Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1

Anttila, Verneri

Collaborators: Dermitzakis, Emmanouil

How to cite

ANTTILA, Verneri. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. In: Nature genetics, 2010, vol. 42, n° 10, p. 869–873. doi: 10.1038/ng.652

This publication URL: https://archive-ouverte.unige.ch/unige:20601

Publication DOI: 10.1038/ng.652

Published in final edited form as:
Nat Genet. 2010 October ; 42(10): 869–873. doi:10.1038/ng.652.

Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1

Verneri Anttila[1,2], **Hreinn Stefansson**[3], **Mikko Kallela**[4], **Unda Todt**[5,6], **Gisela M Terwindt**[7], **M Stella Calafato**[1,8], **Dale R Nyholt**[9], **Antigone S Dimas**[1,10,11], **Tobias Freilinger**[12], **Bertram Müller-Myhsok**[13], **Ville Artto**[4], **Michael Inouye**[1,14], **Kirsi Alakurtti**[1,2], **Mari A Kaunisto**[2,15], **Eija Hämäläinen**[1,2], **Boukje de Vries**[14], **Anine H Stam**[7], **Claudia M Weller**[14], **Axel Heinze**[16], **Katja Heinze-Kuhn**[16], **Ingrid Goebel**[5,6], **Guntram Borck**[5,6], **Hartmut Göbel**[16], **Stacy Steinberg**[3], **Christiane Wolf**[13], **Asgeir Björnsson**[3], **Gretar Gudmundsson**[17], **Malene Kirchmann**[18], **Anne Hauge**[18], **Thomas Werge**[19], **Jean Schoenen**[20], **Johan G Eriksson**[15,21,22,23], **Knut Hagen**[24], **Lars Stovner**[24], **H-Erich Wichmann**[25,26,27], **Thomas Meitinger**[28,29], **Michael Alexander**[30,31], **Susanne Moebus**[32], **Stefan Schreiber**[33,34], **Yurii S Aulchenko**[35], **Monique M B Breteler**[35], **Andre G Uitterlinden**[36], **Albert Hofman**[35], **Cornelia M van Duijn**[35], **Päivi Tikka-Kleemola**[37], **Salli Vepsäläinen**[4], **Susanne Lucae**[13], **Federica Tozzi**[38], **Pierandrea Muglia**[38,39], **Jeffrey Barrett**[1], **Jaakko Kaprio**[2,23,40], **Markus Färkkilä**[4], **Leena Peltonen**[1,2,41,*], **Kari Stefansson**[3], **John-Anker Zwart**[23,42], **Michel D Ferrari**[7], **Jes Olesen**[18], **Mark Daly**[41], **Maija Wessman**[2,15], **Arn M J M van den Maagdenberg**[7,14], **Martin Dichgans**[12], **Christian Kubisch**[5,6,43], **Emmanouil T Dermitzakis**[11], **Rune R Frants**[14], and **Aarno Palotie**[1,2,41,44,45] **on behalf of the International Headache Genetics Consortium**

[1] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK. [2] Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki.Finland. [3] Department of Population Genomics, deCODE genetics, Reykjavik, Iceland. [4] Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland. [5] Institute of Human Genetics, University of Cologne, Cologne, Germany. [6] Institute for Genetics and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany. [7] Department of Neurology, Leiden University Medical Centre, Leiden, the Netherlands. [8] National Institute for Health Research, Cambridge Biomedical Research Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK. [9] Neurogenetics Laboratory, Queensland Institute of Medical Research, Brisbane, Australia. [10] Wellcome Trust Center for Human Genetics, University of Oxford, Oxford, UK. [11] Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland. [12] Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-Universität München, Munich, Germany. [13] Max Planck Institute of Psychiatry, Munich, Germany. [14] Department of Human Genetics, Leiden University Medical Centre, Leiden, the Netherlands. [15] Folkhälsan Research Center, Helsinki, Finland. [16] Kiel Pain and Headache Center, Kiel, Germany. [17] Department of Neurology, Landspitali University Hospital, Reykjavik, Iceland. [18] Department of Neurology, Glostrup Hospital and the

Correspondence and requests for materials should be addressed to V.A. (verneri.anttila@sanger.ac.uk) or A.P. (aarno.palotie@sanger.ac.uk)..
*Deceased

Author contributions. All authors contributed to the current version of the paper.

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: A Supplementary Note accompanies the paper on the Nature Genetics website.

Accession numbers Migraine - OMIM 157300.

Competing financial interests The authors declare no competing financial interests.

Danish Headache Center, Glostrup, Denmark. [19] Research Institute of Biological Psychiatry, University of Copenhagen, Roskilde, Denmark. [20] Headache Research Unit. Department of Neurology and GIGA-Neurosciences, Liège University, Liège, Belgium. [21] Department of General Practice, Helsinki University Central Hospital, Helsinki, Finland. [22] Vaasa Central Hospital, Vaasa, Finland. [23] National Institute for Health and Welfare, Helsinki, Finland. [24] Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway. [25] Institute of Epidemiology, HelmholtzCenter Munich, Neuherberg, Germany. [26] Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie, Ludwig-Maximilians-Universität München, Munich, Germany. [27] Klinikum Großhadern, Ludwig-Maximilians-Universität München, Munich, Germany. [28] Institute of Human Genetics, HelmholtzCenter Munich, Neuherberg, Germany. [29] Institute of Human Genetics, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany. [30] Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany. [31] Institute of Human Genetics, University of Bonn, Bonn, Germany. [32] Institute of Medical Informatics, Biometry and Epidemiology, University Hospital of Essen, University Duisburg-Essen, Essen, Germany. [33] Department of Clinical Molecular Biology, Christian Albrechts University, Kiel, Germany. [34] Department Internal Medicine I, Christian Albrechts University, Kiel, Germany. [35] Department of Epidemiology, Erasmus University Medical Center, Rotterdam, the Netherlands. [36] Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands. [37] Research Program in Molecular Medicine, University of Helsinki, Helsinki, Finland. [38] Drug Discovery, GlaxoSmithKline R&D, Verona, Italy. [39] Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto, Canada. [40] Department of Public Health, University of Helsinki, Helsinki, Finland [41] The Broad Institute of MIT and Harvard, Boston, MA, USA. [42] Department of Neurology, Oslo University Hospital and University of Oslo, Oslo, Norway. [43] Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany. [44] Department of Medical Genetics, University of Helsinki, Helsinki, Finland [45] Department of Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland

