markers due to varying LD structure. After replication, all six study sets (two discovery and four replication samples) were included in the overall meta-analysis. Reasonable genomic inflation was observed ($\lambda = 1.095$, $\lambda 1000 = 1.031$). Consistency of allelic effects across studies was examined utilising the Cochran's Q[43] and I^2 metrics[44]. Between-study (effect) heterogeneity was indicated by Q-statistic p-values ($P \leq 0.1$) and moderate (25-50%) or larger I^2 values[45]. Meta-analysis of SNPs associated with $P \leq 1 \times 10^{-5}$ and showing evidence of effect heterogeneity were also analysed using a random-effects model[46]. Manhattan and quantile-quantile plots were generated from the resulting data of 1,246,388 SNPs (Supplementary Fig. 2).

In the gender effects analysis, we analysed the effect of including gender information in the association analysis for the directly genotyped SNPs at each of the newly identified loci. We analysed the SNPs in PLINK[47] using a logistic regression model assuming additive effects and covariate adjustment for population identity, and compared the output with results from a males- and females-only analyses. In addition, we compared the results to those of a regression analysis where an additional interaction component between gender and genotype was included in the model.

eQTL analysis

In the expression QTL (eQTL) analysis, we assessed publicly available data from two published eQTL studies[7,8]. In these datasets, as described in the original publications, association between the genotypes of the most interesting SNPs and gene expression were analysed using Spearman rank correlation for all genes within a 2-Mb window surrounding the SNP of interest. Significance was assessed by comparing the observed *P*-value at a 0.001 threshold with the minimum *P*-values from each of the 10,000 permutations of the expression values relative to genotypes[7,8,48]. As an additional approach to eQTL, we explored the NIH Genotype-Tissue Expression (GTEx) database. The GTEx data did not provide any association (data not shown).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Web resources
GWAMA - www.well.ox.ac.uk/gwama
NCBI36 - www.ncbi.nlm.nih.gov
Hapmap3 data - www.hapmap.org
Impute 2 - http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
SNPTEST - http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html
Locuszoom - http://csg.sph.umich.edu/locuszoom/
GTEx - www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi

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References

1. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology. 1999; 53:537–542. [PubMed: 10449117]
2. Stovner LJ, Zwart JA, Hagen K, Terwindt GM, Pascual J. Epidemiology of headache in Europe. Eur. J. Neurol. 2006; 13:333–345. [PubMed: 16643310]
3. Stovner L, et al. The global burden of headache: a documentation of headache prevalence and disability worldwide. Cephalalgia. 2007; 27:193–210. [PubMed: 17381554]
4. Olesen J, Lekander I, Andlin-Sobocki P, Jönsson B. Funding of headache research in Europe. Cephalalgia. 2007; 27:995–999. [PubMed: 17727472]
5. Anttila V, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. Nat. Genet. 2010; 42:869–873. [PubMed: 20802479]
6. Chasman DI, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. Nat. Genet. 2011; 43:695–698. [PubMed: 21666692]
7. Dimas AS, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science. 2009; 325:1246–1250. [PubMed: 19644074]
8. Nica AC, et al. The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. PLoS Genet. 2011; 7:e1002003. [PubMed: 21304890]
9. Lin X, Shah S, Bulleit RF. The expression of MEF2 genes is implicated in CNS neuronal differentiation. Brain Res. Mol. Brain Res. 1996; 42:307–316. [PubMed: 9013788]
10. Flavell SW, et al. Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science. 2006; 311:1008–1012. [PubMed: 16484497]
11. Aurora SK, Wilkinson F. The brain is hyperexcitable in migraine. Cephalalgia. 2007; 27:1442–1453. [PubMed: 18034688]
12. Ferrari MD, Odink J, Bos KD, Malessy MJ, Bruyn GW. Neuroexcitatory plasma amino acids are elevated in migraine. Neurology. 1990; 40:1582–1586. [PubMed: 1977102]

