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Genome-wide meta-analysis identifies new susceptibility loci for migraine

Migraine is the most common brain disorder, affecting approximately 14% of the adult population, but its molecular mechanisms are poorly understood. We report the results of a meta-analysis across 29 genome-wide association studies, including a total of 23,285 individuals with migraine (cases) and 95,425 population-matched controls. We identified 12 loci associated with migraine susceptibility ($P < 5 \times 10 - 8$). Five loci are new: near *AJAP1* at 1p36, near *TSPAN2* at 1p13, within *FHL5* at 6q16, within *C7orf10* at 7p14 and near *MMP16* at 8q21. Three of these loci were identified in disease subgroup analyses. Brain tissue expression quantitative trait locus analysis suggests potential functional candidate genes at four loci: *APOA1BP*, *TBC1D7*, *FUT9*, *STAT6* and *ATP5B*.

Q6 Recently, significant progress has been made in the identification of common genetic variants associated with migraine susceptibility through genome-wide association studies (GWAS) of clinic-based migraine with aura[1], migraine in the general population[2,3] and Q7 clinic-based migraine without aura[4]. We performed a meta-analysis of 23,285 migraine cases from 29 clinic- and population-based studies (**Fig. 1**, **Supplementary Fig. 1** and **Supplementary Note**). The samples included 5,175 cases from 5 clinic-based collections of affected individuals matched to 13,972 population-based controls (**Supplementary Table 1**), as well as 18,110 cases from 14 population-based studies and 81,453 migraine-free or control individuals from the same studies (**Supplementary Table 2**). Results from GWAS of the five clinic-based collections[1,4] and four of the population-based collections[2,3] have been previously reported (**Supplementary Fig. 2**).

In addition to the primary meta-analysis using all available genotype data, three subgroup analyses were performed in those cohorts where sufficient additional clinical information was available (**Supplementary Table 3**). The first two subgroups consisted of migraine cases fulfilling the International Headache Society diagnostic criteria[5] for either migraine with aura or migraine without aura. The third subgroup included only the clinic-based samples, under the hypothesis that they represented a group of migraineurs more enriched for severe migraines than cases identified from the general population.

Results from the primary meta-analysis and for the 3 subgroups identified 142 SNPs, at a total of 12 loci, that were significantly associated with migraine susceptibility (**Table 1** and **Supplementary Figs. 3** and **4**). Eight of those loci contain SNPs that lie within a known transcript. In addition, 1,168 SNPs at 134 loci (**Supplementary Table 4**) showed suggestive association with migraine when combining the primary analysis and the 3 subgroup analyses. The single most significant *P* value overall was observed for rs11172113 in the primary analysis ($P = 2.69 \times 10^{-19}$; **Fig. 2a** and **Table 1**) in the *LRP1* locus at 12q13.

Five of the 12 genome-wide significant loci were new (near *AJAP1*, near *TSPAN2*, in *FHL5*, in *C7orf10* and near *MMP16*), and seven confirmed previously reported loci associated with migraine (in *PRDM16* (ref. 2), in *MEF2D*[4], in *TRPM8* (refs. 1,2), near *TGFBR2* (ref. 4), in *PHACTR1* (ref. 4), in *ASTN2* (ref. 4) and in *LRP1* (ref. 2)). All seven previously reported loci seen in this study remained significant (all $P < 6.25 \times 10^{-3}$, correcting for eight previously reported loci) in analyses that excluded the samples used in previous reports[1,2,4] (**Supplementary Fig. 5** and **Supplementary Table 5**). Of the newly identified loci, two contain significantly associated SNPs that are located within known transcripts. At 6q16, *FHL5* encodes a transcription factor that regulates the cyclic AMP (cAMP)-responsive elements *CREM* and *CREB*[6], which have a role in synaptic plasticity[7] and memory formation[8]. The associated locus also overlaps *UFL1* (also known as *KIAA0776*), which encodes a hypothetical protein. At 7p14, mutations in *C7orf10* have Q8 been found in phenotypically mild or even clinically asymptomatic forms of glutaric aciduria type III (ref. 9), a rare metabolic abnormality leading to persistent excretion of glutaric acid.