Abstract

Migraine is a common episodic neurological disorder, typically presenting with recurrent attacks of severe headache and autonomic dysfunction. Apart from rare monogenic subtypes, no genetic or molecular markers for migraine have been convincingly established. We identified the minor allele of rs1835740 on chromosome 8q22.1 to be associated with migraine ($p=5.12 \times 10^{-9}$, OR 1.23 [1.150-1.324]) in a genome-wide association study of 2,748 migraineurs from three European headache clinics and 10,747 population-matched controls. The association was replicated in 3,202 cases and 40,062 controls for an overall meta-analysis p-value of 1.60×10^{-11} (OR 1.18 [1.127 – 1.244]). rs1835740 is located between the astrocyte elevated gene 1 (*MTDH/AEG-1)* and plasma glutamate carboxypeptidase (*PGCP*). In an expression quantitative trait study in lymphoblastoid cell lines transcript levels of the *MTDH/AEG-1* were found to have a significant correlation to rs1835740. Our data establish rs1835740 as the first genetic risk factor for migraine.

The recent boom of genome-wide association (GWA) studies has had a major impact on our current view of genetic susceptibility to common traits and complex disorders. However, the number of loci identified in central nervous system disorders (CNS) is underrepresented (www.genome.gov/gwastudies [1]). To our knowledge no GWA studies or any common, robustly established variants have been reported for major episodic neurological disorders (ICD G40-44, migraine, epilepsy, ataxias). However, there is substantial genetic information for rare Mendelian forms of migraine, epilepsy and ataxia, which classify them as channelopathies associated with compromised neurotransmitter homeostasis[2]. So far there is no evidence for the contribution of ion channel variants in common forms of these diseases[3,4].

Migraine is an episodic neurological disorder with complex pathophysiology, affecting 8% of males and 17% of females[5]. Migraine ranks among the 20 most disabling diseases and has been estimated the most costly neurological disorder for society with a considerable impact on public health[6]. Clinically, the International Classification of Headache Disorders (ICHD-II[7]) recognizes two main common forms: i) migraine with aura (MA), (ii) migraine without aura (MO). The two forms are distinguised based on the presence of aura, a period of variable and diverse neurological symptoms that precede the headache phase. Patient may have attacks of only MO, or only MA, or a combination of both types in variable proportions. There is debate whether MA and MO attacks represent two distinct disorders, or merely are variations of a single disease entity on a common complex genetic background. Migraine headache is believed to be caused by activation of the trigeminovascular system and the aura by cortical spreading depression (CSD), a slowly propagating wave of neuronal and glial depolarization[8-10]. However, these are considered to be downstream events, and it is unknown how migraine attacks are initiated.

To identify variants associated with the common forms of migraine we carried out a two-stage GWA study in six clinic-based and one population-based European migraine samples (Supplementary Figure 1). In the discovery stage, we studied 3,279 migraineurs (1,124 Finns, 1,276 Germans and 879 Dutch) recruited from headache clinics and genotyped using Illumina arrays, against population-matched controls (10,747) recruited from pre-existing population-based GWA studies (see Supplementary Note for details). In the replication stage, a further 3,202 patients and 40,062 population-matched controls from Iceland, Denmark, the Netherlands and Germany were studied.

Diagnoses were made by headache experts using a combination of questionnaire and individual interviews that are based on the ICHD-II[7]. Due to the overlap between MA and MO, we analyzed the following groups: i) “all migraine”, i.e. all migraine patients irrespective of subtype, ii) “MA only”, i.e. patients who only have attacks where aura is present, iii) “both MA and MO”, i.e. patients with attacks both with and without aura and iv) “MO only”, i.e. patients with only attacks of migraine without aura.

A multi-population Cochran-Mantel-Haenszel (CMH) association analysis and a significance threshold of $p \leq 5 \times 10^{-8}$ were applied. In the initial GWA study, 2,748 cases and 10,747 controls (Table 1) passed quality control steps, and 429,912 markers were successfully genotyped (see Online Methods for details). A quantile-quantile plot of the CMH analysis (Supplementary Figure 2) and an overall inflation factor ($\lambda = 1.08$) were used as final quality control measures.

Only one marker, rs1835740 on chromosome 8q22.1, showed significant association to migraine in the multi-population CMH analysis (Figure 1, Supplementary Figure 3). Further 11 loci were found with p-values $\leq 5 \times 10^{-5}$ (Supplementary Table 1). The minor allele (A) of marker rs1835740 was associated with migraine with a p-value of 5.12×10^{-9} and odds ratios ranging between 1.21 – 1.33 (Table 2). Two nearby markers with the highest linkage disequilibrium (LD) to rs1835740 (rs982502: $r^2=0.59$, $p=1.54 \times 10^{-4}$ and rs2436046: $r^2=0.68$, $p=3.83 \times 10^{-5}$) also showed association to migraine (Supplementary Table 2). Haplotype analysis detected a 27 kb haplotype ($p=1.15 \times 10^{-7}$) (Supplementary Figure 4 and Supplementary Table 3). We analysed the HapMap Phase II data[11] to demonstrate that no long-range LD to rs1835740 exists within a 5 Mb window using the ssSNPer program[12], strongly suggesting that the causative variant is tagged by the minor allele of rs1835740 located between two close recombination hotspots (at 98.199 Mb and 98.309 Mb, Figure 1). The 2 Mb window around rs1835740 was also imputed against the 1000 Genomes data (August 2009 release), but no other marker exceeded (Figure 1) the evidence of rs1835740 for association. Conditional analysis of the SNPs around rs1835740 showed no additional independent signals

(Supplementary Table 2). The proportion of genetic variance explained by the rs1835740 variant was estimated to be 1.5-2.5% depending on the heritability estimate used and the population attributable risk to be 10.7% using the methodology of Risch et al.[13]

To confirm and extend our results, we performed a replication study on the only marker with genome-wide significance in the initial study, rs1835740. The replication samples were divided into the phenotypic subgroups similar to the discovery sample. Replication was successful in two "MA only" subsets (Danish: p=0.015, OR 1.29; Icelandic: p=0.038, OR 1.36), the Icelandic MO set (p=0.0292, OR 1.18) as well as in the Icelandic "all migraine" group (p=0.010, OR 1.18) (Table 2). Overall, the A allele of marker rs1835740 was overrepresented (OR 1.05 – 1.36, Table 2) in each subset of all replication samples except in the Danish "MA, MO" group (OR 0.99). The effect was stronger in the MA groups than other migraine subgroups (Figure 2). It should be noted that the majority of the groups which did not reach formal replication were small with limited power. Meta-analysis was conducted using the CMH test for each diagnosis subgroup alone as well as for all migraine samples, with the latter showing a final p-value of 1.60×10^{-11} (Table 2).

