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

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

Migraine without aura is the most common form of migraine, characterized by recurrent disabling headache and associated autonomic symptoms. To identify common genetic variants associated with this migraine type, we analyzed genome-wide association data of 2,326 clinic-based German and Dutch individuals with migraine without aura and 4,580 population-matched controls. We selected SNPs from 12 loci with 2 or more SNPs associated with *P* values of $<1 \times 10^{-5}$ for replication testing in 2,508 individuals with migraine without aura and 2,652 controls. SNPs at two of these loci showed convincing replication: at 1q22 (in *MEF2D*; replication $P = 4.9 \times 10^{-4}$; combined $P = 7.06 \times 10^{-11}$) and at 3p24 (near *TGFBR2*; replication $P = 1.0 \times 10^{-4}$; combined $P = 1.17 \times 10^{-9}$). In addition, SNPs at the *PHACTR1* and *ASTN2* loci showed suggestive evidence of replication ($P = 0.01$; combined $P = 3.20 \times 10^{-8}$ and $P = 0.02$; combined $P = 3.86 \times 10^{-8}$, respectively). We also replicated associations at two previously reported migraine loci in or near *TRPM8* and *LRP1*. This study identifies the first susceptibility loci for migraine without aura, thereby expanding our knowledge of this debilitating neurological disorder.

Migraine is a disabling episodic neurovascular brain disorder affecting 12% of the general population[1–4]. Migraine attacks are typically characterized by severe, throbbing unilateral headache and nausea, vomiting and photo- and phonophobia (migraine without aura). In up to one-third of affected individuals, attacks may be associated with neurological aura symptoms (migraine with aura). Previous genome-wide association studies (GWAS) identified a migraine susceptibility locus on chromosome 8q22, close to *MTDH*, in the clinic-based International Headache Genetics Consortium (IHGC) migraine with aura study[5] and at three other loci in or near *PRDM16*, *LRP1* and *TRPM8* in the population-based Women's Genome Health Study (WGHS) of migraine[6]. Suggestive evidence for association was also found for *TRPM8* ($P < 1 \times 10^{-5}$) in the clinic-based IHGC migraine with aura GWAS[5]. Here, we report the first GWAS of migraine without aura, the most common form of migraine. We analyzed two large samples from headache centers in Germany and The Netherlands, including 2,326 individuals with migraine without aura and 4,580 population-matched controls (**Supplementary Fig. 1** and **Supplementary Note**). A quantile-quantile plot of the joint analysis (**Supplementary Fig. 2**) and an overall genomic inflation factor ($\lambda_{1,000}$) of 1.03 were used as final quality control measures. In the discovery data set, we identified 1 locus associated at genome-wide significance ($P < 5 \times 10^{-8}$) on chromosome 1q22, as well as 11 additional loci containing multiple SNPs with suggestive evidence for association ($P < 1 \times 10^{-5}$) (**Supplementary Table 1**). Eighteen SNPs from these 12 loci were taken forward to the replication stage in 4 independent clinic-based European migraine without aura samples (2,508 cases and 2,652 controls) (**Supplementary Fig. 1** and **Supplementary Table 1**). Eight SNPs in six loci showed *P* values of <0.05 in the replication study, and five of these SNPs also showed *P* values of $<5 \times 10^{-8}$ in the meta-analysis combining the discovery and replication cohorts (**Fig. 1**, **Table 1** and **Supplementary Fig. 3**). Associations were replicated for four loci (at 1q22, 3p24, 6p24 and 9q33), although replication was less convincing for the loci at 6p24 and 9q33, with replication *P* values of 0.012 and 0.018, respectively, even though association *P* values for these loci were $<5 \times 10^{-8}$ in the overall meta-analysis. In addition, we tested top SNPs of the four previously identified migraine-associated loci (at 1p36, 2q37, 8q22 and 12q13)[5,6] in the replication stage (**Fig. 2** and **Supplementary Table 1**),