The newly associated loci at 1p36, 1p13 and 8q21 are located outside of known transcripts. At 1p36, rs10915437 is located approximately 500 kb telomeric of *AJAP1* and approximately 300 kb centromeric of a gene cluster encoding the apoptosis-related proteins DFFB and TP73 as well as centrosomal protein CEP104. *AJAP1* is expressed in the brain (**Supplementary Fig. 6**) and has been associated with tumor invasion and the regulation of metalloproteinase activity[10]. At 1p13, rs12134493 is located 87 kb upstream of *TSPAN2*, a member of the tetraspanin family, encoding a cell surface protein that mediates signal transduction events involved in the regulation of cell development, activation, growth and motility[11]. *TSPAN2* has further been shown to act as a regulator of metalloproteinase activity[11]. At 8q21, rs10504861 is located 200 kb telomeric to the matrix metalloproteinase *MMP16*. Members of the metalloproteinase family are widely expressed in human tissues and are involved in the breakdown of extracellular matrix in normal physiological processes. Notably, the protein encoded by *MMP16* (MT-MMP2) cleaves LRP1 (ref. 12), encoded by a previously reported migraine-associated gene[2]. In addition, *MMP16* has recently been shown to be involved in basal NgR1 (Nogo-66 receptor) shedding in cortical neurons, thereby increasing axonal and synaptic plasticity[13].

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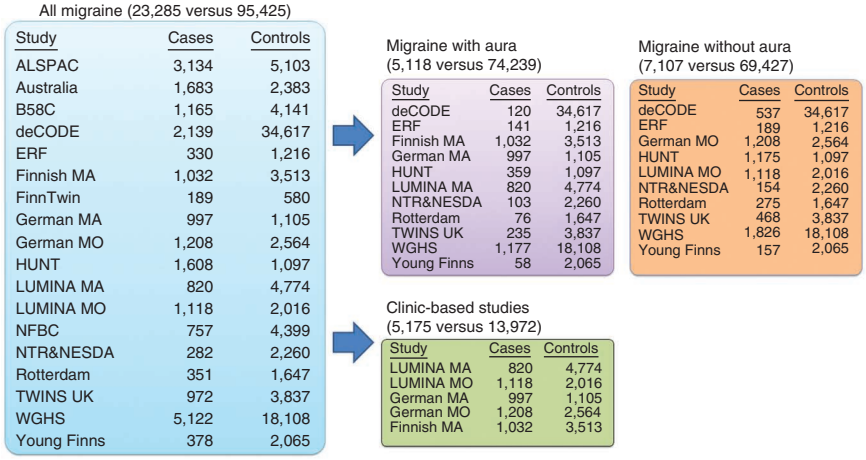

Figure 1 Description of the studies comprising the International Migraine Genetics Meta-analysis Consortium and their sample contributions to each analysis. Each colored box corresponds to one analyzed phenotype and lists the total number of cases and controls, as well as the sample contributions of individual cohorts. Participation in each analysis depended on the availability of the data in question and the recruitment method.

Four of the 12 migraine-associated loci (near *AJAP1*, near *TGFBR2*, in *PHACTR1* and near *MMP16*), including 2 of the new associations, were identified exclusively in the subgroup analyses (**Table 1** and **Supplementary Table 4**). Two of the loci (rs9349379 in *PHACTR1* at 6p21 and rs10504861 near *MMP16* at 8q21) had associations that reached genome-wide significance only in migraine without aura, whereas no SNPs had signals that reached genome-wide significance in migraine with aura (**Fig. 2c**,**d**). The lowest *P* value in migraine with aura was observed for rs7015657 ($P = 7.88 \times 10^{-8}$), which is located approximately 582 kb downstream of *GFRA2*, a member of the glial cell line–derived neurotrophic factor family.

A similar subgroup analysis was performed in only those samples that originated from specialized migraine clinics. Two loci with suggestive associations in the primary analysis, rs6790925 (near *TGFBR2*) and rs6478241 (in *ASTN2*), reached genome-wide significance in the clinic-based subgroup (**Table 1** and **Supplementary Tables 6** and **7**). All 12 of the genome-wide significant loci associated with migraine showed larger estimated effect sizes in the clinic-based subgroup compared to the primary analysis of all samples (**Supplementary Table 6** and **7**). A two-tailed binomial test showed that the chance of observing larger effects at all 12 loci was significantly different from that expected by chance ($P = 4.88 \times 10^{-4}$). When all reported loci ($P < 1 \times 10^{-5}$) were considered, only the clinic-based group showed a number of associated SNPs with higher effect sizes (odds ratio (OR) > 1.2) at low frequency (minor allele frequency (MAF) < 0.05) (**Supplementary Fig. 7**). Thus, clinic-based migraine samples may represent a promising subgroup to help prioritize loci in the search for low-frequency variants with moderate effects. Overall, of the 146 loci identified, there were twice as many that had causative minor alleles than had protective ones (with the ratio of these alleles as MAF decreased).

