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

V. Anttila[1,2,*], H. Stefansson[3], M. Kallela[4], U. Todt[5,6], G. M. Terwindt[7], M. S. Calafato[1,8], D.R. Nyholt[9], A.S. Dimas[1,10,11], T. Freilinger[12], B. Müller-Myhsok[13], V. Artto[4], M. Inouye[1,14], K. Alakurtti[1,2], M.A. Kaunisto[2,15], E. Hämäläinen[1, 2], B. de Vries[14], A.H. Stam[7], C.M. Weller[14], A. Heinze[16], K. Heinze-Kuhn[16], I. Goebel[5,6], G. Borck[5,6], H. Göbel[16], S. Steinberg[3], C. Wolf[13], A. Björnsson[3], G. Gudmundsson[17], M. Kirchmann[18], A. Hauge[18], T. Werge[19], J. Schoenen[20], J.G. Eriksson[15,21,22,23], K. Hagen[24], L. Stovner[24], H.-E. Wichmann[25,26,27], T. Meitinger[28,29], M. Alexander[30,31], S. Moebus[32], S. Schreiber[33], Y. S. Aulchenko[34], M.M.B. Breteler[34], A.G. Uitterlinden[35], A. Hofman[34], C. M. van Duijn[34], P. Tikka-Kleemola[36], S. Vepsäläinen[4], S. Lucae[13], F. Tozzi[37], P. Muglia[37,38], J. Barrett[1], J. Kaprio[2,23,39], M. Färkkilä[4], L. Peltonen[1, 2,40],*, K. Stefansson[3], J.-A. Zwart[23,41], M.D. Ferrari[7], J. Olesen[18], M. Daly[40], M. Wessman[2,15], A.M.J.M. van den Maagdenberg[7, 14], M. Dichgans[12], C. Kubisch[5,6,42], E.T. Dermitzakis[11], R.R. Frants[14], A. Palotie[1,2, 40] on behalf of the International Headache Genetics Consortium

** A full list of author affiliations appears at the end of the paper.*

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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 ($P = 5.38\times10^{-9}$, odds ratio = 1.23, 95% CI 1.150–1.324) in a genome-wide association study of 2,731 migraine cases ascertained 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 1.69×10^{-11} (odds ratio = 1.18, 95% CI 1.127–1.244). rs1835740 is located between *MTDH* (astrocyte elevated gene 1, also known as AEG-1) and *PGCP* (encoding plasma glutamate carboxypeptidase). In an expression quantitative trait study in lymphoblastoid cell lines, transcript levels of the MTDH were found to have a significant correlation to rs1835740 ($P = 3.96\times10^{-5}$, permuted threshold for genome-wide significance 7.7×10^{-5}). To our knowledge, our data establish rs1835740 as the first genetic risk factor for migraine.

Introduction

The recent boom of genome-wide association studies (GWAS) has had a major impact on our current view of genetic susceptibility to common traits and complex disorders. However, central nervous system disorders are under-represented among the conditions for which such associations have been found[1]. To our knowledge, no GWAS or common, robustly established linked genetic variants have been reported for major episodic neurological disorders (ICD-10 codes G40–G44, migraine, epilepsy and ataxias). However, there is substantial genetic information for rare Mendelian forms of migraine, epilepsy and ataxia, which classifies 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] in the European population. Migraine ranks among the 20 most disabling diseases and has been estimated as the most costly neurological disorder, with a considerable impact on public health[6]. Clinically, the International Classification of Headache Disorders (ICHD-II[7]) recognizes two main common forms of migraine: migraine with aura and migraine without aura. The two forms are distinguished from each other based on the presence of aura, a period of variable and diverse neurological symptoms that precede the headache phase. Individuals may have attacks of only migraine without aura, or only migraine with aura, or they may have a combination of both types in variable proportions. There is debate among the scientific community whether migraine with aura and migraine without aura attacks represent two distinct disorders or if they are merely variations of a single disease having 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, 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 GWAS in seven European migraine case collections (six clinic-based and one population-based) (Supplementary Fig. 1). In the discovery stage, we studied 3,279 migraineurs (1,124 Finnish, 1,276 German and 879 Dutch individuals) recruited from headache clinics and genotyped using Illumina arrays against population-matched controls (10,747 individuals) recruited from preexisting population-based GWAS (Supplementary Note). In the replication stage, a further 3,202 cases and 40,062 population-matched controls from Iceland, Denmark, The Netherlands and Germany were studied.

Results

Diagnoses were made by headache experts using a combination of questionnaires and individual interviews that were based on the ICHD-II guidelines[7]. Due to the overlap between individuals having migraine with aura and those having migraine without aura, we analyzed the following diagnostic subgroups: (i) 'all migraine', defined as all individuals with migraine irrespective of subtype; (ii) 'migraine with aura only', defined as individuals who only have attacks where aura is present; (iii) 'both migraine with aura and migraine without aura', defined as individuals with attacks both with and without aura; and (iv) 'migraine without aura only', defined as individuals with only attacks of migraine without aura.