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

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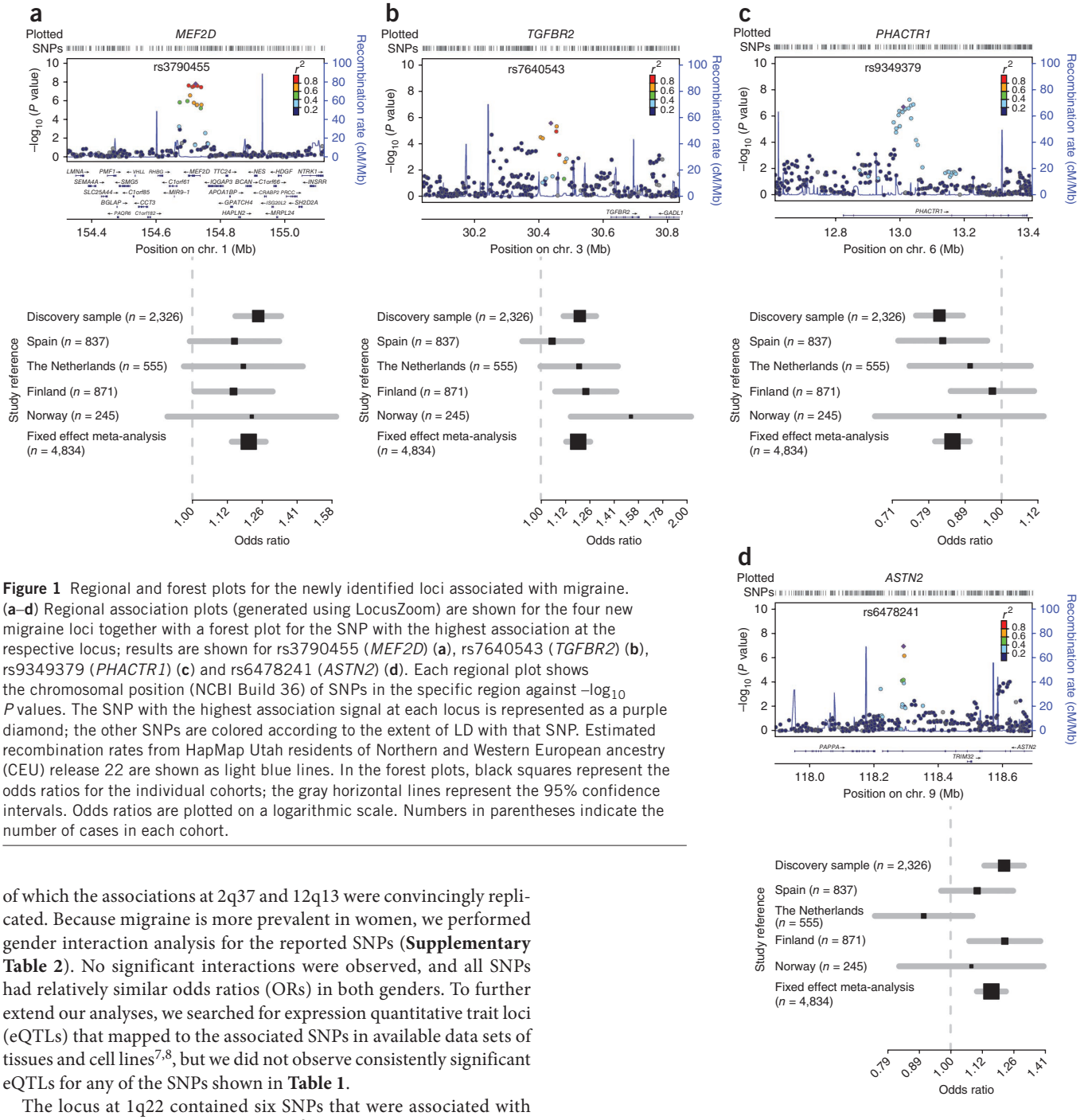