Marker rs1835740 is located between two potentially interesting candidate genes, *MTDH/AEG-1* and *PGCP*. We analyzed the effect of the marker genotype on the expression of genes within a 2 Mb window in fibroblasts, primary T-cells and lymphoblastoid cell lines (LCL) established from umbilical cords[14]. In the expression quantitative trait locus (eQTL) analysis, the rs1835740 genotype was found to have significant correlation to the transcript levels of the nearby *MTDH/AEG-1* gene in LCLs (see Table 3 and Supplementary Table 4), with the risk allele A being associated with higher expression levels (Figure 3). This is in line with previous studies, which have proven expression analyses in LCL cells to be informative in neurological and neuropsychiatric traits[15-17]. No significant association was detected in fibroblasts or primary T-cells. The eQTL analysis suggests rs1835740 to be a *cis* regulator of *MTDH/AEG-1* in LCLs.

The location of the associating sequence variant, rs1835740, between two genes involved in glutamate homeostasis, *PGCP* and *MTDH/AEG-1*, suggests that this region contains elements that could regulate either or both of these flanking genes, the eQTL analysis pointing to the latter gene. Although *MTDH/AEG-1* has mainly been studied in carcinogenesis[18], previous studies in cultured astrocytes have shown that *MTDH/AEG-1* down-regulates EAAT2/GLT1[18-22], the major glutamate transporter in the brain. Furthermore, mice lacking the EAAT2 gene have been shown to suffer from lethal spontaneous epileptic seizures[23]. Despite the limitations to extrapolate eQTL findings from LCL cells directly to brain tissue the data suggests a plausible link between the identified variant and glutamate regulation. This is a tempting hypothesis as this neurotransmitter has long been suspected to play a key role in migraine pathophysiology[24].

Although the evidence provided here is indirect, accumulation of excess glutamate in the synaptic cleft through down-regulation of EAAT2/GLT1 or through increased *PGCP* activity (or both), would provide an intriguing putative mechanism for the occurrence of migraine attacks. It is reasonable to speculate that this accumulation can increase susceptibility to migraine through increased sensitivity to CSD, the likely mechanism for the migraine aura[9, 10], as well as through glutamate involvement in central sensitization, which has been postulated to be the underlying mechanism of allodynia during a migraine attack[25].

This and our previous study[3] did not yield evidence for association of ion channel genes to common forms of migraine. Thus, even if the contribution of ion channel genes is well established in Mendelian forms of paroxysmal neurological disorders, such as familial hemiplegic migraine (FHM)[26-29], their direct role in more common forms remains open.

Interestingly, previous studies suggested that the imbalance of glutamate release and clearance is a key component of the pathogenesis of FHM, where the underlying mutation is in *CACNA1A, ATP1A2 or SCN1A*[30,31]. The results of the present study support the hypothesis that complementary pathways such as the glutamate system may tie the Mendelian channelopathies with pathogenetic mechanisms of more common forms of episodic neurological disorders, such as migraine. Mutations in the functionally related EAAT1 transporter have been identified in other episodic phenotypes (such as episodic ataxia 6[32], and a non FHM1/2 hemiplegic migraine/episodic ataxia/seizure phenotype[33]), providing an example of the link between EAAT transporters to episodic disorders. Future studies should be conducted to specifically test this hypothesis.

In summary, we have identified the first robust genetic association to migraine. As our cases were mainly selected from specialized headache clinics, subsequent studies are needed to establish the contribution of rs1835740 in population-based migraine cohorts. These population based cohorts may represent a different severity spectrum and thus, possibly, also a somewhat different underlying combination of genetic susceptibility variants. The effect of rs1835740 is stronger in MA than MO, but further studies are needed to confirm the role of the variant in different migraine subgroups. The variant explains only a small fraction of the overall genetic variance in migraine and future GWA studies, perhaps with different ascertainment schemes, will likely identify additional loci explaining more of the genetic variance.

Online Methods

Study design

We jointly analyzed patient samples from three migraine with aura collections from Finland, Germany and the Netherlands with population-matched controls obtained from pre-existing studies. This initial phase was followed by a replication study of the top SNP, rs1835740, in migraine samples from Denmark, Iceland, the Netherlands and Germany. Characteristics of each study sample are described in Table 1, and the recruitment and ascertainment of cases and controls are described in the Supplementary Note.

Initial genome-wide association (GWA) study genotyping

DNA was extracted from patient blood samples using standard methods. Genotyping of the GWA study samples was done at the Wellcome Trust Sanger Institute on the Illumina 610K (Finns, Germans) and 550K (Dutch) single nucleotide polymorphisms (SNP) microarrays following the Infinium II protocol from the manufacturer (Illumina Inc., San Diego, USA). Genotype calling was performed using the Illuminus software[34].

Replication study genotyping

For the replication study, Danish cases and 459 migraine-free controls were genotyped using the Centaurus platform (Nanogen Inc., San Diego, CA, USA), and 904 additional controls were genotyped at deCODE genetics using Illumina HumanHap650 BeadArrayTM. The Icelandic cases and controls were genotyped using the Illumina HumanHap 317K, 370K, 610K or 1M bead arrays at deCODE genetics. The Dutch replication cohort was genotyped using the TaqMan technology (Applied Biosystems, Life Technologies, Foster City, CA, USA) at Leiden University Medical Center. The German replication cases were genotyped using Illumina HumanHap 610K at Munich with external replication.