We used a multipopulation Cochran-Mantel-Haenszel (CMH) association analysis and a significance threshold of $P \leq 5\times10^{-8}$ in our analyses. In the discovery sample, 2,731 cases and 10,747 controls (Table 1) passed quality control steps, and 429,912 markers were successfully genotyped (Online Methods). A quantile-quantile plot of the CMH analysis (Supplementary Fig. 2) and an overall inflation factor (λ) of 1.08 were used as final quality control measures.

Table 1 *Study populations used in the two stages of the study*

		Total	Men (%)	Women (%)	Individuals with both MA and MO (%)	Individuals with MA only (%)	Individuals with MO only (%)
Discovery stage							
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	2,731	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	Cases	5,933	19.0	81.0	54.4	23.7	22.1
meta-analysis	Controls	50,809	53.2	46.8	–	–	–

MA, migraine with aura; MO, migraine without aura.

Only one marker, rs1835740 on chromosome 8q22.1, showed significant association with migraine in the multipopulation CMH analysis (Fig. 1 and Supplementary Fig. 3). Eleven further loci were found with $P \leq 5\times10^{-5}$ (Supplementary Table 1). The minor allele (A) of marker rs1835740 was associated with migraine with $P = 5.38\times10^{-9}$ and odds ratios ranging between 1.21 and 1.33 (Table 2). Two nearby markers with the highest linkage disequilibrium (LD) to rs1835740 (rs982502, $r^2 = 0.59$, $P = 1.34\times10^{-4}$ and rs2436046, $r^2 = 0.69$, $P = 1.78\times10^{-5}$) also showed association with migraine (Supplementary Table 2). Haplotype analysis detected a 27-kb haplotype ($P = 5.35\times10^{-8}$) (Supplementary Fig. 4 and Supplementary Table 3). In the HapMap Phase II data[11], the variant is located between two close recombination hotspots, and analysis using the ssSNPer program[12] demonstrated that no long-range LD to rs1835740 exists within a 5-Mb window, strongly suggesting that the causative variant in this region is tagged by the minor allele of rs1835740 (Fig. 1). The 2-Mb window around rs1835740 was also imputed against the 1000 Genomes data (August 2009 release), but no other marker showed evidence of association exceeding that for rs1835740 (Fig. 1). 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 between 1.5% and 2.5%, depending on the heritability estimate used, and the population attributable risk was estimated to be 10.7% using previous methodology[13].

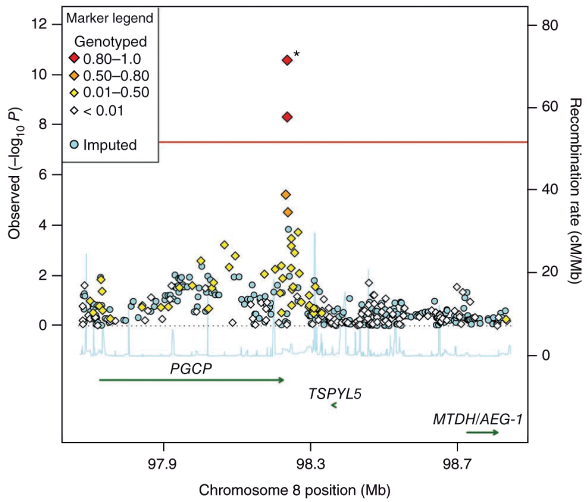

Figure 1 *Cochran-Mantel-Haenszel association results for combined analysis of the three study populations between 97.5 Mb and 99.0 Mb on chromosome 8q22.1. Diamonds show the position and P value for each marker in the region, with colors representing the extent of linkage disequilibrium (measured in r^2) with the marker rs1835740, and blue circles indicate the locations and P-values of the imputed markers. For rs1835740, P-values are shown for both the original GWAS 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]. The red line denotes the threshold for genome-wide significance ($P \leq 5\times10^{-8}$). This figure was generated using a modified version of the script available at http://www.broadinstitute.org/node/555.*

To confirm and extend our results, we performed a replication study on the only marker with genome-wide significance in the discovery stage: rs1835740. The diagnostic subgroups used in the discovery stage were also applied to the replication stage. Replication was successful in two 'migraine with aura only' subsets (Danish, *P* = 0.015, OR = 1.29 and Icelandic, *P* = 0.038, OR = 1.36), in the Icelandic 'migraine without aura' set (*P* = 0.0292, OR = 1.18) and 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 'both migraine with aura and migraine without aura' group (OR = 0.99). The effect was consistently stronger in the 'migraine with aura only' groups than other migraine subgroups (Fig. 2). It should be noted that the majority of the groups that did not reach formal replication were small and had limited power. Meta-analysis was conducted using the CMH test for each diagnosis subgroup alone as well as for all migraine samples together, with the latter group showing a final $P = 1.69\times10^{-11}$ (Table 2).