13. Martínez F, Castillo J, Rodríguez JR, Leira R, Noya M. Neuroexcitatory amino acid levels in plasma and cerebrospinal fluid during migraine attacks. Cephalalgia. 1993; 13:89–93. [PubMed: 8098663]
14. Tottene A, et al. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. Neuron. 2009; 61:762–773. [PubMed: 19285472]
15. Flavell SW, et al. Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron. 2008; 60:1022–1038. [PubMed: 19109909]
16. Morrow EM, et al. Identifying Autism Loci and Genes by Tracing Recent Shared Ancestry. Science. 2008; 321:218–223. [PubMed: 18621663]
17. Pfeiffer BE, et al. Fragile X Mental Retardation Protein Is Required for Synapse Elimination by the Activity-Dependent Transcription Factor MEF2. Neuron. 2010; 66:191–197. [PubMed: 20434996]
18. Schytz HW, et al. PACAP38 induces migraine-like attacks in patients with migraine without aura. Brain. 2009; 132:16–25. [PubMed: 19052139]
19. Markovics A, et al. Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice. Neurobiol. Dis. 2011; 45:633–644. [PubMed: 22033344]
20. Goadsby PJ, Lipton RB, Ferrari MD. Migraine - current understanding and treatment. N. Engl. J. Med. 2002; 346:257–270. [PubMed: 11807151]
21. Lin HY, Wang XF, Ng-Eaton E, Weinberg RA, Lodish HF. Expression cloning of the TGF-beta type 2 receptor, a functional transmembrane serine/threonine kinase. Cell. 1992; 68:775–785. [PubMed: 1310899]
22. Law C, et al. Clinical features in a family with an R460H mutation in transforming growh factor β receptor 2 gene. J. Med. Genet. 2006; 43:908–916. [PubMed: 16885183]
23. Rist PM, Diener HC, Kurth T, Schürks M. Migraine, migraine aura, and cervical artery dissection: a systematic review and meta-analysis. Cephalalgia. 2011; 31:886–896. [PubMed: 21511950]
24. Allen PB, Greenfield AT, Svenningsson P, Haspeslagh DC, Greengard P. Phactrs 1-4: a family of protein phosphatise 1 and actin regulatory proteins. Proc. Nat. Acad. Sci. 2004; 101:7187–7192. [PubMed: 15107502]
25. Greengard P, Allen PB, Nairn AC. Beyond the Dopamine Receptor: the DARPP-32/Protein Phosphatase-1 Cascade. Neuron. 1999; 23:435–447. [PubMed: 10433257]
26. Jarray R, et al. Depletion of the novel protein PHACTR-1 from human endothelial cells abolishes tube formation and induces cell death receptor apoptosis. Biochemie. 2011; 93:1668–1675.
27. Myocardial Infarction Genetics Consortium. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat. Genet. 2009; 41:334–341. [PubMed: 19198609]
28. Tietjen GE. Migraine as a systemic vasculopathy. Cephalalgia. 2009; 29:987–996. [PubMed: 19689607]
29. Wilson PM, Fryer RH, Fang Y, Hatten ME. Astn2, a novel member of the astrotactin gene family, regulates the trafficking of ASTN1 during glial-guided neuronal migration. J. Neurosci. 2010; 30:8529–8540. [PubMed: 20573900]
30. DaSilva AF, Granziera C, Snyder J, Hadjikhani N. Thickening in the somatosensory cortex of patients with migraine. Neurology. 2007; 69:1990–1995. [PubMed: 18025393]
31. Kruit MC, et al. Migraine as a risk factor for subclinical brain lesions. JAMA. 2004; 291:427–434. [PubMed: 14747499]
32. Proudfoot CJ, et al. Anagesie mediated by the TRPM8 cold receptor in chronic neuropathic pain. Curr. Biol. 2006; 16:1591–1605. [PubMed: 16920620]
33. Caspani O, Zurborg S, Labuz D, Heppenstall PA. The contribution of TRPM8 and TRPA1 channels to cold allodynia and neuropathic pain. PLoS One. 2009; 4:e7383. [PubMed: 19812688]
34. D'Agostino VC, Francia E, Licursi V, Cerbo R. Clinical and personality features of allodynic migraine. Neurol. Sci. 2010; 31(Suppl1):S159–161. [PubMed: 20464611]

35. Lillis AP, van Duyn LB, Murphy-Ullrich JE, Strickland DK. LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies. Physiol. Rev. 2008; 88:887–918. [PubMed: 18626063]
36. May P, et al. Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice. J. Mol Cell Biol. 2004; 24:8872–8883.
37. Russell MB, Rasmussen BK, Fenger K, Olesen J. Migraine without aura and migraine with aura are distinct clinical entities: a study of four hundred and eighty-four male and female migraineurs from the general population. Cephalalgia. 1996; 16:239–245. [PubMed: 8792035]
38. Kallela M, Wessman M, Havanka H, Palotie A, Färkkilä M. Familial migraine with and without aura: clinical characteristics and co-occurrence. Eur. J. Neurol. 2001; 8:441–449. [PubMed: 11554907]
39. Frazer KA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449:851–861. [PubMed: 17943122]
40. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 2009; 5:e1000529. [PubMed: 19543373]
41. Altshuler DM, et al. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467:52–58. [PubMed: 20811451]
42. Magi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. BMC bioinformatics. 2010; 11:288. [PubMed: 20509871]
43. Cochran WG. The combination of estimates from different experiments. Biometrics. 1954; 10:101–129.
44. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat. Med. 2002; 21:1539–1558. [PubMed: 12111919]
45. Ioannidis JP, Patsopoulos NA, Evangelou E. Heterogeneity in meta-analyses of genome-wide association investigations. PLoS One. 2007; 2:e841. [PubMed: 17786212]
46. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin. Trials. 1986; 7:177–188. [PubMed: 3802833]
47. Purcell S, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 2007; 81:559–575. [PubMed: 17701901]
48. Stranger BE, et al. Population genomics of human gene expression. Nat. Genet. 2007; 39:1217–1224. [PubMed: 17873874]