Expression study

The GenCord resource, a collection of cell lines derived from umbilical cords of 75 newborns of Western European origin born at the maternity ward of the University of Geneva Hospital, was used. Sample collection was performed on full term or near full term pregnancies to ensure homogeneity for sample age. Three cell types were derived: 1) primary fibroblasts, 2) LCLs and 3) primary T-cells[14]. Total RNA was extracted from these cells and two one-quarter scale Message Amp II reactions (Ambion) were performed for each extraction with 200 ng of total RNA. 1.5 μg of cRNA was hybridized to Illumina's WG-6 v3 Expression BeadChip array to quantify transcript abundance[35]. Intensity values were log2 transformed and normalized independently for each cell type using quantile normalization for sample replicates, and median normalization across all individuals. Each cell type was renormalized using the mean of the medians of each cell type expression values. DNA samples were extracted from umbilical cord tissue LCLs with the Puregene cell kit (Gentra-Qiagen, Venlo, the Netherlands) and genotyping was performed using the Illumina 550K SNP array (Illumina Inc., San Diego, USA) to obtain the SNP genotypes for the samples.

Statistical analysis of initial genome-wide scan data

Stringent per-SNP and per-sample limits were implemented in order to obtain high-quality data. Quality control measures were: exclusion of samples with call rates <97%, non-comparable ancestry as measured using multidimensional scaling plots from PLINK[36], possible contamination as identified by being an extreme heterozygosity outlier, and cryptic relatedness (low-level relatedness to a large number of samples), and non-cryptic relatedness of pi-hat>12.5%. From the initial 3,279 cases and 12,369 controls, altogether 2,748 cases and 10,747 controls passed all quality control criteria, while 531 cases and 1,622 controls were excluded. The majority of case exclusions were due to quality issues on the 550K chips, and the majority of control exclusions were due to low-level relatedness in the Dutch control set. SNPs were excluded for having a minor allele frequency of <1% or for departing from Hardy-Weinberg equilibrium with $p < 10^{-6}$ in cases or controls. Only completely overlapping SNPs from the three populations were used, leaving a total of 429,912 SNPs for analysis. To ascertain whether the control samples were properly matched to the cases, a population-specific and overall genomic inflation factors (λ) was estimated using the median χ^2 value from a 1-degree of freedom allelic χ^2 test. For the Finns, $\lambda = 1.05$, for Germans $\lambda = 1.07$, for the Dutch $\lambda = 1.09$, and overall $\lambda = 1.08$, suggesting reasonably well-matched controls in each case. Differences between cases and controls were assessed between each SNP and disease using a two-tailed Cochran-Mantel-Haenszel (CMH) test for 2x2xK stratified data (K = 3), as implemented in PLINK v1.06. To exclude long-range LD for the identified variant, we used the program ssSNPer[12] to demonstrate that no SNP within a 5 Mb window had high LD to rs1835740 in HapMap Phase II data.

Conditional analysis for secondary effects

In addition to rs1835740, two other SNPs on 8q22.1, rs2436046 and rs982502, showed a CMH p-value $< 10^{-3}$ (main paper Table 2 and Figure 2). Based on our data, rs2436046 ($r^2 = 0.68$) and rs982502 ($r^2 = 0.59$) are in moderate LD with rs1835740. To evaluate whether these signals were independent from the top SNP association signal, the association between migraine and SNP alleles was tested using logistic regression and conditioning on rs1835740 as implemented in PLINK v1.06. Conditioning on rs1835740, no evidence of additional independent signals was found either for rs2436046 or rs982502 ($p = 0.89$ and $p = 0.47$) (Supplementary Table 3), suggesting that the moderate association of rs2436046 and rs982502 observed in the CMH test is the result of these SNPs being in LD with rs1835740.

UKPMC Funders Group Author Manuscript

Meta-analysis of initial and replication samples

The CMH test was used for meta-analysis, with a nominal covariate used to distinguish each sample collection from the others. For the replication in Icelandic and Danish samples, association analysis was carried out using a likelihood procedure[37], and results were adjusted for relatedness by dividing the chi-square statistics by an inflation factor estimated through simulation[38].

Imputation

For each cohort, imputation of the untyped markers in the 2 Mb region around rs1835740 was carried out using IMPUTE v2 with recommended options[39]. Haplotypes from the 1,000 Genomes Project (August 2009 release) and haplotypes from HapMap Phase 3 (www.hapmap.org) were used as reference panels.

eQTL analysis

Association between genotypes and expression was analyzed with Spearman rank correlation for all SNPs with a 2 Mb window centered on the transcription start site of the gene. Significance was assessed by comparing the observed p-values at a 0.001 threshold with minimum p-values from each of 10,000 permutations of the expression values relative to genotypes[35].

URLs

Control populations: Finland – Health2000 study, www.nationalbiobanks.fi; Finland – Helsinki Birth Cohort study, www.nationalbiobanks.fi; Germany – KORA S4/F4 study, www.helmholtz-muenchen.de/kora; Germany – PopGen study, www.popgen.de; Germany – HNR study, www.recall-studie.uni-essen.de; Illumina iControlDB – www.illumina.com; the Netherlands – Rotterdam I and III studies, www.epib.nl/research/ergo.htm; the Netherlands – Lumina study, www.lumc.nl/hoofdpijn. Other URLs: International Headache Genetics Consortium – www.headachegenetics.org; ssSNPer – http://gump.qimr.edu.au/general/daleN/ssSNPer/; GWAS plotter – broadinstitute.org/node/555; HapMap Phase 2 and 3 data – www.hapmap.org