Figure 1 Regional and forest plots for the newly identified loci associated with migraine. (**a**–**d**) Regional association plots (generated using LocusZoom) are shown for the four new migraine loci together with a forest plot for the SNP with the highest association at the respective locus; results are shown for rs3790455 (*MEF2D*) (**a**), rs7640543 (*TGFBR2*) (**b**), rs9349379 (*PHACTR1*) (**c**) and rs6478241 (*ASTN2*) (**d**). Each regional plot shows the chromosomal position (NCBI Build 36) of SNPs in the specific region against $-\log_{10}$ *P* values. The SNP with the highest association signal at each locus is represented as a purple diamond; the other SNPs are colored according to the extent of LD with that SNP. Estimated recombination rates from HapMap Utah residents of Northern and Western European ancestry (CEU) release 22 are shown as light blue lines. In the forest plots, black squares represent the odds ratios for the individual cohorts; the gray horizontal lines represent the 95% confidence intervals. Odds ratios are plotted on a logarithmic scale. Numbers in parentheses indicate the number of cases in each cohort.

of which the associations at 2q37 and 12q13 were convincingly replicated. Because migraine is more prevalent in women, we performed gender interaction analysis for the reported SNPs (**Supplementary Table 2**). No significant interactions were observed, and all SNPs had relatively similar odds ratios (ORs) in both genders. To further extend our analyses, we searched for expression quantitative trait loci (eQTLs) that mapped to the associated SNPs in available data sets of tissues and cell lines[7,8], but we did not observe consistently significant eQTLs for any of the SNPs shown in **Table 1**.

The locus at 1q22 contained six SNPs that were associated with genome-wide significance ($P < 5 \times 10^{-8}$) in the discovery stage of the analysis and that were all in close linkage disequilibrium (LD; $r^2 > 0.98$). The rs1050316 and rs3790455 SNPs were taken forward to the replication stage, and associations were successfully replicated (overall meta-analysis *P* values of 3.21×10^{-10} (OR = 1.19) and 7.06×10^{-11} (OR = 1.20), respectively) (**Fig. 1**, **Table 1** and **Supplementary Fig. 3**). All associated SNPs were located within the *MEF2D* gene (encoding myocyte enhancer factor 2D) (intronic and within the 3′ UTR). The MEF2D protein is a transcription factor that is highly expressed in brain. MEF2D regulates neuronal differentiation by supporting the survival of newly formed neurons[9]. Perhaps even more relevant to migraine, neuronal activity–dependent activation of MEF2D restricts the number of excitatory synapses[10]. As the brain in migraine is hyperexcitable[11], it is tempting to speculate that MEF2D dysregulation might affect neuronal excitatory neurotransmission in individuals with migraine without aura. There is some evidence for increased levels of glutamate (the main brain excitatory neurotransmitter) in individuals with migraine[12,13], and increased glutamatergic neurotransmission was reported in a transgenic knock-in mouse model with a pathogenic mutation in the human *CACNA1A* gene associated with hemiplegic migraine[14]. A role for MEF2D dysregulation in migraine is also plausible given that several MEF2 transcriptional targets have been associated with other neurological disorders, such as epilepsy[15–17]. Notably, pituitary adenylate cyclase–activating polypeptide-38 (PACAP-38), which is encoded by one of the