To explore the biological contexts of the identified loci, we examined the properties of the most proximal genes to the 12 top-associated SNPs reaching genome-wide significance (**Table 1**). In expression data from 55,269 samples profiled using the Affymetrix HG-U133 Plus 2.0 microarray (including 1,990 brain and 384 endothelial samples), 11 of the 12 genes nearest to the identified loci (all except *FHL5*) were at least moderately expressed (with >20% of the tissue samples showing a normalized $\log_2$ expression value greater than 6; Online Methods) in disease-relevant brain regions (**Supplementary Fig. 6**). In contrast, only *TGFBR2* and *MEF2D* showed moderate or greater expression in the endothelial samples. Possibly reflecting known comorbidity between migraine and cardiovascular disease[14], 2 of the 12 most proximal genes (*TGFBR2* and *PHACTR1*) have also been associated with cardiovascular traits: *TGFBR2* mutations have been reported to cause monogenic Marfan syndrome[15] and to be involved in abdominal aortic aneurysms[16], and *PHACTR1* is associated with early-onset myocardial infarction[17]. *TSPAN2* (ref. 18), *MEF2D*[19], *TRPM8* (ref. 20), *TGFBR2* (ref. 21), *PHACTR1* (ref. 22), *MMP16* (ref. 23), *ASTN2* (ref. 24) and *LRP1* (ref. 25) have been suggested to

Table 1 Results of association analyses

SNP	Chr.	Position (bp)	Location	Gene[a]	Minor allele	MAF	*P* value	OR (95% CI)	*q* value	I^2	Group or subgroup with the lowest *P* value	Additional subgroup with significant association
rs2651899	1	3073572	Genic	*PRDM16*[b]	C	0.41	3.28×10^{-14}	1.09 (1.07–1.12)	0.214	20%	All samples	
rs10915437	1	4082866	Intergenic	Near *AJAP1*	G	0.36	2.81×10^{-8}	0.86 (0.82–0.91)	0.108	47%	Clinics only	
rs12134493	1	115479469	Intergenic	Near *TSPAN2*	A	0.46	6.71×10^{-14}	1.14 (1.10–1.18)	0.480	14%	All samples	
rs2274316	1	154712866	Genic	*MEF2D*[b]	C	0.37	3.14×10^{-8}	1.07 (1.04–1.09)	0.021	45%	All samples	
rs7577262	2	234483608	Genic	*TRPM8*[b]	A	0.10	3.27×10^{-13}	0.87 (0.84–0.90)	0.070	33%	All samples	Clinics only, MO only
rs6790925	3	30455089	Intergenic	Near *TGFBR2*[b]	T	0.38	2.16×10^{-8}	1.15 (1.10–1.21)	0.780	0%	Clinics only	
rs9349379	6	13011943	Genic	*PHACTR1*[b]	G	0.40	2.81×10^{-10}	0.86 (0.82–0.90)	0.443	0%	MO only	All samples
rs13208321	6	96967075	Genic	*FHL5*	A	0.22	2.15×10^{-12}	1.18 (1.13–1.24)	0.168	0%	All samples	MO only
rs4379368	7	40432725	Genic	*c7orf10*	T	0.12	1.46×10^{-9}	1.11 (1.08–1.15)	0.441	2%	All samples	MO only
rs10504861	8	89617048	Intergenic	Near *MMP16*	T	0.16	1.32×10^{-8}	0.86 (0.81–0.90)	0.755	0%	MO only	
rs6478241	9	118292450	Genic	*ASTN2*[b]	A	0.38	1.04×10^{-9}	1.16 (1.11–1.22)	0.646	0%	Clinics only	All samples
rs11172113	12	55813550	Genic	*LRP1*[b]	C	0.43	2.69×10^{-19}	0.90 (0.88–0.92)	0.188	22%	All samples	MO only

When multiple subgroups showed significant association, *P* values and ORs are shown for the analysis with the lowest *P* value. ORs are reported for the minor allele. Chromosomal positions are based on NCBI Build 36. Chr., chromosome; CI, confidence interval; *q* value, *P* value from Cochran's heterogeneity statistic; I^2, heterogeneity index; MO, migraine without aura.

[a]For genic SNPs (located within the coding region of a gene), the relevant gene is listed; for intergenic SNPs, the gene closest to the locus is listed. [b]Previously identified migraine-associated locus.