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank all patients of the respective cohorts for their generous participation. This work was supported by the Wellcome Trust [grant number WT089062] and among others by the Academy of Finland (200923 to AP, 00213 to MW), the Helsinki University Central Hospital (to MK, MF, VAr, SV), Academy of Finland Center of Excellence for Complex Disease Genetics, the EuroHead project (LSM-CT-2004-504837), the Helsinki Biomedical Graduate School (to VA, PT-K), the Finnish Cultural Foundation (to VA), the Finnish Neurology Foundation, Biomedicum Helsinki Foundation (to VA, PT-K and VAr), Cambridge Biomedical Research Centre (SC); the NHMRC Fellowship (339462 and 613674) and ARC Future Fellowship (FT0991022) schemes (DRN); the German Federal Ministry of Education and Research (BMBF) (BMBF Grant 01GS08121 to MD, to HEW in context of the German National Genome Research Network (NGFN-2 and NGFN-plus) for the HNR study, and to CK (EMINet - 01GS08120) for the National Genome Network (NGFN-1 and NGFN-Plus)), and the Center for Molecular Medicine Cologne to CK, Heinz Nixdorf Foundation (Chairman: Dr. jur. G. Schmidt) for the HNR study, Deutsche Forschungsgemeinschaft (DFG) to CK and HG; Netherlands Organization for the Health Research and Development (ZonMw) nr. 90700217 (to GMT) and to the Rotterdam study, the Netherlands Organisation for Scientific Research (NWO) VICI (918.56.602) and Spinoza (2009) grants (to MDF), the Center for Medical Systems Biology (CMSB) established by the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (NGI/NWO) nr. 050-060-409 (to RRF, MDF, and AMJMvdM), project nr 050-060-810 and nr. 175.010.2005.011, 911-03-012 to the Rotterdam study. We thank the Health 2000 study for providing Finnish control genotypes. The Broad Institute Center for Genotyping and Analysis is supported by grant from the National Center for Research Resources. The KORA research platform was initiated

and financed by the Helmholtz Center Munich, German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria, and supported within the Munich Center of Health Sciences (MC Health) as part of LMUinnovativ. The Rotterdam Study is supported by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/N, Erasmus Medical Center and Erasmus University, Rotterdam, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam and the Netherlands Consortium for Healthy Ageing (NCHA). We wish to thank Sarah Hunt, Rhian Gwillian, Pamela Whittaker, Simon Potter and Avazeh Tashakkori-Ghanbarian as well as Pablo Marin-Garcia for their invaluable help for this study. Finally, we wish to collectively thank everyone who has contributed to the collection, genotyping and analysis of the individual cohorts.

References

1. Hindorff, LA.; Junkins, HA.; Mehta, JP.; Manolio, TA. A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/gwastudies. Accessed Feb 16th 2010
2. Hanna MG. Genetic neurological channelopathies. Nat Clin Pract Neurol 2006;2:252–263. [PubMed: 16932562]
3. Nyholt DR, et al. A high-density association screen of 155 ion transport genes for involvement with common migraine. Hum Mol Genet 2008;17:3318–3331. [PubMed: 18676988]
4. Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. Trends in Genet 2009;25:361–367. [PubMed: 19665252]
5. Stovner LJ, Zwart JA, Hagen K, Terwindt GM, Pascual J. Epidemiology of headache in Europe. Eur J Neurol 2006;13:333–345. [PubMed: 16643310]
6. Stovner LJ, et al. The global burden of headache: a documentation of headache prevalence and disability worldwide. Cephalalgia 2007;27:193–210. [PubMed: 17381554]
7. International Headache Society. The International Classification of Headache Disorders: 2nd edition. Cephalalgia 2004;24(Suppl 1):9–160. [PubMed: 14979299]
8. Goadsby PJ, Lipton RB, Ferrari MD. Migraine--current understanding and treatment. New Engl J Med 2002;346:257–270. [PubMed: 11807151]
9. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. Brain 1994;117:199–210. [PubMed: 7908596]
10. Hadjikhani N, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci U S A 2001;98:4687–4692. [PubMed: 11287655]
11. Frazer KA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851–861. [PubMed: 17943122]
12. Nyholt DR, et al. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–769. [PubMed: 14997420]
13. Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000;405:847–856. [PubMed: 10866211]
14. Dimas AS, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science 2009;325:1246–1250. [PubMed: 19644074]
15. Hu VW, et al. Gene expression profiling differentiates autism case-controls and phenotypic variants of autism spectrum disorders: evidence for circadian rhythm dysfunction in severe autism. Autism Res 2009;2:78–97. [PubMed: 19418574]
16. Nishimura Y, et al. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. Hum Mol Genet 2007;16:1682–98. [PubMed: 17519220]
17. Martin MV, et al. Exon expression in lymphoblastoid cell lines from subjects with schizophrenia before and after glucose deprivation. BMC Med Genomics 2009;2:62. [PubMed: 19772658]
18. Emdad L, et al. Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. Proc Natl Acad Sci U S A 2009;106:21300–21305. [PubMed: 19940250]
19. Kang DC, et al. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 2005;353:8–15. [PubMed: 15927426]
20. Noch E, Khalili K. Molecular mechanisms of necrosis in glioblastoma: the role of glutamate excitotoxicity. Cancer Biol Ther 2009;8:1791–1797. [PubMed: 19770591]

21. Boycott HE, Wilkinson JA, Boyle JP, Pearson HA, Peers C. Differential involvement of TNF alpha in hypoxic suppression of astrocyte glutamate transporters. Glia 2008;56:998–1004. [PubMed: 18381653]
22. Dallas M, et al. Hypoxia suppresses glutamate transport in astrocytes. J Neurosci 2007;27:3946–3955. [PubMed: 17428968]
23. Tanaka K, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 1997;276:1699–1702. [PubMed: 9180080]
24. Goadsby PJ, Charbit AR, Andreou AP, Akerman S, Holland PR. Neurobiology of migraine. Neurosci 2009;161:327–41.
25. Burstein R, Cutrer MF, Yarnitsky D. The development of cutaneous allodynia during a migraine attack clinical evidence for the sequential recruitment of spinal and supraspinal nociceptive neurons in migraine. Brain 123;2000;(Pt 8):1703–1709.
26. Ophoff RA, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell 1996;87:543–552. [PubMed: 8898206]
27. De Fusco M, et al. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 2003;33:192–196. [PubMed: 12539047]
28. Dichgans M, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet 2005;366:371–377. [PubMed: 16054936]
29. Pietrobon D. Familial hemiplegic migraine. Neurotherapeutics 2007;4:274–284. [PubMed: 17395138]
30. de Vries B, Frants RR, Ferrari MD, van den Maagdenberg AM. Molecular genetics of migraine. Hum Genet 2009;126:115–132. [PubMed: 19455354]
31. 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]
32. de Vries B, et al. Episodic ataxia associated with EAAT1 mutation C186S affecting glutamate reuptake. Arch Neurol 2009;66:97–101. [PubMed: 19139306]
33. Jen JC, Wan J, Palos TP, Howard BD, Baloh RW. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. Neurology 2005;65:529–534. [PubMed: 16116111]
34. Teo YY, et al. A genotype calling algorithm for the Illumina BeadArray platform. Bioinformatics 2007;23:2741–6. [PubMed: 17846035]
35. Stranger BE, et al. Population genomics of human gene expression. Nat Genet 2007;39:1217–24. [PubMed: 17873874]
36. Purcell S, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75. [PubMed: 17701901]
37. Gretarsdottir S, et al. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 2003;35:131–138. [PubMed: 14517540]
38. Grant SF, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320–3. [PubMed: 16415884]
39. 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:e1000529. [PubMed: 19543373]