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)
GWAS							
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)
Discovery stage							
	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	2,731	10,747	1,384/4,052 (0.255)	4,637/16,385 (0.216)	**5.38×10^{-9}**	1.23 (1.150–1.324)
Replication stage							
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	1,116	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[a]	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 "All migraine"	5,933	50,809	2,877/8,963 (0.243)	20,949/80,647 (0.206)	**1.69×10^{-11}**	1.18 (1.127–1.244)

MA, migraine with aura; MO, migraine without aura. Genome-wide significant values and successful replications are shown in boldface.
[a]Values in this row were calculated after excluding an outlier control sample. The German replication control set consisted of several small samples. The largest of these had a considerably deviating minor allele frequency (MAF) (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 in the case allele and control allele columns. The meta-analysis value includes all control samples (without the outlier control group, "all migraine without aura samples," P = 0.00107, OR = 1.18, 95% CI 1.068–1.298 and "all migraine samples," $P = 8.43 \times 10^{-13}$, OR = 1.20, 95% CI 1.143–1.264.

Marker rs1835740 is located between two potentially interesting candidate genes, *MTDH* and *PGCP*. We analyzed the effect of this marker's genotype on the expression of genes within a 2-Mb window in fibroblasts, primary T cells and lymphoblastoid cell lines (LCL) obtained 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 gene in LCLs (Table 3 and

Supplementary Table 4), with the risk allele A being associated with higher expression levels (Fig. 3). This is in line with previous studies, which have proven that expression analyses in LCL cells are informative in neurological and neuropsychiatric traits[15–17]. No significant association was detected in fibroblasts or primary T cells. The eQTL analysis suggested that rs1835740 is a cis regulator of MTDH in LCLs.

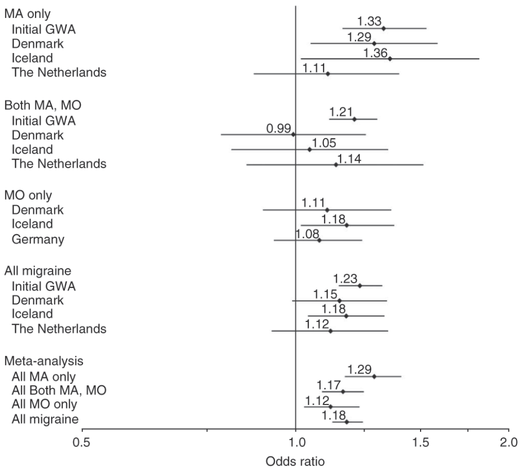

Figure 2 *For each dataset, the horizontal line indicates the 95% CI, and the number above the line indicates the point estimate of the odds ratio. MA only, individuals whose attacks are always accompanied with aura; both MA, MO, individuals with attacks with and without aura; MO only, individuals whose attacks never include aura.*

Table 3 *Association of rs1835740 genotype with gene expression levels*

SNP	Gene	Strand SNP	coordinate	Gene start	Distance	SRC *P*
rs1835740	UQCRB	–	98,236,089	97,311,911	924,178	0.0013226
rs1835740	MTDH	+	98,236,089	98,725,583	489,494	0.0000396[a]
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.
[a]This value surpassed the significance threshold 7.7×10^{-5} (corresponding to a 0.001 permutation threshold after 10,000 permutations). Gene start refers to the location of 5′ end of the gene if on the positive strand and the 3′ end if on the negative strand. Locations and distances are given in base pairs and are according to NCBI build 36. SRC, Spearman rank correlation.

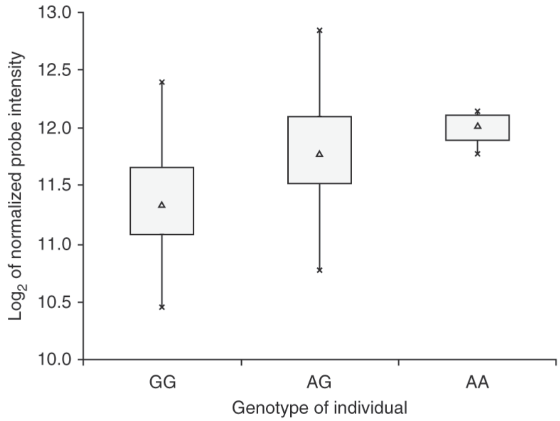

Figure 3. *A box-plot of the quantified expression values for MTDH/AEG-1, ordered based on sample genotype of rs1835740. Normalised expression levels in lymphoblastoid cell lines using Illumina's WG-6 v3 Expression BeadChip array shown. In each group, the small pyramid indicates median value, the shaded area represents the lower and upper quartiles, and the crosses show the minimum and maximum values in the expression data.*

Discussion

The location of the associating sequence variant, rs1835740, between two genes involved in glutamate homeostasis, *PGCP* and *MTDH*, suggests that this region contains elements that could regulate either or both of these flanking genes; the eQTL analysis pointed to the latter. Although *MTDH* has mainly been studied in relation to carcinogenesis[18], previous studies in cultured astrocytes have shown that *MTDH* downregulates *SLC1A2* (also known as EAAT2 and GLT-1)[18–22], the gene encoding the major glutamate transporter in the brain. Furthermore, knock-out mice lacking the EAAT2 protein from their brains have been shown to suffer from lethal spontaneous epileptic seizures[23]. Despite the limitations in extrapolating eQTL findings from LCL cells directly to brain tissue, these data suggest 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 downregulation of EAAT2 or an increase in PGCP activity (or both) would provide a 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 cortical spreading depression, 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].