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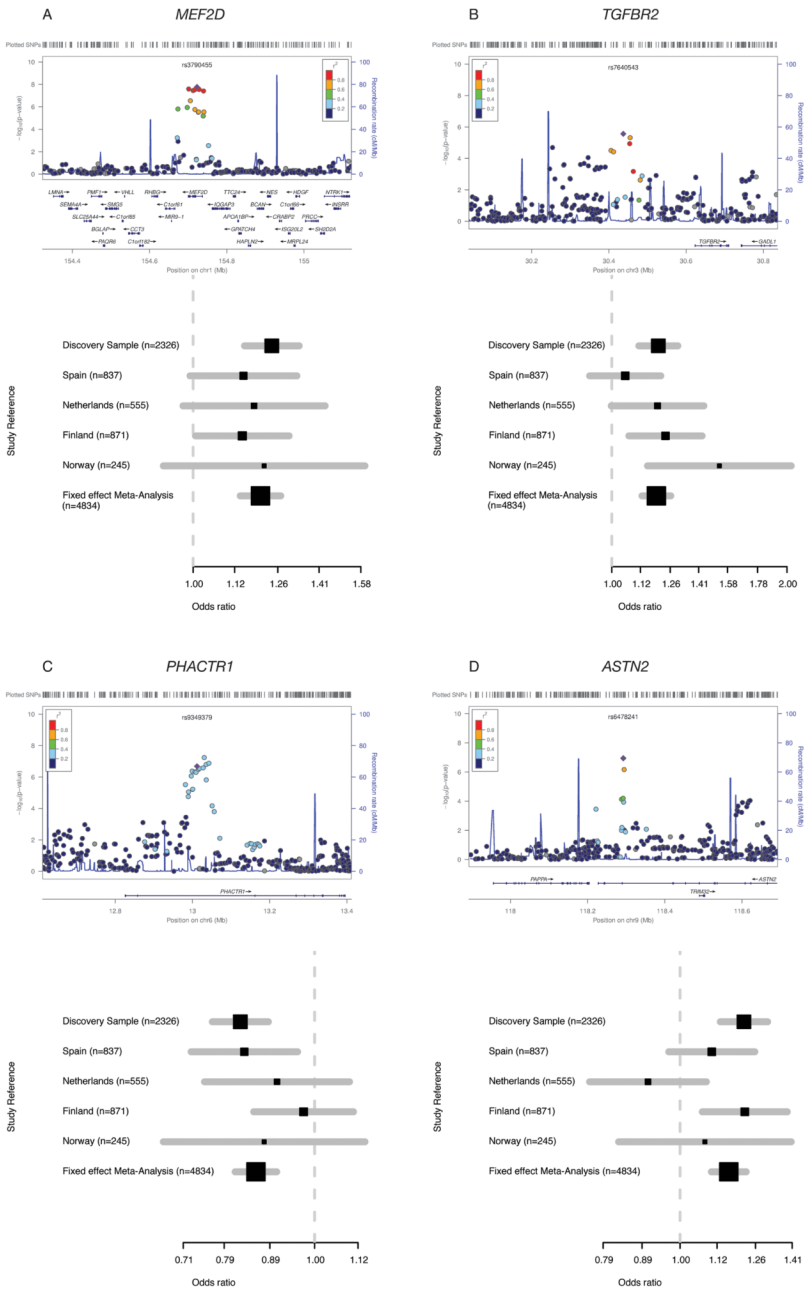

Figure 1. Regional and forest plots for the novel migraine loci

Regional association plots (generated using LocusZoom (see Web resources)) are shown for the four novel migraine loci together with a forest plot for the SNP with highest association for the respective locus (with the number of cases for each sample indicated) (**a-d**). Each regional plot shows the chromosomal position (NCBI build 36) of SNPs in the specific region against the $-\log_{10}$ *P*-values. The SNP with the highest association signal in each locus is represented as a purple diamond; the additional SNPs are color-coded according to the extent of LD with that SNP. Estimated recombination rates (cM/Mb) from HapMap CEU release 22 are shown as light blue lines. Forest plots are shown for: rs3790455

(*MEF2D*) (**a**), rs7640543 (*TGFBR2*) (**b**), rs9349379 (*PHACTR1*) (**c**), and rs6478241 (*ASTN2*) (**d**). Black squares represent the odds ratios for the individual cohorts; the horizontal lines represent the 95% confidence intervals. Numbers in parentheses indicate the number of cases in each cohort.