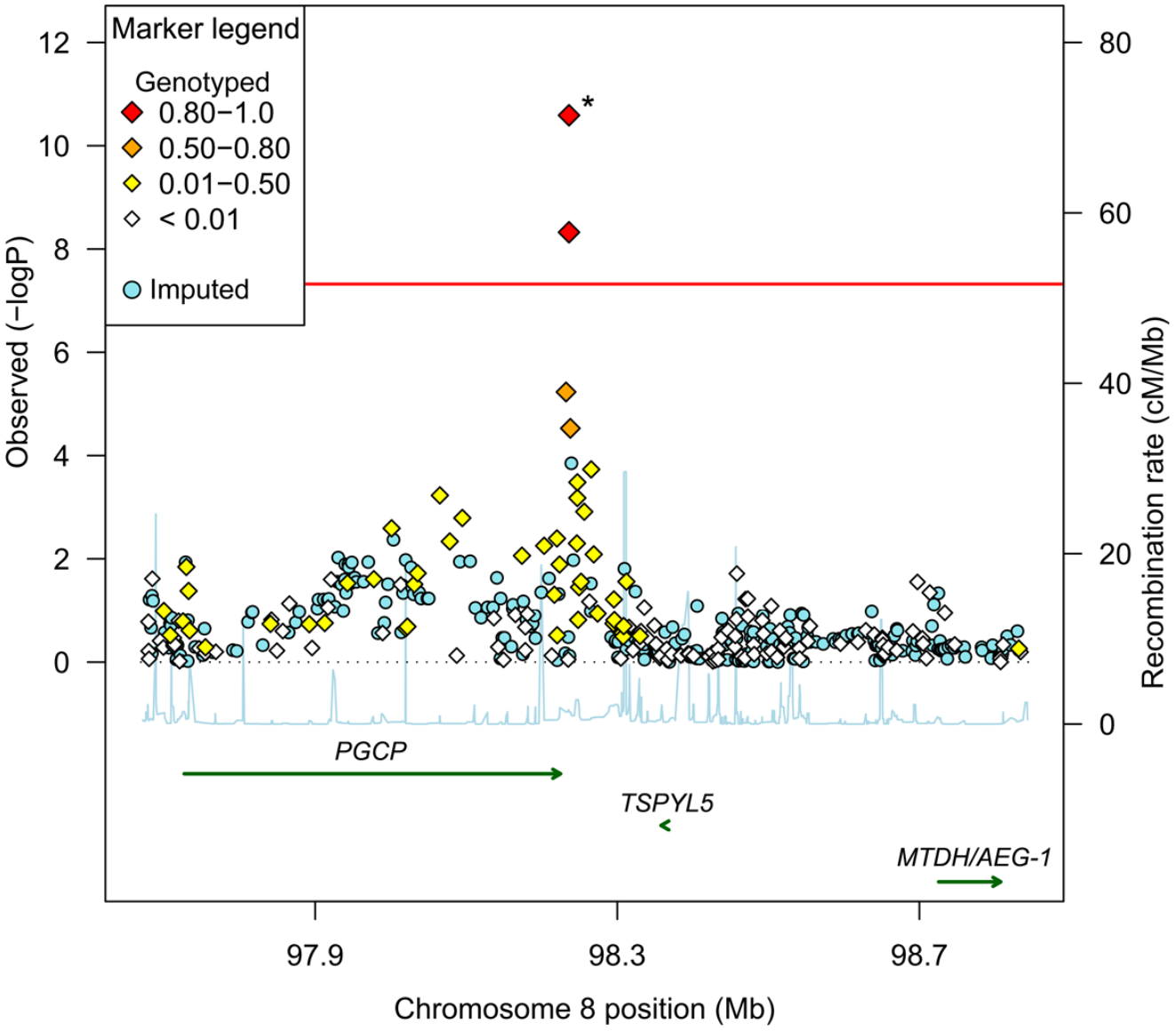

Figure 1. Cochran-Mantel-Haenszel association results for combined analysis of the three study populations between 97.5 and 98.5 Mb on chromosome 8q22.1

Diamonds show position and p-value for each marker in the region, with colors representing extent of linkage disequilibrium (measured in r^2) with marker rs1835740, and blue circles indicate locations and p-values of imputed markers. For rs1835740, p-values are shown for both the original genome-wide association study and the meta-analysis of all migraine samples in the study (denoted by asterisk). The blue graph shows the local recombination rate based on HapMap Phase II data[11]. Red line denotes the threshold for genome-wide significance ($p \leq 5 \times 10^{-8}$). Figure was generated using a modified version of the script available at broadinstitute.org/node/555.