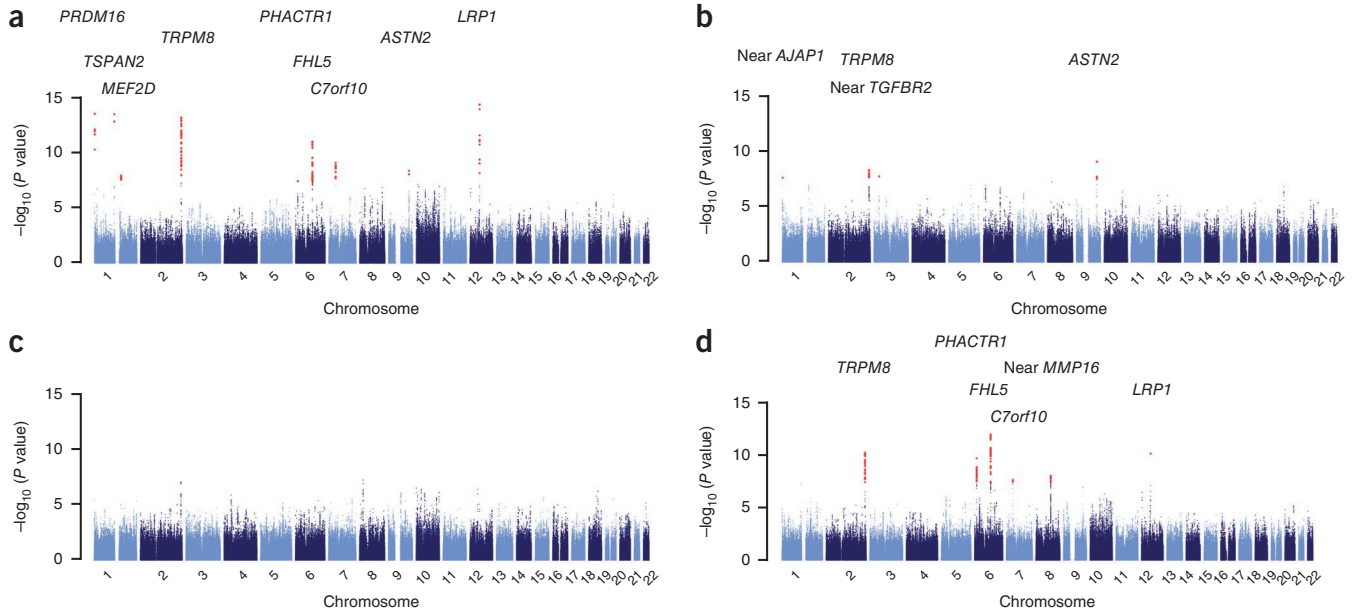

Figure 2 Manhattan plot of the results of the meta-analysis. Results are shown for the meta-analysis of all migraine cases, of any migraine subtype and with any recruiting method, versus all available controls, adjusting for sex. Red circles indicate SNPs with significant *P* values ($<5 \times 10^{-8}$). (**a**) Analysis of all migraine samples. (**b**) Analysis of clinic-based studies only. (**c**) Analysis of migraine with aura only. (**d**) Analysis of migraine without aura only.

have functions in synapse formation or regulation, *PRDM16* has been linked to the oxidative stress response, and *AJAP1* has been implicated in maintaining tissue borders (**Supplementary Fig. 8**).

To identify possible genes underlying these associations that were not included in proximity-based analyses, we examined expression quantitative trait locus (eQTL) data for 394 samples of brain tissue from the North American Brain Expression Consortium and the UK Brain Expression Consortium. Of the 12 regions with significant associations with migraine, 4 were found to contain significant eQTLs (Online Methods) identified in the SNP-probe pairs in the tested brain samples (4 in the frontal cortex and 1 in the cerebellum; **Table 2**). On chromosome 1, rs12136718 is an eQTL for *APOA1BP*, rather than the gene closest to it, *MEF2D*. *APOA1BP* is widely expressed and is potentially linked to cholesterol efflux from cells[26]. On chromosome 6, rs35128104 is an eQTL for *FUT9*, encoding α1,3-fucosyl transferase IX. The FUT9 enzyme synthesizes the Lewis X carbohydrate structure, which has been implicated in neurite outgrowth in several types of neuronal cells in the brain[27–29]. At the chromosome 12 locus, two different eQTLs in brain tissue were found within the association peak, including for *STAT6* (rs4559) and *ATP5B* (rs113953523), the former at a very robust *P* value (2.16×10^{-22}). STAT family members are known for transducing activation signals to transcription factors in macrophages[30], and STAT6 phosphorylation has recently been shown to sense oxidative stress in astrocytes, resulting in prostaglandin release[31]. The second eQTL-regulated gene at the same locus, *ATP5B*, is the β subunit of the mitochondrial ATP synthase, but a specific role in neuronal or vascular cells is not known. Finally, on chromosome 6, rs9349379 is an eQTL in cerebellar tissue for *TBC1D7*, which potentially downregulates the tuberous sclerosis gene *TSC1* through positive regulation of the mTOR signaling pathway[32]. In the central nervous system, *TSC1-TSC2* signaling contributes to neural connectivity through multifaceted roles[33]. On the basis of the available data, it is not possible to decide whether the gene identified by eQTL analysis or the gene closest to the strongest association is the most relevant gene contributing to migraine pathogenesis.

