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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[12,13], Bertram Müller-Myhsok[14], Ville Artto[4], Michael Inouye[1,15], Kirsi Alakurtti[1,2], Mari A Kaunisto[2,16], Eija Hämäläinen[1,2], Boukje de Vries[15], Anine H Stam[7], Claudia M Weller[15], Axel Heinze[17], Katja Heinze-Kuhn[17], Ingrid Goebel[5,6], Guntram Borck[5,6], Hartmut Göbel[17], Stacy Steinberg[3], Christiane Wolf[14], Asgeir Björnsson[3], Gretar Gudmundsson[18], Malene Kirchmann[19], Anne Hauge[19], Thomas Werge[20], Jean Schoenen[21], Johan G Eriksson[16,22–24], Knut Hagen[25], Lars Stovner[25], H-Erich Wichmann[26–28], Thomas Meitinger[29,30], Michael Alexander[31,32], Susanne Moebus[33], Stefan Schreiber[34,35], Yurii S Aulchenko[36], Monique M B Breteler[36], Andre G Uitterlinden[37], Albert Hofman[36], Cornelia M van Duijn[36], Päivi Tikka-Kleemola[38], Salli Vepsäläinen[4], Susanne Lucae[14], Federica Tozzi[39], Pierandrea Muglia[39,40], Jeffrey Barrett[1], Jaakko Kaprio[2,24,41], Markus Färkkilä[4], Leena Peltonen[1,2,42,48], Kari Stefansson[3], John-Anker Zwart[25,43], Michel D Ferrari[7], Jes Olesen[19], Mark Daly[42], Maija Wessman[2,16], Arn M J M van den Maagdenberg[7,15], Martin Dichgans[12,13], Christian Kubisch[5,6,44,45], Emmanouil T Dermitzakis[11], Rune R Frants[15] & Aarno Palotie[1,2,42,46,47] for the International Headache Genetics Consortium

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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 ($P = 5.38 \times 10^{-9}$, 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 ($P = 3.96 \times 10^{-5}$, 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.

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

1

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 meta-analysis	Cases	5,933	19.0	81.0	54.4	23.7	22.1
	Controls	50,809	53.2	46.8	–	–	–

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

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.

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 $P \leq 5 \times 10^{-8}$ 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.

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 $P \leq 5 \times 10^{-5}$ (**Supplementary Table 1**). The minor allele (A) of marker rs1835740 was associated with migraine with $P = 5.38 \times 10^{-9}$ and odds ratios ranging between 1.21 and 1.33 (**Table 2**). Two nearby markers with the highest linkage disequilibrium (LD) to rs1835740 (rs982502, $r^2 = 0.59$, $P = 1.34 \times 10^{-4}$ and rs2436046, $r^2 = 0.69$, $P = 1.78 \times 10^{-5}$) also showed association with migraine (**Supplementary Table 2**). Haplotype analysis detected a 27-kb haplotype ($P = 5.35 \times 10^{-8}$) (**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
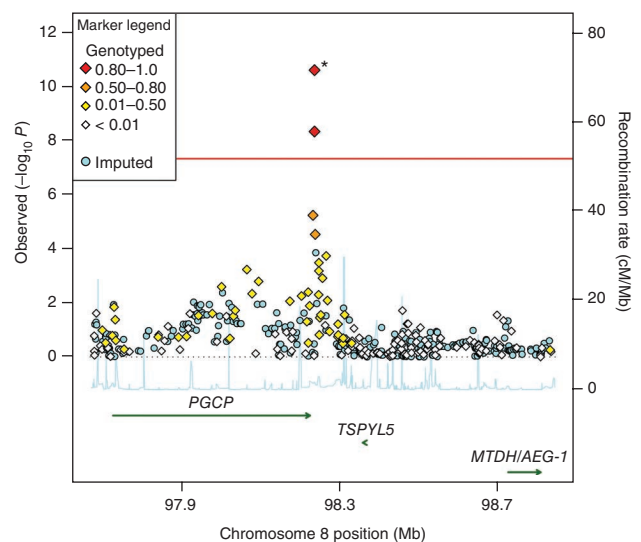
Figure 1 Cochran-Mantel-Haenszel association results for combined analysis of the three study populations between 97.5 Mb and 99.0 Mb on chromosome 8q22.1. Diamonds show the position and *P* value for each marker in the region, with colors representing the extent of linkage disequilibrium (measured in *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 ($P \leq 5 \times 10^{-8}$). This figure was generated using a modified version of the script available at http://www.broadinstitute.org/node/555.