Table 1 SNPs at the six loci associated with migraine without aura

General SNP information								Discovery samples			All replications			Overall meta-analysis			
SNP	Chr.	Position[a]	Location	Gene[b]	Minor allele	Minor allele frequency	Sequence type[c]	*P* value	OR (95% CI)	I^2	*P* value	OR (95% CI)	I^2	*P* value	OR (95% CI)	I^2	r^2 with top SNP
Locus 1																	
rs1050316	1	154701327	Intragenic	*MEF2D*	G	0.34	Imputed	**2.59×10^{-8}**	1.24 (1.15–1.33)	0.00	1.15×10^{-3}	1.14 (1.06–1.24)	0.00	**3.21×10^{-10}**	1.19 (1.13–1.26)	0.00	0.987
rs2274316	1	154712866	Intragenic	*MEF2D*	C	0.35	Genotyped	**3.60×10^{-8}**	1.23 (1.14–1.33)	0.00	–	–	–	–	–	–	0.986
rs1925950	1	154717364	Intragenic	*MEF2D*	G	0.35	Imputed	**2.97×10^{-8}**	1.24 (1.15–1.33)	0.00	–	–	–	–	–	–	0.988
rs3790455	1	154722925	Intragenic	*MEF2D*	C	0.34	Genotyped	**1.71×10^{-8}**	1.24 (1.15–1.34)	0.00	4.85×10^{-4}	1.16 (1.07–1.26)	0.00	**7.06×10^{-11}**	1.20 (1.14–1.27)	0.00	–
rs3790459	1	154728331	Intragenic	*MEF2D*	A	0.35	Imputed	**2.85×10^{-8}**	1.24 (1.15–1.33)	0.00	–	–	–	–	–	–	0.989
rs12136856	1	154739738	Intergenic	*MEF2D*	C	0.34	Imputed	**3.90×10^{-8}**	1.23 (1.15–1.33)	0.00	–	–	–	–	–	–	0.985
Locus 2																	
rs7640543	3	30437407	Intergenic	*TGFBR2*	A	0.32	Genotyped	2.72×10^{-6}	1.20 (1.11–1.30)	0.00	1.02×10^{-4}	1.18 (1.09–1.29)	0.51	**1.17×10^{-9}**	1.19 (1.13–1.26)	0.19	–
Locus 3																	
rs9349379	6	13011943	Intragenic	*PHACTR1*	G	0.38	Genotyped	2.06×10^{-7}	0.82 (0.77–0.89)	0.00	0.01	0.90 (0.83–0.98)	0.00	**3.20×10^{-8}**	0.86 (0.81–0.91)	0.10	–
Locus 4																	
rs6478241	9	118292450	Intragenic	*ASTN2*	A	0.38	Genotyped	1.14×10^{-7}	1.22 (1.13–1.31)	0.00	0.02	1.10 (1.02–1.19)	0.57	**3.86×10^{-8}**	1.16 (1.10–1.23)	0.56	–
Locus 5																	
rs10166942	2	234489832	Intergenic	*TRPM8*	C	0.18	Genotyped	1.32×10^{-5}	0.82 (0.74–0.89)	0.00	5.62×10^{-9}	0.74 (0.67–0.82)	0.00	**9.83×10^{-13}**	0.78 (0.73–0.84)	0.00	–
rs17862920	2	234492734	Intragenic	*TRPM8*	T	0.10	Genotyped	2.19×10^{-5}	0.78 (0.69–0.87)	0.00	6.44×10^{-5}	0.75 (0.66–0.87)	0.00	**5.97×10^{-9}**	0.77 (0.70–0.84)	0.00	0.520
Locus 6																	
rs11172113	12	55813550	Intragenic	*LRP1*	C	0.40	Genotyped	3.38×10^{-5}	0.86 (0.80–0.92)	0.00	2.33×10^{-4}	0.86 (0.79–0.93)	0.67	**2.97×10^{-8}**	0.86 (0.81–0.91)	0.45	–

SNPs showing significant association either in the discovery stage (locus 1) or after meta-analysis of the discovery and replication samples (loci 2–6). Genome-wide significant *P* values and successful replications are shown in boldface. ORs are reported for the minor allele. Chr., chromosome; I^2, heterogeneity index. r^2 indicates the LD between the SNP and the top SNP at the respective locus.
[a]Chromosomal positions are based on NCBI Build 36. [b]For intragenic SNPs, the gene is listed in which the SNP is located, whereas, for intergenic SNPs, the nearest gene is listed. [c]Indicates whether a SNP was genotyped or imputed.