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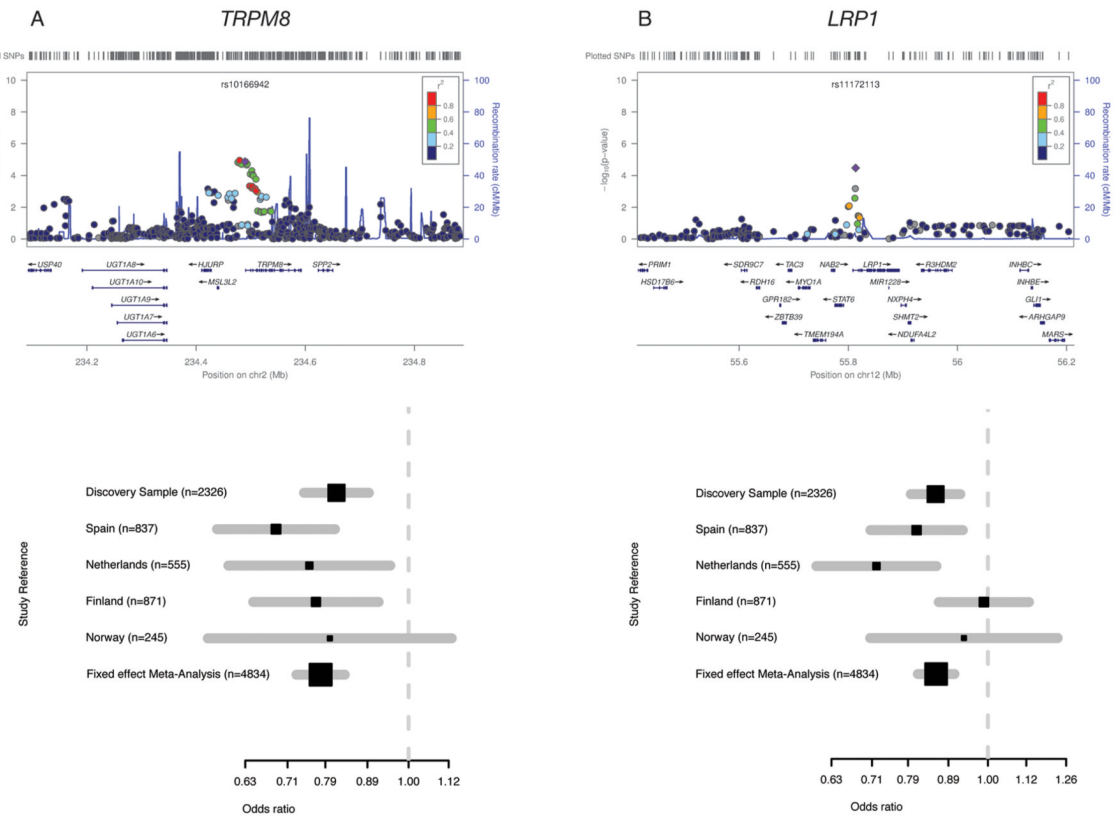

Figure 2. Regional and forest plots for the previously reported migraine loci that replicate in the current MO study

Regional association plots (generated using LocusZoom (see Web resources)) are shown for the two previously reported migraine loci[6] that significantly replicated in the current study as well as forest plots for the SNP with highest association in these loci (with the number of cases for each sample indicated) (**a** and **b**).

Each regional plot shows the chromosomal position (NCBI build 36) of SNPs in the specific region against the $-\log_{10}$ *P*-values. SNP with the highest association signal in each locus is represented as a purple diamond; the additional SNPs are color-coded according to the extent of LD with this SNP. Estimated recombination rates (cM/Mb) from HapMap CEU release 22 are shown as light blue lines. Forest plots are shown for: rs10166942 (*TRPM8*) (**a**) and rs11127113 (*LRP1*) (**b**). Black squares represent the odds ratio for the individual cohorts; the horizontal lines represent the 95% confidence intervals. Numbers in parentheses indicate the number of cases in each cohort.