Neither this study nor our previous study[3] yielded 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 of paroxysmal neurological disorders remains open. Interestingly, previous studies suggested that the imbalance of glutamate release and clearance is a key component of the pathogenesis of FHM; the underlying mutation in FHM lies 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 the pathogenetic mechanisms of more common forms of episodic neurological disorders, such as migraine. Alterations in the functionally related EAAT1 transporter have been identified in other episodic phenotypes (such as episodic ataxia 6 (ref. 32) and a phenotype with episodic ataxia, hemiplegia and seizures[33]), providing an example of the link between EAAT transporters and episodic disorders. Future studies should be conducted to specifically test this hypothesis.

In summary, to our knowledge, 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 possibly also a somewhat different underlying combination of genetic susceptibility variants. The effect of rs1835740 is stronger in individuals with migraine with aura than in those with migraine without aura, but further studies are needed to confirm the role of the variant in different migraine subgroups. This variant explains only a small fraction of the overall genetic variance in migraine, and future GWAS, perhaps with different ascertainment schemes, will likely identify additional loci explaining more of the genetic variance.

Methods

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

Note: Supplementary information is available on the Nature Genetics website.

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Affiliations

[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 National Health Service 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]Institute for Stroke and Dementia Research, Klinikum der Universität München, Munich, Germany. [14]Max Planck Institute of Psychiatry, Munich, Germany. [15]Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands. [16]Folkhälsan Research Center, Helsinki, Finland. [17]Kiel Pain and Headache Center, Kiel, Germany. [18]Department of Neurology, Landspítali University Hospital, Reykjavik, Iceland. [19]Department of Neurology, Glostrup Hospital and the Danish Headache Center, Glostrup, Denmark. [20]Research Institute of Biological Psychiatry, University of Copenhagen, Roskilde, Denmark. [21]Headache Research Unit, Department of Neurology and Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA)-Neurosciences, Liège University, Liège, Belgium. [22]Department of General Practice, Helsinki University Central Hospital, Helsinki, Finland. [23]Vaasa Central Hospital, Vaasa, Finland. [24]National Institute for Health and Welfare, Helsinki, Finland. [25]Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway. [26]Institute of Epidemiology, Helmholtz Center Munich, Neuherberg, Germany. [27]Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie, Ludwig-Maximilians-Universität München, Munich, Germany. [28]Klinikum Großhadern, Ludwig-Maximilians-Universität München, Munich, Germany. [29]Institute of Human Genetics, Helmholtz Center Munich, Neuherberg, Germany. [30]Institute of Human Genetics, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany. [31]Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany. [32]Institute of Human Genetics, University of Bonn, Bonn, Germany. [33]Institute of Medical Informatics, Biometry and Epidemiology, University Hospital of Essen, University Duisburg-Essen, Essen, Germany. [34]Department of Clinical Molecular Biology, Christian Albrechts University, Kiel, Germany. [35]Department of Internal Medicine I, Christian Albrechts University, Kiel, Germany. [36]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. [37]Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands. [38]Research Program in Molecular Medicine, University of Helsinki, Helsinki, Finland. [39]Drug Discovery, GlaxoSmithKline Research and Development, Verona, Italy. [40]Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada. [41]Department of Public Health, University of Helsinki, Helsinki, Finland. [42]The Broad Institute of MIT and Harvard, Boston, Massachusetts, USA. [43]Department of Neurology, Oslo University Hospital and University of Oslo, Oslo, Norway. [44]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany. [45]Institute of Human Genetics, University of Ulm. [46]Department of Medical Genetics, University of Helsinki, Helsinki, Finland. [47]Department of Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland. [48]Deceased.

Online Methods

Study design

We jointly analyzed samples from three migraine with aura collections from Finland, Germany and The Netherlands with population-matched controls obtained from preexisting studies. This discovery phase was followed by a replication study of the top SNP, rs1835740, in samples from individuals with migraine 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.

Discovery stage genotyping

DNA was extracted from the subjects' blood samples using standard methods. Genotyping of the GWAS samples was done at the Wellcome Trust Sanger Institute on the Illumina 610K (for the Finnish and German samples) and the Illumina 550K (for the Dutch samples) SNP microarrays following the Infinium II protocol from the manufacturer (Illumina Inc.). Genotype calling was performed using the Illuminus software[34].