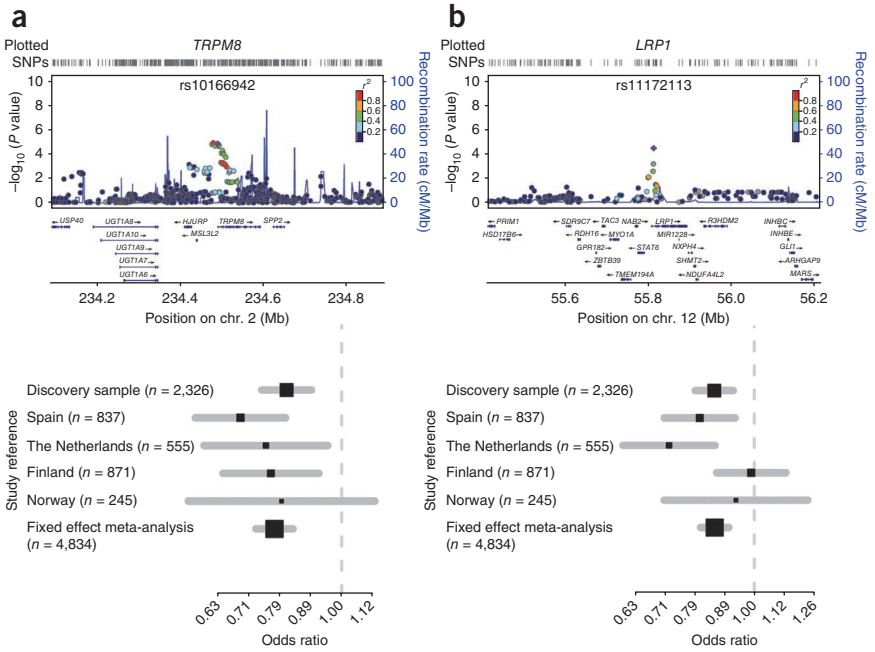

Figure 2 Regional and forest plots for the previously reported loci associated with migraine that replicate in the current migraine without aura study. (**a**,**b**) Regional association plots (generated using LocusZoom) are shown for the two previously reported migraine loci[6] that significantly replicated in the current study together with forest plots for the SNP with the highest association at these loci; results are shown for rs10166942 (*TRPM8*) (**a**) and rs11172113 (*LRP1*) (**b**). Each regional plot shows the chromosomal position (NCBI Build 36) of SNPs in the specific region against $-\log_{10}$ *P* values. The SNP with the highest association signal in each locus is represented as a purple diamond; the other SNPs are colored according to the extent of LD with this SNP. Estimated recombination rates from HapMap CEU release 22 are shown as light blue lines. In the forest plots, black squares represent the odds ratios for the individual cohorts; the gray horizontal lines represent the 95% confidence intervals. Odds ratios are plotted on a logarithmic scale. Numbers in parentheses indicate the number of cases in each cohort.

activity-regulated MEF2D target genes and modulates excitatory synaptic transmission, may trigger migraine-like attacks in persons with migraine without aura[18]. In mice, PACAP-38 is involved in nitroglycerol-induced trigeminovascular activation[19], which is the presumed origin of the migraine headache[20].

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

For the locus at 6p24, the rs9349379 SNP reached genome-wide significance when combining the data from the discovery and replication samples ($P = 3.20 \times 10^{-8}$, OR = 0.86) (**Fig. 1**, **Table 1** and **Supplementary Fig. 3**). Five SNPs (with association at $P < 1 \times 10^{-5}$) were taken forward to the replication stage (**Supplementary Table 1**). All five SNPs were located in *PHACTR1* (encoding phosphatase and actin regulator 1). The encoded protein is a member of the PHACTR/scapinin family, which controls synaptic activity and synapse morphology through regulation of protein phosphatase 1 and actin binding[24,25]. PHACTR1 has further been implicated in endothelial cell function[26] and susceptibility to early-onset myocardial infarction[27]. Thus, the link between *PHACTR1* and migraine could be neuronal, through aberrant synaptic transmission, or vascular, as endothelial dysfunction, cardiovascular disease and myocardial infarction all seemed to be linked with migraine[28]. Like TGFBR2, PHACTR1 may also be involved in systemic vascular disease through a TGF-β signaling pathway.