Table 2 Association results for marker rs1835740 using the CMH test

	Diagnosis	n (cases)	n (controls)	Case alleles (MAF)	Control alleles (MAF)	P	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 and 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)	$\mathbf{5.38 \times 10^{-9}}$	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 and 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 and 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 and 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 and 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)	$\mathbf{1.69 \times 10^{-11}}$	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," $\mathbf{P = 8.43 \times 10^{-13}}$, OR = 1.20, 95% CI 1.143–1.264.

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].

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 = 1.69×10^{-11} (**Table 2**).
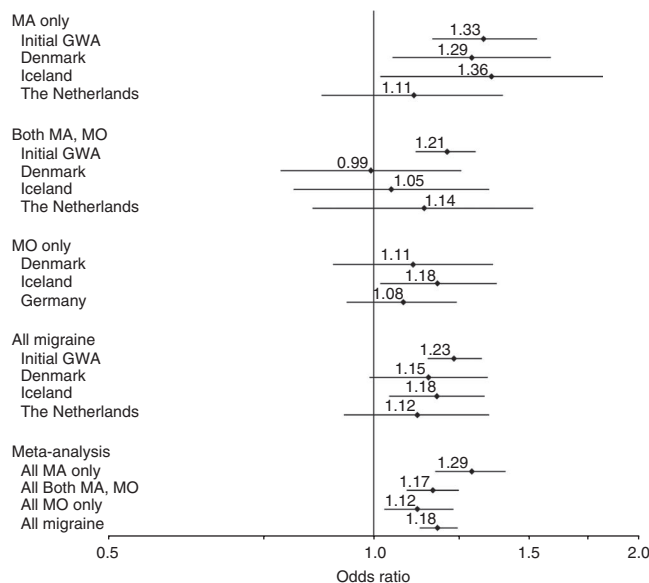
Figure 2 For each dataset, the horizontal line indicates the 95% CI, and the number above the line indicates the point estimate of the odds ratio. MA only, individuals whose attacks are always accompanied with aura; both MA, MO, individuals with attacks with and without aura; MO only, individuals whose attacks never include aura.

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

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.

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.

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].
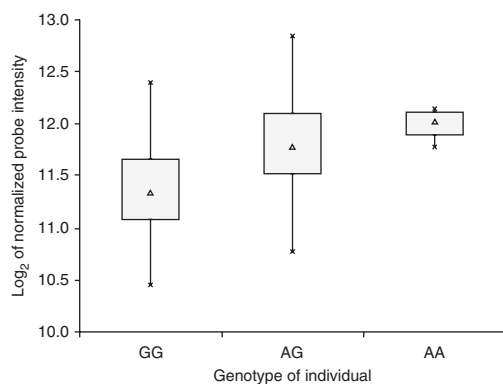

Figure 3 A box-plot of the quantified expression values for *MTDH*, ordered based on the sample genotype of rs1835740. Normalized expression levels in lymphoblastoid cell lines using Illumina's WG-6 v3 Expression BeadChip array are shown. In each group, the small pyramid indicates the median value, the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

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/.

Accession codes. Migraine, OMIM 157300.

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

AUTHOR CONTRIBUTIONS
All authors contributed to the current version of the paper.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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[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. Correspondence should be addressed to V.A. (verneri.anttila@sanger.ac.uk) or A.P. (aarno.palotie@sanger.ac.uk).

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 χ^2 value from a 1 degree-of-freedom allelic χ^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 $2 \times 2 \times K$ 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 χ^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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