Replication stage genotyping

For the replication study, all Danish cases and 459 migraine-free controls were genotyped using the Centaurus platform (Nanogen Inc.), and 904 additional controls were genotyped at deCODE genetics using the Illumina HumanHap650 BeadArray. 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) at Leiden University Medical Center. The German replication cases were genotyped using Illumina HumanHap 610K array at the Institute of Human Genetics at the Helmholtz Zentrum, Munich.

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 for the expression study. Sample collection was performed on full-term or near-full-term pregnancies to ensure homogeneity for sample source age. Three cell types were derived: (i) primary fibroblasts, (ii) LCLs and (iii) primary T cells[14]. Total RNA was extracted from these cells and two one-quarter-scale MessageAmp 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's expression values. DNA samples were extracted from umbilical cord tissue LCLs with the Puregene cell kit (Gentra-Qiagen), and genotyping was performed using the Illumina 550K SNP array (Illumina Inc.) to obtain the SNP genotypes for the samples.

Statistical analysis of the genome-wide scan data

Stringent per-SNP and per-sample limits were implemented in order to obtain high-quality data. Quality control measures were as follows: 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 $\hat{\pi}$ >12.5%. From the initial 3,279 cases and 12,369 controls, 2,731 cases and 10,747 controls passed all quality control criteria, and 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 inflation factor and an overall genomic inflation factor (λ) were estimated using the median $\chi 2$ value from a 1 degree-of-freedom allelic $\chi 2$ test. For the Finnish samples, $\lambda = 1.05$; for the German samples, $\lambda = 1.07$; for the Dutch samples, $\lambda = 1.09$; and the overall $\lambda = 1.08$, suggesting reasonably well matched controls in each case. Differences between cases and controls were assessed between each SNP and disease status using a two-tailed CMH test for 2 × 2 × K stratified data (where 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 $P < 10^{-3}$ (Table 2 and Fig. 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, 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.

Meta-analysis of discovery and replication samples

The CMH test was used for the 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 $\chi 2$ 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 the recommended options[39]. Haplotypes from the 1000 Genomes Project (August 2009 release) and haplotypes from HapMap Phase 3 were used as reference panels.

eQTL analysis

Association between genotypes and expression was analyzed using 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 the minimum P values from each of 10,000 permutations of the expression values relative to genotypes[35].

URLs

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

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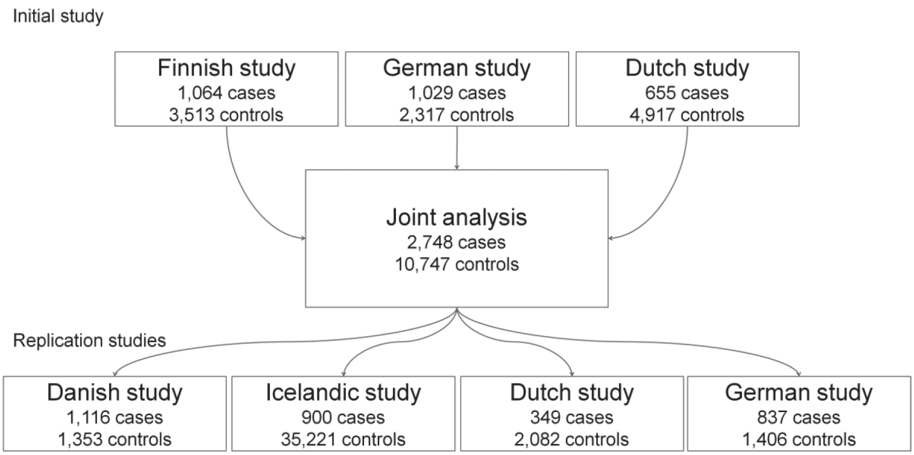
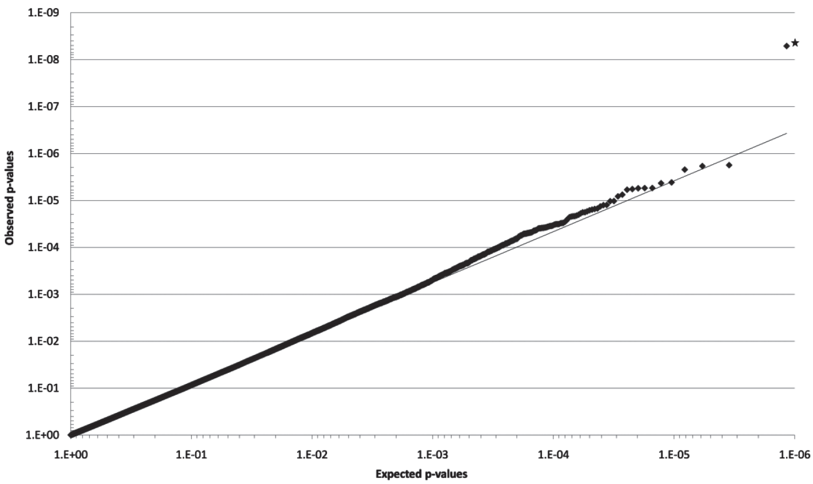
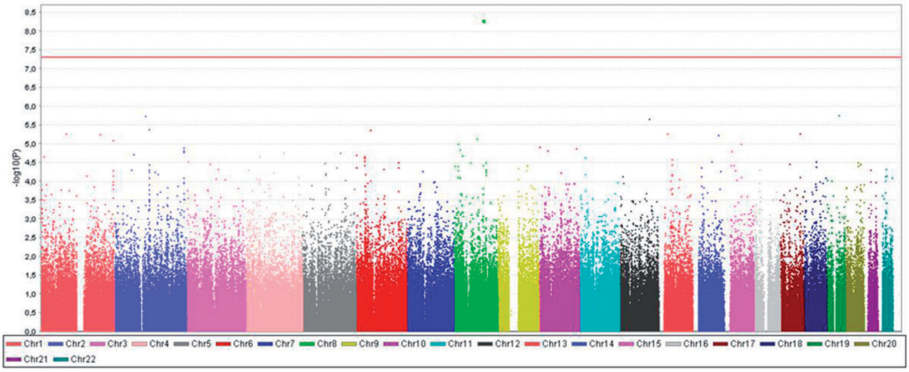
Supplementary Material