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

In addition, two of the four previously reported migraine loci[5,6] showed significant association in the current clinic-based migraine without aura GWAS. Two top SNPs ($r^2 = 0.52$) in the migraine-associated locus at 2q37 reached genome-wide significance in the overall migraine without aura meta-analysis (rs10166942: $P = 9.83 \times 10^{-13}$, OR = 0.78; rs17862920: $P = 5.97 \times 10^{-9}$, OR = 0.77) (**Fig. 2**, **Table 1** and **Supplementary Fig. 3**). The rs10166942 SNP is located approximately 1 kb upstream of the predicted transcriptional start site of *TRPM8* (encoding transient receptor potential melastatin 8), whereas rs17862920 is located in the first intron of *TRPM8*. *TRPM8* encodes a cold- and menthol-activated ion channel that is expressed in sensory neurons. The gene was identified as a migraine susceptibility gene, both in the population-based WGHS migraine GWAS[6] and in the clinic-based IHGC migraine with aura GWAS[5]. The effect direction of these SNPs was the same in all three studies, and the effect size estimates were similar (OR = 0.78 in the clinic-based IHGC migraine with aura study for rs17862920 versus 0.77 in the present study; OR = 0.85 in the population-based WGHS migraine study for rs10166942 versus 0.78 in the present study). TRPM8 could be involved in cutaneous allodynia[32–34], which is defined as pain due to thermal or mechanical stimuli that normally do not provoke pain, and is present in the majority of individuals with migraine.

The top SNP at the previously reported migraine-associated locus at 12q13 (ref. 6) reached genome-wide significance in the overall migraine without aura meta-analysis (rs11172113: overall $P = 2.97 \times 10^{-8}$, OR = 0.86) (**Fig. 2**, **Table 1** and **Supplementary Fig. 3**). rs11172113 is located within the first intron of *LRP1*, which encodes the low-density lipoprotein receptor–related protein 1, a protein that is expressed in multiple tissues, including neurons and vasculature. LRP1 is a cell surface receptor that acts as a sensor of the extracellular environment: it is involved in the proliferation of vascular smooth muscle cells and modulates synaptic transmission[35,36]. A possible role for LRP1 in migraine can be envisaged because of its neuronal and/or vascular function.

Addressing the question of whether migraine with aura and migraine without aura represent different disease entities[37,38], we tested the top SNPs from the six loci from the current migraine without aura study *in silico* in our previous IHGC migraine with aura GWAS data set[5] (**Supplementary Table 3**). Except for the locus at 3p24, all loci showed *P* values below 0.05 in the migraine with aura data set, and ORs were in the same direction in both studies. The *TRPM8* locus showed the most significant *P* value for association (rs1786920: 2.19×10^{-5}, OR = 0.78; rs10166942: 1.32×10^{-5}, OR = 0.82) in the migraine with aura data set. Notably, rs10166942 also showed association in the WGHS migraine study ($P = 2.30 \times 10^{-7}$, OR = 0.86 in the initial scan)[6]. This suggests that *TRPM8* may have a role in various forms of migraine. In contrast, the rs1835740 SNP at 8q22 in the IHGC migraine with aura GWAS[5] pointing at *MTDH* as the putative migraine susceptibility gene[5] did not show association in the present GWAS ($P = 0.70$) nor in the population-based WGHS migraine GWAS[6] ($P = 0.22$). This may suggest that *MTDH* confers more susceptibility to aura than to headache.

In conclusion, we present the first GWAS of migraine without aura, the most common migraine type, and identify several associated loci. Two loci (*MEF2D* and *TGFBR2*) showed convincing association in the replication stage and genome-wide significance levels in the combined meta-analysis. Replication for the *PHACTR1* and *ASTN2* loci was weaker, and additional studies are needed to test their relevance as migraine susceptibility loci. In addition, two of the four previously identified loci associated with migraine (*TRPM8* and *LRP1*) were replicated in this clinic-based migraine without aura study. Functional studies are necessary to dissect the exact underlying molecular pathways in order to identify putative treatment targets for this common debilitating brain disorder.

URLs. GWAMA, http://www.well.ox.ac.uk/gwama/; NCBI, http://www.ncbi.nlm.nih.gov/; HapMap 3 data, http://hapmap.ncbi.nlm.nih.gov/; IMPUTE2, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html; SNPTEST, http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html; LocusZoom, http://csg.sph.umich.edu/locuszoom/; Genotype-Tissue Expression (GTEx) eQTL Browser, http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi.