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Table 1

SNPs of the six migraine without aura loci

SNPs showing significant association either in the discovery stage (i.e. locus 1) or after meta-analysis of the discovery and replication samples (i.e. loci 2-6).

General SNP information								Discovery samples			All replications			Overall meta-analysis			
SNP	Chr	Position[a]	Location	Gene[b]	Minor allele	minor allele frequency	Gen./Imp.[c]	*P*-Value	OR (95% CI)	I^2	*P*-value	OR (95% CI)	I^2	*P*-value	OR (95% CI)	I^2	r^2 with top SNP
Locus 1:																	
rs1050316	1	154.701.327	Intragenic	*MEF2D*	G	0.34	Imputed	**2.59×10^{-8}**	1.24 [1.15-1.33]	0.00	1.15×10^{-3}	1.14 [1.06-1.24]	0.00	**3.21×10^{-10}**	1.19 [1.13-1.26]	0.00	0.987
rs2274316	1	154.712.866	Intragenic	*MEF2D*	C	0.35	Genotyped	**3.60×10^{-8}**	1.23 [1.14-1.33]	0.00	-	-	-	-	-	-	0.986
rs1925950	1	154.717.364	Intragenic	*MEF2D*	G	0.35	Imputed	**2.97×10^{-8}**	1.24 [1.15-1.33]	0.00	-	-	-	-	-	-	0.988
rs3790455	1	154.722.925	Intragenic	*MEF2D*	C	0.34	Genotyped	**1.71×10^{-8}**	1.24 [1.15-1.34]	0.00	4.85×10^{-4}	1.16 [1.07-1.26]	0.00	**7.06×10^{-11}**	1.20 [1.14-1.27]	0.00	-
rs3790459	1	154.728.331	Intragenic	*MEF2D*	A	0.35	Imputed	**2.85×10^{-8}**	1.24 [1.15-1.33]	0.00	-	-	-	-	-	-	0.989
rs12136856	1	154.739.738	Intergenic	*MEF2D*	C	0.34	Imputed	**3.90×10^{-8}**	1.23 [1.15-1.33]	0.00	-	-	-	-	-	-	0.985
Locus 2:																	
rs7640543	3	30.437.407	Intergenic	*TGFBR2*	A	0.32	Genotyped	2.72×10^{-6}	1.20 [1.11-1.30]	0.00	1.02×10^{-4}	1.18 [1.09-1.29]	0.51	**1.17×10^{-9}**	1.19 [1.13-1.26]	0.19	-
Locus 3:																	
rs9349379	6	13.011.943	Intragenic	*PHACTR1*	G	0.38	Genotyped	2.06×10^{-7}	0.82 [0.77-0.89]	0.00	0.01	0.90 [0.83-0.98]	0.00	**3.20×10^{-8}**	0.86 [0.81-0.91]	0.10	-
Locus 4:																	
rs6478241	9	118.292.450	Intragenic	*ASTN2*	A	0.38	Genotyped	1.14×10^{-7}	1.22 [1.13-1.31]	0.00	0.02	1.10 [1.02-1.19]	0.57	**3.86×10^{-8}**	1.16 [1.10-1.23]	0.56	-
Locus 5:																	
rs10166942	2	234.489.832	Intergenic	*TRPM8*	C	0.18	Genotyped	1.32×10^{-5}	0.82 [0.74-0.89]	0.00	5.62×10^{-9}	0.74 [0.67-0.82]	0.00	**9.83×10^{-13}**	0.78 [0.73-0.84]	0.00	-
rs17862920	2	234.492.734	Intragenic	*TRPM8*	T	0.10	Genotyped	2.19×10^{-5}	0.78 [0.69-0.87]	0.00	6.44×10^{-5}	0.75 [0.66-0.87]	0.00	**5.97×10^{-9}**	0.77 [0.70-0.84]	0.00	0.520
Locus 6:																	
rs11172113	12	55.813.550	Intragenic	*LRP1*	C	0.40	Genotyped	3.38×10^{-5}	0.86 [0.80-0.92]	0.00	2.33×10^{-4}	0.86 [0.79-0.93]	0.67	**2.97×10^{-8}**	0.86 [0.81-0.91]	0.45	-

Genome-wide significant *P*-values and successful replications are shown in boldface. ORs are reported for the minor allele.

[a] Chromosomal positions are based on NCBI build 36.

[b] For intragenic SNPs the gene is listed in which the SNP is located, whereas for intergenic SNPs the nearest gene is listed.

[c] Gen./Imp. (Genotyping/imputation) indicates whether a SNP is genotyped or imputed. I^2 = heterogeneity index. r^2 indicates the LD between the SNP and the top SNP in the respective locus.