UKPMC Funders Group Author Manuscript

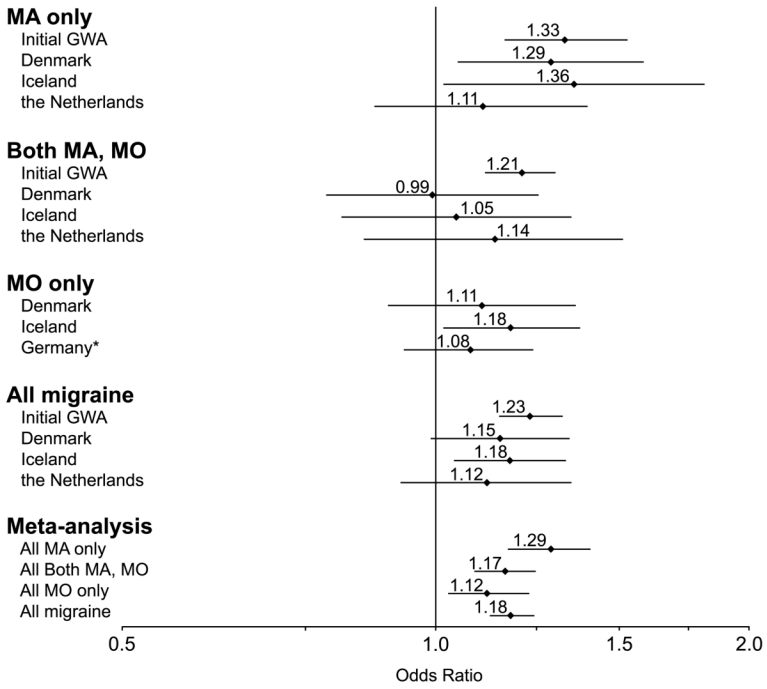

Figure 2. Forest plot of migraine risk for individuals carrying the A allele of marker rs1835740 in each study population

For each dataset, the horizontal line indicates 95% confidence interval, and the number above the line indicates the point estimate of the odds ratio. MA only – patients whose attacks are always accompanied with aura, Both MA, MO – patients with attacks with and without aura, MO only – patients whose attacks never include aura.

UKPMC Funders Group Author Manuscript

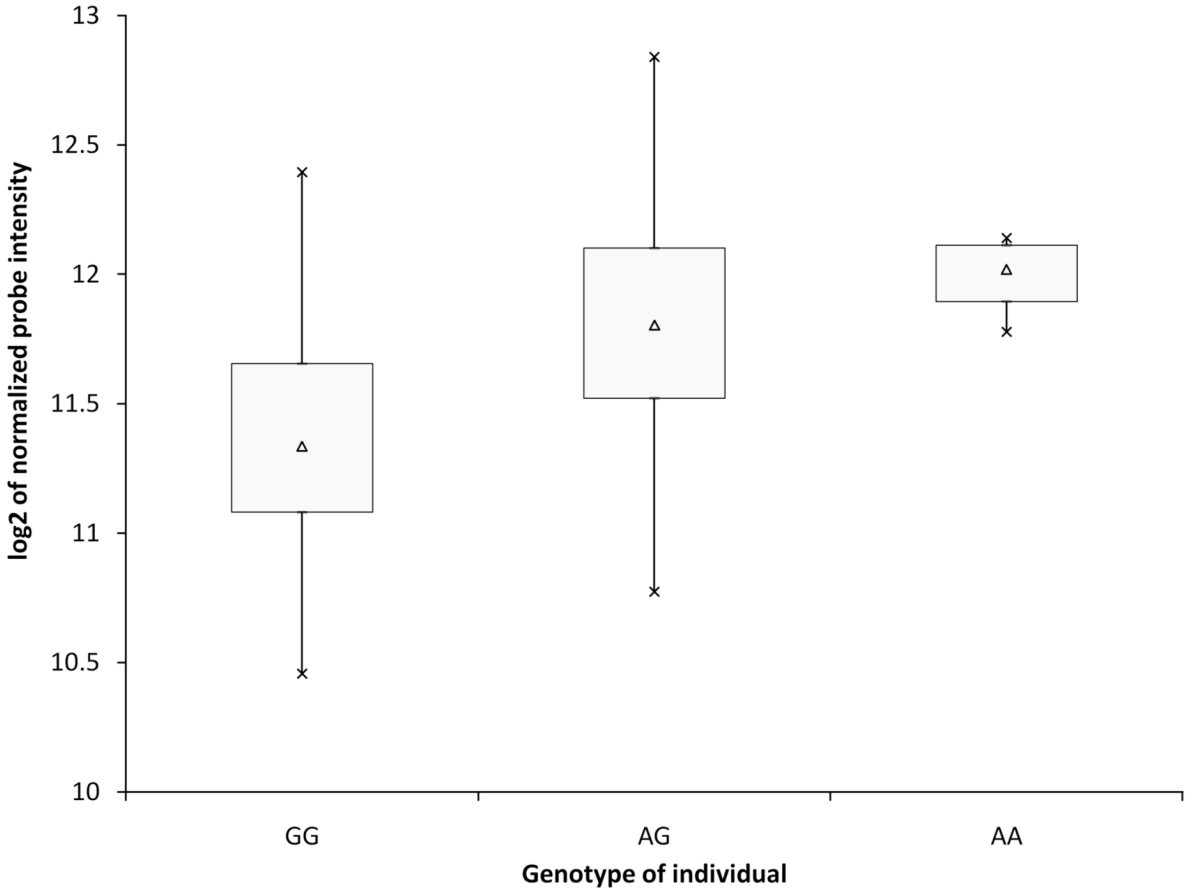

Figure 3. A box-plot of the quantified expression values for *MTDH/AEG-1* ordered based on sample genotype of rs1835740

Normalised expression levels in lymphoblastoid cell lines using Illumina's WG-6 v3 Expression BeadChip array are shown. In each group, the small pyramid indicates median value, the shaded area represents the lower and upper quartiles, and the crosses show the minimum and maximum values in the expression data.

UKPMC Funders Group Author Manuscript

Table 1

Study populations used in the two stages of the study.

		Total	Men	Women	Patients with both MA and MO attacks	Patients with MA attacks only	Patients with MO attacks only
Initial GWA study							
Finland	Cases	1 064	19.8 %	80.2 %	94.4 %	5.6 %	0.0 %
	Controls	3 513	47.4 %	52.6 %	-	-	-
Germany	Cases	1 029	18.9 %	81.1 %	70.2 %	29.8 %	0.0 %
	Controls	2 317	45.1 %	54.9 %	-	-	-
the Netherlands	Cases	655	17.2 %	82.8 %	65.9 %	34.1 %	0.0 %
	Controls	4 917	41.7 %	58.3 %	-	-	-
Total GWAS	Cases	2 748	18.8 %	81.2 %	78.5 %	21.5 %	0.0 %
	Controls	10 747	44.3 %	55.7 %	-	-	-
Replication stage							
Iceland	Cases	900	22.5 %	77.5 %	63.0 %	21.8 %	15.2 %
	Controls	35 221	57.4 %	42.6 %	-	-	-
Denmark	Cases	1 116	22.4 %	77.6 %	26.3 %	43.3 %	30.5 %
	Controls	1 353	44.5 %	55.5 %	-	-	-
the Netherlands	Cases	349	18.3%	81.7%	59.8%	40.2%	0.0 %
	Controls	2 082	43.9%	56.1%	-	-	-
Germany	Cases	837	11.6 %	88.4 %	0.0 %	0.0 %	100.0 %
	Controls	1 406	37.3 %	62.7 %	-	-	-
Total replication	Cases	3 202	19.1 %	80.9 %	33.8 %	25.6 %	41.0 %
	Controls	40 062	55.6 %	44.4 %	-	-	-
Overall meta-analysis	Cases	5 950	19.0 %	81.0 %	54.4 %	23.7 %	22.1 %
	Controls	50 809	53.2 %	46.8 %	-	-	-