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

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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[1]Institute for Stroke and Dementia Research, Klinikum der Universität München, Munich, Germany. [2]Department of Neurology, Klinikum der Universität München, Munich, Germany. [3]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK. [4]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland. [5]Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands. [6]Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland. [7]Department of Neurology, Leiden University Medical Centre, Leiden, The Netherlands. [8]Department of Neurology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain. [9]Headache Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain. [10]Department of Neurology, Oslo University Hospital and University of Oslo, Oslo, Norway. [11]Neurogenetics Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia. [12]Institute of Human Genetics, University of Ulm, Ulm, Germany. [13]Department of Psychiatry, Leiden University Medical Centre, Leiden, The Netherlands. [14]Folkhälsan Research Center, Helsinki, Finland. [15]Headache Research Unit, Department of Neurology and Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA)-Neurosciences, Liège University, Liège, Belgium. [16]Department of Clinical Physiology, University of Turku and Turku University Central Hospital, Turku, Finland. [17]Department of Clinical Chemistry, Tampere University Hospital and University of Tampere, Tampere, Finland. [18]Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain. [19]Kiel Pain and Headache Center, Kiel, Germany. [20]Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany. [21]Department of Genetics, University of Barcelona, Barcelona, Spain. [22]Biomedical Network Research Centre on Rare Diseases (CIBERER), Barcelona, Spain. [23]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. [24]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. [25]Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway. [26]Department of Public Health, University of Helsinki, Helsinki, Finland. [27]Department of Mental Health and Alcohol Research, National Institute for Health and Welfare, Helsinki, Finland. [28]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain. [29]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany. [30]Institute of Human Genetics, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany. [31]Max Planck Institute of Psychiatry, Munich, Germany. [32]The Broad Institute of MIT and Harvard, Boston, Massachusetts, USA. [33]Department of Medical Genetics, University of Helsinki, Helsinki, Finland. [34]Department of Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland. [35]A full list of members and affiliations is provided in the **Supplementary Note**. [36]These authors contributed equally to this work. [37]These authors jointly directed this work. Correspondence should be addressed to A.P. (ap8@sanger.ac.uk) or A.M.J.M.v.d.M. (maagdenberg@lumc.nl).

ONLINE METHODS

Overall study design. The discovery stage of the study was based on an analysis of *de novo* genotyping in two large migraine without aura sample sets from headache clinics in Germany (Munich and Kiel) and The Netherlands (Leiden) (**Supplementary Fig. 1**). Population-matched controls were recruited from studies with existing genotyping data (details of study cohorts and controls are given in the **Supplementary Note**). For both sample sets, raw data were imputed to approximately 1.4 million SNPs using HapMap 3 release 2 (ref. 39) as a reference panel. As an initial step, genome-wide logistic regression analysis was performed independently in both samples, and meta-analysis was then performed for the two data sets. Subsequently, the top SNPs from the meta-analysis were tested for replication in four smaller, clinic-based migraine without aura samples from Finland (Helsinki), Spain (Barcelona), Norway (Trondheim) and The Netherlands (Leiden) (**Fig. 1** and **Supplementary Fig. 1**).

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

Discovery-stage genotyping. Genomic DNA was extracted from peripheral blood samples according to standard protocols. Genotyping of the German GWAS sample was performed at the Genome Analysis Center at Helmholtz Zentrum München using the Illumina Human 610-Quad v1 (n = 838) or Illumina Human 660W-Quad v1 (n = 391) SNP microarrays according to the Infinium II protocol from the manufacturer. Genotype calling was performed using Illumina Gencall data analyses software. Genotyping of the complete Dutch GWAS sample was performed at the Wellcome Trust Sanger Institute using Illumina 660W technology. Genotype calling was performed using Illuminus software[40].

Replication-stage genotyping. For the replication study, all cases and controls were genotyped at the Wellcome Trust Sanger Institute using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry with Sequenom MassARRAY methodology. Amplification reactions and parameters were based on the manufacturer's instructions. Each 384-well plate contained positive (Centre d'Etude du Polymorphism Humain (CEPH) DNA) and negative controls to check for assay performance and contamination, respectively. Spectrocaller software supplied by the manufacturer was used to automatically call the genotypes. Clusters were checked manually, and all doubtful calls were evaluated.