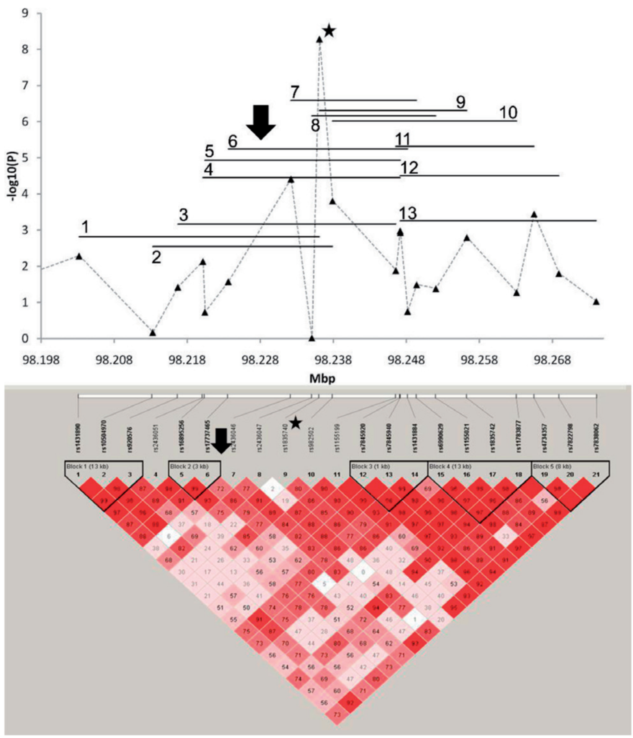
Supplementary Figure 1. *Study design. In the initial study, migraine with aura (MA) patients from three clinic-based collections were analyzed in a joint genome-wide association analysis. The most significant association signal was replicated in an independent Danish clinic-based sample and an Icelandic population-based sample, containing MA and migraine without aura (MO) samples, as well as in a German clinic-based MO-specific sample.*



Supplementary Figure 2. *Quantile-quantile plot of the results in the Cochran-Mantel-Haenszel analysis Asterisk denotes marker rs1835740. Black line represents the distribution of p-values under the null given study inflation factor lambda of 1.08.*



Supplementary Figure 3. *Genome-wide Cochran-Mantel-Haenszel results for association between each marker and migraine with aura in the combined analysis of the three initial study populations. Red line denotes the threshold of genome-wide significance ($p \leq 5{\times}10^{-8}$). Only marker rs1835740 on 8q22.1 exceeded this threshold.*



Supplementary Figure 4. *Nine SNP sliding window haplotype analysis and local haplotype structure around marker rs1835740 on chromosome 8q22.1. In the upper part of the figure, the black pyramids show single-marker association results for each marker. The horizontal lines show the length and overall P-values for the nine marker sliding windows in the haplotype analysis. The lower part of the figure shows the Haploview D' matrix in the GWA study analysis data, with estimated LD blocks using the Gabriel et al. method1. Black stars denote the location of rs1835740 and the black arrows denote the 3' end of PGCP in either part of the figure*

Supplementary tables

Supplementary Table 1. *Association signals with $p \leq 5\times10^{-5}$ and with multiple nearby associating SNPs*