In an analysis of hypersensitivity sites, associated SNPs in the loci for migraine were found to occur significantly more often in DNase I hypersensitivity sites in a number of tissues (**Supplementary Fig. 9**). This finding suggests that the loci associated with migraine are enriched for actively transcribed regions, supporting the notion

Table 2 Significant eQTLs observed in 394 samples of 2 different brain tissues at loci significantly associated with migraine

					Frontal cortex				Cerebellum			
Chr.	Position (bp)	Locus	Probe	Gene name	SNPs with significant eQTL with probe	SNP with lowest eQTL *P* value	Lowest eQTL *P* value	Highest LD with reported SNP[a]	SNPs with significant eQTL with probe	SNP with lowest eQTL *P* value	Lowest *P* value	Highest LD with reported SNP
1	154830352	*MEF2D*	ILMN_1729533	*APOA1BP*	1	rs12136718	2.18×10^{-5}	0.38	0	–	–	–
6	13413333	*PHACTR1*	ILMN_1661622	*TBC1D7*	0	–	–	–	1	rs9395224	2.06×10^{-4}	0.451
6	96769539	*FHL5*	ILMN_1878007	*FUT9*	2	rs35128104	5.96×10^{-5}	0.74	0	–	–	–
12	55775568	*LRP1*	ILMN_1763198	*STAT6*	40	rs4559	2.16×10^{-22}	1	–	–	–	–
12	55318337	*LRP1*	ILMN_1772132	*ATP5B*	1	rs113953523	1.62×10^{-4}	0.39	0	–	–	–

Chr., chromosome. All base-pair positions are given for NCBI Build 36 (hg18). Owing to multiple overlapping signals, the human leukocyte antigen (HLA) region was excluded from the analysis.

[a]Maximum extent of LD observed between a significant eQTL SNP-probe pair (Online Methods) and a SNP passing the reporting threshold ($P < 1 \times 10^{-5}$) for association with migraine.

that the variants have regulatory roles. Both neuronal and vascular tissue types carry an enriched set of sites within the detected loci. In addition, in querying the RegulomeDB (see URLs), several of the associated SNPs were found to overlap directly with known transcription factor binding motifs (**Supplementary Table 8**).

The number of significantly associated loci is still modest for forming a broad understanding of disease susceptibility, and any proposed functional hypothesis from the identified loci must thus be approached with caution. Some functional hypotheses could be inferred from the results of this study, as the majority of the identified loci harbor genes that can be linked to neuronal function.

The eQTL analysis further supported the notion that regulatory effects in brain tissue may underlie several of the association signals. However, the lack of replication across brain regions suggests that our ability to use eQTL data to pinpoint the most functionally significant gene within each locus is limited.

The observed difference between the number of significant loci in the group with migraine without aura and the group with migraine with aura (6 versus 0, respectively; **Fig. 2c**,**d**), despite reasonably similar sample sizes, was somewhat unexpected. Migraine with aura has been shown to have a considerably higher heritability estimate and sibling recurrence risk than migraine without aura (3.8 versus 1.9) and has thus been considered to be the more heritable of the two common migraine types[34]. One possible explanation for the observed difference is that genetic susceptibility to migraine with aura is mediated more by rare variants with larger effect sizes, although this notion remains speculative. Another explanation for the difference may be a higher degree of heterogeneity among the cases of migraine with aura (due to genetically distinct subgroups, for example). No common variants specifically predisposing to migraine with aura were identified by this study (the lowest observed association P value was 7.88×10^{-8}).