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

Quality control. To ensure high data quality, the data sets from the primary study were subjected to per-SNP and per-sample quality control analysis before and after imputation. In both types of analysis, cutoffs of 1% for minor allele frequency and 1×10^{-6} for Hardy-Weinberg equilibrium (HWE) P values were used for cases and controls independently (as the cases and controls were genotyped independently in both populations), and the latter was also used for the combined set of cases and controls. Further, SNPs with call rates of <97% were excluded. Subjects with a genotyping rate of <97% and subjects who were closely related to each other (π_hat > 0.15) were removed, as were those with cryptic relatedness and those considered to be heterozygosity outliers. In addition, population outliers were excluded by manual selection from a multidimensional scaling plot of the genome-wide identity-by-state (IBS) pairwise distance matrix in PLINK.

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

Imputation. Imputation of the German and Dutch discovery samples was performed using IMPUTE2 (v2.1.0 for the German samples and v2.1.2 for the Dutch samples)[41]. For the phased haploid reference panel, we used HapMap 3 release 2 February 2009) samples (n = 1,011)[42] as provided by the IMPUTE2 website (see URLs). We followed the recommended parameters for imputation, with the exception that we used different numbers of copying states (κ = 60 for the German and 80 for the Dutch samples) and a larger buffer size for the Dutch samples (500 kb instead of 250 kb). After imputation, we used individual call posterior probability of >0.9 and info measure I(A) of >0.6 as cutoffs to ensure high imputation data quality. From 1,411,821 SNPs after imputation, 165,433 SNPs were excluded on the basis of quality control analysis.

Statistical analysis. For the GWAS data from the two initial study samples, we analyzed the imputed allele dosage data with SNPTEST software (version 2.2.0; see URLs) to generate population-specific summary statistics. We used the presence of migraine as a binary phenotype and assumed an additive model. In accordance with our previous clinic-based migraine with aura GWAS[5], association analysis was not corrected for age or gender. The primary reason for not using age as a covariate was that age information was not available for some of the control cohorts. However, the majority of the individuals in the cohorts were of working age, similar to our case samples. The missing data score likelihood option was used to handle missing data. For the replication studies, genotyped markers were analyzed using the same model as was used for the discovery samples for the population-specific results.

A fixed-effect meta-analysis of the summary statistics was conducted using GWAMA[43] (see URLs), first on the two discovery samples for the primary results. In the discovery sample meta-analysis, only SNPs that were present in both data sets were retained and filtered for having heterogeneity measure I^2 of <0.5. This moderately high threshold for I^2 was chosen to reflect the expectation of some differences in association signals of common markers due to varying LD structure. After replication, all six study sets (two discovery and four replication samples) were included in the overall meta-analysis. Reasonable genomic inflation was observed (λ = 1.095, λ_{1000} = 1.031). Consistency of allelic effects across studies was examined using the Cochran's Q[44] and I^2 (ref. 45) metrics. Between-study (effect) heterogeneity was indicated by Q-statistic P values ($P \leq 0.1$) and moderate (25–50%) or larger I^2 values[46]. Meta-analysis of SNPs associated with $P \leq 1 \times 10^{-5}$ and showing evidence of effect heterogeneity was also performed using a random-effects model[47]. Manhattan and quantile-quantile plots were generated from the resulting data of 1,246,388 SNPs (**Supplementary Fig. 2**).

In gender effects analysis, we analyzed the effect of including gender information in the association analysis for the directly genotyped SNPs at each of the newly identified loci. We analyzed the SNPs in PLINK[48] using a logistic regression model assuming additive effects and with covariate adjustment for population identity and compared the output with results from male- and female-only analyses. In addition, we compared the results to those from a regression analysis where an additional interaction component between gender and genotype was included in the model.

eQTL analysis. In the eQTL analysis, we assessed publicly available data from two published eQTL studies[7,8]. In these data sets, as described in the original publications, association between the genotypes of the most interesting SNPs and gene expression were analyzed using Spearman rank correlation for all genes within a 2-Mb window surrounding the SNP of interest. Significance was assessed by comparing the observed P value at a 0.001 threshold with the minimum P values from each of the 10,000 permutations of the expression values relative to genotypes[7,8,49]. In an additional approach to identify eQTLs, we explored the US National Institutes of Health (NIH) Genotype-Tissue

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