SNP	Chr	Location	*P*-value	OR	95% CI	Location	Gene
rs12084862	1	244269837	8.20×10^{-6}	1.17	1.09-1.25	intragenic	*SMYD3*
rs17528324	2	118572626	4.13×10^{-6}	1.27	1.15-1.41	intragenic	*INSIG2*
rs17862920	2	234492734	1.26×10^{-5}	0.776	0.693-0.870	intragenic	*TRPM8*
rs2038761	6	2625766	2.02×10^{-5}	0.865	0.809-0.925	intragenic	*MYLK4*
rs6456880	6	29071227	2.18×10^{-5}	0.873	0.819-0.929	intragenic	*ZNF311*
rs7753655	6	49644523	4.29×10^{-6}	0.852	0.796-0.912	intergenic	-
rs10888075	8	13804790	1.04×10^{-5}	1.21	1.11-1.31	intergenic	near *SGCZ*
rs10111769	8	21003036	1.49×10^{-5}	1.15	1.08-1.23	intergenic	-
rs2042600	11	19709275	2.28×10^{-5}	0.876	0.824-0.932	intragenic	*NAV2*
rs3794331	13	44951545	2.70×10^{-5}	1.28	1.14-1.43	intragenic	*COG3*
rs473422	15	56453633	1.03×10^{-5}	0.864	0.820-0.922	intergenic	near *AQP9*

Footnote: Locations and distances in basepairs, according to NCBI build 36. Only the SNP with the lowest p-value is reported for each locus.

Supplementary Table 2. *Conditional analyses for the two SNPs with moderate linkage disequilibrium to rs1835740 in chromosome 8q22.1*

Chr	SNP A	SNP B	r2	SNP A *P*-value	SNP B *P*-value	SNP B given A
8	rs1835740	rs2436046	0.69	5.12×10^{-9}	1.78×10^{-5}	0.892
8	rs1835740	rs982502	0.59	5.12×10^{-9}	1.34×10^{-4}	0.4

Supplementary Table 3. *Nine SNP sliding window haplotype analysis on the chromosome 8q22.1 associated region from Supplementary Figure 2*

Haplotype	First SNP	Last SNP	Chi-sq.	D.f.	Overall *P*-value
1	rs1431890	rs1835740	43.07	16	2.730×10^{-04}
2	rs10504970	rs982502	43.10	17	4.643×10^{-04}
3	rs920576	rs1155199	41.37	13	8.291×10^{-05}
4	rs2436051	rs7845920	48.48	14	1.093×10^{-05}
5	rs16895256	rs7845940	47.68	12	3.553×10^{-06}
6	rs17737465	rs1431884	46.52	10	1.156×10^{-06}
7	**rs2436046**	**rs6990629**	**51.62**	**9**	$\mathbf{5.327 \times 10^{-08}}$
8	rs2436047	rs1155021	48.93	10	4.196×10^{-07}
9	rs1835740	rs1835742	53.46	10	6.107×10^{-08}
10	rs982502	rs11783877	45.23	8	3.327×10^{-07}
11	rs1155199	rs4734357	41.91	8	1.410×10^{-06}
12	rs7845920	rs7822798	39.34	9	9.995×10^{-06}
13	rs7845940	rs7838062	32.98	8	6.208×10^{-05}

The nine SNP window in bold is the one referred to in the text. N.B. haplotype value shown in text is for the single haplotype, above values for the association of the whole haplotype distribution.

Supplementary Table 4. *SNPs with nominal or higher p-values for association with expression levels of MTDH/AEG-1*

SNP	Gene SNP	coordinate	Gene start	Distance	SRC *P*-value
rs11783750	*MTDH/AEG-1*	98 865 219	98 725 583	139 636	0.0018741
rs10105830	*MTDH/AEG-1*	98 307 895	98 725 583	417 688	0.0004235
rs1835740	*MTDH/AEG-1*	98 236 089	98 725 583	489 494	**0.0000396***
rs7845920	*MTDH/AEG-1*	98 247 132	98 725 583	478 451	0.0014652

*Footnote: * indicates surpassing the significance threshold 7.7×10^{-5} (corresponding to a 0.001 permutation threshold after 10,000 permutations). SRC = Spearman rank correlation. Locations and distances in basepairs, according to NCBI build 36. Numbers in bold are statistically significant.*

Supplementary Note: Clinical subject ascertainment and control samples

Ethical aspects

Written informed consent was obtained from all participants, and the study was approved by the respective local research ethics committees of the Helsinki University Central Hospital, Pain Clinic Kiel in Kiel, the Department of Neurology at Klinikum Großhadern, Ludwig-Maximilians-University in Munich, and the University of Leiden Medical Centre. Informed consent was obtained from all patients.

Initial study

The initial genome-wide association study consisted of three patient samples, collected from headache clinics in Finland, Germany and the Netherlands.

In Finland, 1,124 Finnish migraine with aura (MA, and MA/MO) patients were recruited. Each patient belongs to a multigenerational family with at least three family members with migraine. Patients were examined by a neurologist, and fulfilled the validated Finnish Migraine Specific Questionnaire for Family Studies ($FMSQ_{FS}$[2]). In cases of insufficient or conflicting information, a follow-up interview was conducted by telephone. All patients were diagnosed by the same headache specialist (M. Kallela) according to the current International Headache Society diagnostic criteria (ICHD-II)[3].