In summary, we conducted a large migraine meta-analysis and identified 12 loci significantly associated with migraine susceptibility, including 5 new loci, as well as 134 additional suggestive loci. An eQTL analysis of brain tissue highlighted a further five genes potentially implicated in migraine susceptibility. Two of the 12 loci were showed association only in the clinic-based sample group, suggesting higher specificity for severe migraine headache, and only two loci were identified in the migraine without aura group. Seven previously reported loci for migraine susceptibility were replicated in independent samples in this study. Observed differences in the number of identified loci and the strength of association suggest that the genetic background of migraine with aura is considerably less influenced by common variants than that of migraine without aura, contrary to previous expectations. Finally, although pathway analysis of the 146 loci showed no concentration of candidate causal genes in any particular pathway or tissue, 8 of the 12 identified loci are located in or immediately adjacent to genes with known functions in synaptic or neuronal regulation, and several exert regulatory control over one another.

URLs. DAPPLE, http://www.broadinstitute.org/mpg/dapple/dapple.php/; GRAIL, http://www.broadinstitute.org/mpg/grail/; GWAMA, http://www.well.ox.ac.uk/gwama/; IMPUTE2, http://mathgen.stats.ox.ac.uk/impute/; International Headache Genetics Consortium, http://www.headachegenetics.org/; MAGENTA, http://www.broadinstitute.org/mpg/magenta/; ProbABEL, http://www.genabel.org/packages/ProbABEL; RegulomeDB, http://regulome.stanford.edu/; SNAP, http://www.broadinstitute.org/mpg/snap/; SNPTEST, https://mathgen.stats.ox.ac.uk/genetics_software/snptest/.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A. Palotie, D.C., D.R.N., M.J.D., K.S., G.D.S., M.W., M.D., A.M.J.M.v.d.M., M.D.F., C.K., T.K., D.P.S., L.C., J.-A.Z., M.-R.J., C.v.D., D.I.B., J.K., L.Q. and G.T. jointly supervised research. A. Palotie, D.C., D.R.N., V. Anttila, B.S.W., M.J.D., M.W., M.D., A.M.J.M.v.d.M., C.K., L.C., J.-A.Z., M.-R.J., C.v.D., D.I.B., J.K., T.K., M. Kallela, R.M., B.d.V., G.T., L.Q., M.A.I., L.L., E.H., M.S., H.S., K.S., F.J., T.F. and B.M.-M. conceived and designed the study. V. Anttila, B.S.W., A. Palotie, D.R.N., G.D.S., M.W., M.D., A.M.J.M.v.d.M., C.K., J.-A.Z., M.-R.J., O.R., C.v.D., D.I.B., J.K., E.B., M. Kallela, B.d.V., G.T., E.H., T.F., R.R.F., N.G.M., A.G.U., T.M. and J.G.E. performed the experiments. V. Anttila, B.S.W., P.G., D.C., D.R.N., M.J.D., D.P.S., E.B., J.R.G., S.B.R.J., T.K., F.B., G.M., R.M., B.d.V., L.Q., M.A.I., L.L., I.D., P.P., M.S., S. Steinberg, T.F. and B.M.-M. performed statistical analysis. V. Anttila, B.S.W., P.G., A. Palotie, D.C., D.R.N., L.C., J.R.G., S.B.R.J., K.L., T.K., F.B., G.M., R.M., B.d.V., S.E.M., L.Q., M.A.I., L.L., J.W., P.P., M.S., S. Steinberg, H.S., T.F., N.A., B.M.-M. and D.T. analyzed the data. D.C., D.R.N., M.J.D., A. Palotie, G.D.S., M.W., M.D., A.M.J.M.v.d.M., M.D.F., C.K., D.P.S., L.C., J.-A.Z., M.-R.J., O.R., C.v.D., D.I.B., B.W.P., J.K., E.B., J.R.G., K.L., E.R., V. Anttila, B.S.W., P.G., T.K., F.B., G.M., M. Kallela, R.M., B.d.V., G.T., U.T., W.L.M., L.Q., M. Koiranen, M.A.I., T.L., A.H.S., L.L., I.D., B.M.N., M.S., L.M.R., J.E.B., P.M.R., S. Steinberg, H.S., F.J., D.A.L., D.M.E., S.M.R., M.F., V. Artto, M.A.K., T.F., J.S., R.R.F., N.P., C.M.W., R.Z., A.C.H., P.A.F.M., G.W.M., N.G.M., G.B., H.G., A. Heinze, K.H.-K., F.M.K.W., A.-L.H., A. Pouta, J.v.d.E., A.G.U., A. Hofman, J.-J.H., J.M.V., K.H., M.A., B.M.-M., S. Schreiber, T.M., H.E.W., A.A., J.G.E., B.T. and D.T. contributed reagents and/or materials and/or analysis tools. V. Anttila, B.S.W., A. Palotie, D.C., D.R.N., A.M.J.M.v.d.M. and C.K. wrote the manuscript. All authors contributed to the final version of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Overall study design. For this meta-analysis, we used SNP marker data from 23,285 cases and 95,425 controls of European descent from 29 studies, including 5 clinic-based studies, compared to population-matched control samples with unknown migraine status, as well as 14 entirely population-based cohorts. Four of the population-based cohorts (B58C, NFBC, Young Finns and FinnTwin; see **Supplementary Note** for further details) were birth cohorts. The data sets for the meta-analysis included previously genotyped GWAS data from migraine-specific studies by the International Headache Genetics Consortium (see URLs) and the Women's Genome Health Study, as well as a number of pre-existing population-based GWAS (for a complete list of references, see **Supplementary Table 2**). Local research ethics committees approved the individual studies, and informed consent was obtained from all participants when necessary (see the **Supplementary Note** for full details of ethics and consent procedures for each study). Additional details on sample recruitment and phenotypes and summary details for each collection are given in the **Supplementary Note** and in **Supplementary Tables 1** and **2**. Genome-wide SNP genotyping was performed independently in each cohort with the use of various standard genotyping technologies, and genotypes were imputed for each study with reference to HapMap release 21 or 22 CEU (Utah residents of Northern and Western European ancestry) phased genotypes[35].