UKPMC Funders Group Author Manuscript

Table 2

Association results for marker rs1835740 using the CMH test.

	Diagnosis	n (cases)	n (controls)	Case alleles (MAF)	Control alleles (MAF)	P-value	OR	95% CI
GWA study								
Finland	All migraine	1 064	3 513	548/1 576 (0.258)	1 553/5 461 (0.221)	0.000447	1.22	1.093-1.368
Germany	All migraine	1 029	2 317	515/1 537 (0.251)	998/3 632 (0.216)	0.00142	1.22	1.079-1.378
the Netherlands	All migraine	655	4 917	329/963 (0.255)	2 086/7 742 (0.212)	0.000876	1.26	1.098-1.437
Combined GWA								
	MA only	589	10 747	313/859 (0.267)	4 637/16 385 (0.216)	3.07×10^{-5}	1.33	1.164-1.528
	Both MA,MO	2 142	10 747	1 071/3 193 (0.251)	4 637/16 385 (0.216)	2.69×10^{-6}	1.21	1.115-1.304
	All migraine	2 748	10 747	1 392/4 076 (0.255)	4 637/16 385 (0.216)	$\mathbf{5.12 \times 10^{-9}}$	1.23	1.150-1.324
Replication study								
Denmark	MA only	483	1 353	244/722 (0.253)	562/2 144 (0.208)	**0.015**	1.29	1.050-1.583
	Both MA,MO	293	1 353	121/465 (0.206)	562/2 144 (0.208)	0.951	0.99	0.785-1.255
	MO only	340	1 353	153/527 (0.225)	562/2 144 (0.208)	0.333	1.11	0.900-1.362
	All migraine	1116	1 353	518/1 714 (0.232)	562/2 144 (0.208)	0.069	1.15	0.989-1.344
Iceland	MA only	137	35 221	70/204 (0.255)	14 212/56 230 (0.202)	**0.0380**	1.36	1.017-1.812
	Both MA,MO	196	35 221	82/310 (0.209)	14 212/56 230 (0.202)	0.7256	1.05	0.812-1.350
	MO only	567	35 221	261/873 (0.230)	14 212/56 230 (0.202)	**0.0292**	1.18	1.017-1.376
	All migraine	900	35 221	413/1 387 (0.229)	14 212/56 230 (0.202)	**0.010**	1.18	1.041-1.334
the Netherlands	MA only	212	2 082	100/324 (0.236)	909/3 255 (0.218)	0.406	1.11	0.873-1.399
	Both MA,MO	137	2 082	66/208 (0.241)	909/3 255 (0.218)	0.382	1.14	0.853-1.513
	All migraine	349	2 082	166/532 (0.238)	909/3 255 (0.218)	0.250	1.12	0.925-1.350
Germany	MO only	837	1 406	396/1 278 (0.240)	629/2 183 (0.224)	0.3206	1.08	0.932-1.241
	MO only *	837	541	396/1 278 (0.240)	218/864 (0.201)	**0.0307**	1.23	1.019-1.480
Meta-analysis								
	All MA only	1 421	49 403	727/2 109 (0.256)	20 320/78 464 (0.206)	6.98×10^{-8}	1.29	1.173-1.408
	All Both MA,MO	2 768	49 403	1 340/4 176 (0.243)	20 320/78 464 (0.206)	1.09×10^{-5}	1.17	1.089-1.248
	All MO only	1 744	37 980	810/2 678 (0.232)	15 403/60 557 (0.203)	0.0105	1.12	1.028-1.230
	All migraine	5 950	50 809	2 885/8 987 (0.243)	20 949/8 0647 (0.206)	$\mathbf{1.60 \times 10^{-11}}$	1.18	1.127-1.244

UKPMC Funders Group Author Manuscript

Genome-wide significant values and successful replications in bold.

*The German replication control set consists of several small samples. The largest of these had a considerably deviating MAF (0.238, n=865) compared to other German (average MAF 0.216, n=3,260) and Central European control sets (average MAF 0.212, n=9,560). Thus values with both including and excluding the outlier control sample are presented. The meta-analysis value includes all control samples (without outlier control group, “All MO samples” p-value 0.00107, OR 1.18 [1.068-1.298] and “All migraine” $\mathbf{8.43 \times 10^{-13}}$, OR 1.20 [1.143 - 1.264]. Values on line denoted by * are calculated after excluding the outlier control sample.

UKPMC Funders Group Author Manuscript

Table 3

Association of rs1835740 genotype with gene expression levels.

SNP	Gene	Strand	SNP coordinate	Gene start	Distance	SRC p-value
rs1835740	*UQCRB*	−	98 236 089	97 311 911	924 178	0.0013226
rs1835740	*MTDH/AEG-1*	+	98 236 089	98 725 583	489 494	**0.0000396***
rs1835740	*HRSP12*	−	98 236 089	99 183 743	947 654	0.0028748

Genes with nominal or higher p-values of expression association to rs1835740 genotype in the Spearman rank correlation test are shown.

* indicates surpassing the significance threshold 7.7×10^{-5} (corresponding to a 0.001 permutation threshold after 10,000 permutations). SRC = Spearman rank correlation. Gene start refers to the location of 5′ end of the gene, if on positive strand, 3′ end if on negative. Locations and distances in basepairs, according to NCBI build 36. P-values in bold surpass the level of genome-wide significance.