In Germany, patient recruitment was done at two sites, in Kiel and in Munich. At the Pain Clinic in Kiel, a total of 994 German MA and MA/MO patients were recruited to a patient collection maintained at the Universities of Bonn and Cologne. All patients were diagnosed according to the ICHD-II[3] by headache specialists[4]. The detailed migraine anamnesis was obtained either by face-to-face interviews or by telephone interviews standardized by using a comprehensive migraine questionnaire. The second German set of 282 MA and MA/MO cases were recruited and examined by a headache specialist at the Klinikum Großhadern of the Ludwig-Maximilians-University, Munich. Phenotyping was based on a German translation of the $FMSQ_{FS}$[2]. Whenever the information was insufficient or conflicting, an additional telephone interview was performed. Information was obtained on all aspects of the ICHD-II[3] criteria as well as on other aspects (such as age at onset, prodromal symptoms, triggers, acute and prophylactic medication, family history, general past medical history, co-morbidity and place of birth).

In the Netherlands, 879 MA and MA/MO patients were available from the clinic-based Leiden University Migraine Neuro Analysis (LUMINA) study. Self-reported migraineurs were recruited via the project's website. A set of screening questions validated previously in a population-based study[5] was used first. Participants fulfilling the screening criteria completed then the extended questionnaire focusing on signs and symptoms of migraine headache and aura as outlined in ICHD-II[3]. Individual diagnoses were made using an algorithm based on these criteria. The algorithm diagnosis was validated by a semi-structured telephone interview performed by experienced study physicians or by well-trained medical students. Specific attention was paid to migraine aura. A subset of the patients was asked to participate upon visiting the outpatient clinic.

Replication studies

The replication phase of the study consisted of four separately recruited migraine patient samples from Denmark, Iceland, the Netherlands and Germany.

The Danish replication sample comprised 825 MA subjects of which 776 were successfully genotyped. Of these, 483 patients suffered from only MA attacks and 293 from both MA and MO attacks. Patients were selected from the Danish National Patient Register and from case files from neurological clinics, 1,365 took part in a screening telephone interview. If the proband was diagnosed with MA, the proband and selected relatives were diagnosed according to the ICHD-I[6] in a validated telephone interview (M. Kirchmann or A.H.). 305 Danish MO patients were selected from case files at the Danish Headache Center and diagnosed as mentioned above (ICHD-II[3]) in an extensive semi-structured telephone interview performed by trained physicians. In addition 81 MO subjects were identified during recruitment of the MA families. Thus, 386 MO patients were recruited and 340 successfully genotyped.

The Icelandic replication samples were recruited from three sources: first, a list of patients provided by two neurologists (401 potential participants), second, responses to an advertisement in the newsletter of the Icelandic Migraine Society (137 participants), and third, responses to a brief screening questionnaire mailed to a random sample of 20,000 Icelanders, aged 18–50 years and living in the Reykjavik area. All Icelandic recruits were asked to answer the comprehensive validated deCODE Migraine Questionnaire 2 or 3 (DMQ2 or DMQ3[7]). The questionnaire was designed based on ICHD-II3. The reliability of the MA and MO diagnoses based on the DMQ3 was assessed using a physician-conducted interview as an empirical index of validity. In total 1,612 subjects reporting five or more headache attacks were genotyped. Of them, 712 subjects reported atypical symptoms, preventing reliable IHS classification through questionnaire data only, and were excluded from the analysis. In total, the Icelandic sample consists of 567 MO patients, and 333 MA patients either with or without the MO attacks.

The German replication cohort includes 837 MO cases from the Department of Neurology of the Ludwig-Maximilians-University, Munich, Germany. Phenotyping followed the same protocol as described for the Munich patient sample. The Dutch replication sample includes 356 Dutch MA or MA/MO patients that were recently recruited through the clinic-based Leiden University Migraine Neuro Analysis (LUMINA) study. The diagnosis and classification followed the same procedure as in the initial Dutch sample. Nature

Control samples

Population-matched control samples were obtained from previously genotyped studies (for links to studies, see URL section of Online Methods). 1,881 Finnish controls originated from the Helsinki Birth Cohort study[8] and 2,173 controls from the Health2000 study, genotyped on the Illumina 660K or 610K platforms. 840 German controls were obtained from the KORA S4/F4 study[9], 380 controls from the HNR study[10] and 677 from PopGen study[11], all genotyped on the Illumina 550K platform. In addition, 444 controls were obtained from Illumina iControlDB by querying all Caucasian samples genotyped on the Illumina 550K platform on June 30th, 2008 and filtering these samples based on stratification as observed from multidimensional scaling plots of all existing German samples, and keeping only those identified as being of German descent. 974 Dutch controls were obtained from the Rotterdam study I[12], genotyped on the Illumina 550K platform and imputed to cover all markers on the 610K platform. For each replication study, the group providing a replication dataset supplied a matched control cohort; the controls for the Danish and Icelandic replications were provided by deCODE, and German controls were obtained from the MARS study[13] and from GlaxoSmithKline[14] and Rotterdam study III.

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