Study phenotypes. The primary phenotype analyzed was migraine of any type, regardless of source. This initial step was followed by a subgroup analysis consisting of (i) only the clinical samples, (ii) only samples satisfying criteria for migraine with aura and (iii) only samples satisfying criteria for migraine without aura. Population-based samples were not analyzed across the genome as a subgroup because they constituted 78% of cases and 85% of controls in the main analysis, but associations were calculated for significant SNPs for comparative purposes. In the clinical cohorts, a headache specialist assigned a migraine diagnosis on the basis of an interview in person or over the telephone or through the use of an extensive questionnaire. For the population studies, migraine status for individuals in a study sample was determined using a questionnaire (**Supplementary Note**).

Statistical analysis of GWAS data. Each study contributed summary statistic data from an association analysis performed using a frequentist additive model based on an expected allelic dosage model for SNP markers, adjusting for sex (using either SNPTEST or ProbABEL (see URLs)). SNPs were filtered on the study level with inclusion criteria of MAF of >0.1% and imputation quality measure (I_A) of >0.6 (IMPUTE 2)[36] or r^2 of >0.3 (MACH)[37]. Four of the included studies contained new genotyping (HUNT) or imputation (the Finnish, German and Dutch MA studies and HUNT). In the meta-analysis, combined association data for ~2.3 million imputed and genotyped autosomal SNPs were analyzed in a fixed-effects model using GWAMA. At this stage, SNPs with a heterogeneity coefficient I^2 exceeding 75% or that were present in four or fewer studies were filtered out. In the meta-analysis, there was little evidence for population stratification at the study level (each genomic inflation factor λ was ≤1.1), although moderate inflation was observed at the meta-analysis level (λ = 1.15; **Supplementary Fig. 4**). For estimating genome-wide significance, we used the commonly accepted thresholds of 5×10^{-8} for primary loci[38] and 1×10^{-5} for secondary loci, in accordance with the reporting threshold for the GWAS catalog[39]. At secondary loci, to limit spurious associations, at least two SNPs within a 50-kb window were required to pass the significance threshold. We also estimated the robustness of this threshold using the false discovery rate (FDR) method of Benjamini and Hochberg[40], showing that the *P*-value threshold corresponding to FDR of <0.05 was 2.33×10^{-5}. The quantile-quantile plot of the meta-analysis *P* values (**Supplementary Fig. 4**) showed a marked excess of association signals, well beyond the number expected by chance, below the threshold for suggestive association used for reporting. The loci with significant associations were visualized using the LocusZoom interface[41]. For the heterogeneity analyses for migraine type, owing to shared controls in some of the sets, the available study samples were divided into groups that were as equally sized as possible in terms of effective study size, and the data were then analyzed using the sex heterogeneity analysis method[42] (–sex option) of GWAMA[43], with a dummy variable coding for migraine with aura and migraine without aura instead of sex. For the heterogeneity analyses for sex, the same method was used to compare *P* values from male-only and female-only analyses.

Pathway analyses. *MAGENTA*. MAGENTA software[44] was used to conduct an analysis to evaluate whether *P* values for association with migraine were enriched for particular biological networks, using pathway lists from GO, Panther, Ingenuity, KEGG, Reactome and Biocarta. In gene set enrichment analysis, *P* values were estimated via 10,000 permutations of genes evaluated at the 75th percentile of FDR (owing to the assumption of high polygenicity), manually corrected to account for FDR across all pathway sets. *DAPPLE*. Using a refined database of high-confidence protein-protein interactions (InWeb)[45,46], we employed DAPPLE[47] to assess the amount of physical interactions connecting the genes within 50 kb of the 146 reported migraine loci, as well as an analysis of only the 16 proteins from the 12 genome-wide significant loci. Both direct and indirect (through first-order common interaction partners) were measured and compared to a random expectation over 10,000 permutations, and the resulting network was plotted.

GRAIL. The GRAIL web interface[48] was used to explore similarities in published PubMed articles (August 2012 freeze), using data from HapMap release 22 CEU samples and with gene size correction set to on. From the GRAIL results, only genes with significant ($P < 0.05$) are shown, and the list of similar genes was capped to include only genes within the top 200 highest rankings.

Overlap with DNase I hypersensitivity sites. The positions of SNPs in migraine-associated loci were overlapped with DNase I hotspot regions from the Encyclopedia of DNA Elements (ENCODE) Project that mark generalized chromatin accessibility, mapped for each of 125 diverse cell lines and tissues[49]. To assess the significance of overlap for the set of SNPs as a whole, 100 background sets of SNPs were chosen from the genome so that each migraine-associated SNP was matched in each set by a SNP within the same decile for MAF, distance to the nearest transcriptional start site and GC content of the 100-base region surrounding the SNP. Background SNP sets were overlapped with the DNase I hotspots, and the enrichment for overlap with the migraine-associated SNP set was expressed as the *z* score relative to the distribution of the background SNP set overlaps, on a per-cell-line basis. In addition, migraine-associated SNPs were analyzed for other overlap with ENCODE data, including with transcription factor motifs, using RegulomeDB (see URLs)[50].

Tissue-based gene expression analysis. For the tissue analysis, a microarray-based analysis of gene expression was performed on a data set of 55,269 samples in the Gene Expression Omnibus (GEO) that were measured on the Affymetrix U133 Plus 2.0 array. Each sample in the raw expression data was first linearly transformed using a modified invariant set normalization method[51] based on a set of 80 control genes with stable expression on the U133 Plus 2.0 array. Expression data were $\log_2$ transformed to stabilize the variance and distribution of expression levels. Finally, data were quantile normalized[52] to match the expression distribution of each sample. Expression values for genes with multiple probe sets were calculated by taking the median value of all probe sets for that gene. After normalization, a $\log_2$ expression value of four was considered baseline, and a $\log_2$ expression value of greater than six was considered to indicate expression. Sample annotations were curated on the basis of GEO descriptions provided by depositors. To account for variation in the number of samples representing each tissue in the data set, expression of a gene was plotted to show the fraction of samples of a tissue that exceeded a $\log_2$ expression value of six, with higher fractions indicating more ubiquitous expression in the tissue in question.

eQTL analysis. On the basis of the meta-analysis results for association with migraine, 146 regions of interest were queried against eQTL results from the North American Brain Expression Consortium and UK Brain Expression Consortium studies (GEO accession GSE36192 and database of Genotypes and Phenotypes (dbGaP) accession phs000249). These eQTL results are based on mRNA expression levels in cerebellum and frontal cortex tissue from 394 human subjects (**Supplementary Note**). Of the 146 associated regions, 134 were represented in the eQTL analysis. Within these regions, 831 mRNA expression traits and 222,668 *cis* SNP–expression trait pairs were considered in

cerebellum, and 864 mRNA expression traits and 230,660 *cis* SNP–expression trait pairs were considered in the frontal cortex. Forty-five SNPs within the migraine-associated loci were found to have significant correlation (evaluated at FDR-corrected thresholds: 0.0001668 for frontal cortex and 0.0002187 for cerebellum) with the expression of 12 mRNA transcripts in both the cerebellum and the frontal cortex. A more detailed methods description can be found in the **Supplementary Note**. The extent of linkage disequilibrium between the SNPs associated with migraine and the SNPs in the tested eQTL SNP-probe pairs was evaluated using SNAP (see URLs).

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