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Meta-analysis of genome-wide association for migraine in six population-based European cohorts

Lannie Ligthart,^{1,2*} Boukje de Vries,^{3*} Albert V Smith,⁴ M Arfan Ikram,⁵ Najaf Amin,⁶ Jouke-Jan Hottenga,¹ Stephany C Koelewijn,³ V Mathijs Kattenberg,¹ Marleen HM de Moor,¹ A Cecile JW Janssens,⁶ Yurii S Aulchenko,⁶ Ben A Oostra,⁶ Eco JC de Geus,¹ Johannes H Smit,⁷ Frans G Zitman,⁸ André G Uitterlinden,⁹ Albert Hofman,⁵ Gonneke Willemsen,¹ Dale R Nyholt,¹⁰ Grant W Montgomery,¹¹ Gisela M Terwindt,¹² Vilmondur Gudnason,^{4,13} Brenda WJH Penninx,^{7,8,14} Monique Breteler,⁵ Michel D Ferrari,¹² Lenore J Launer,¹⁵ Cornelia M van Duijn,^{6*} Arn MJM van den Maagdenberg,^{3*} and Dorret I Boomsma^{1*} for the Dutch Icelandic migraine genetics consortium (DICE)

* Authors contributed equally

¹Department of Biological Psychology, VU University Amsterdam, The Netherlands

²EGMO Institute for Health and Care Research (EMGO+), Amsterdam, The Netherlands

³Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands

⁴Icelandic Heart Association, Heart Preventive Clinic and Research Institute, 201 Kopavogur, Iceland.

⁵Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, The Netherlands

⁶Genetic Epidemiology Unit, Departments of Epidemiology and Clinical Genetics, Erasmus University Medical Centre, The Netherlands

⁷Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, the Netherlands

⁸Department of Psychiatry, Leiden University Medical Centre, Leiden, The Netherlands

⁹Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

¹⁰Neurogenetics Laboratory, Queensland Institute of Medical Research, Brisbane, Australia

¹¹Molecular Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia

¹²Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands

¹³University of Iceland, 101 Reykjavik, Iceland.

¹⁴Department of Psychiatry, University Medical Center Groningen, Groningen, the Netherlands

¹⁵National Institute of Aging, Laboratory for Epidemiology, Demography, and Biometry, Bethesda, Maryland, USA

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Corresponding author:

Lannie Ligthart

Van der Boechorststraat 1

1081 BT Amsterdam, The Netherlands

Tel: +31 (0)20 598 6649

Fax: +31 (0)20 598 8832

Email: rsl.ligthart@psy.vu.nl

Conflict of interest

The authors report no conflict of interest.

Acknowledgments

See supplemental data.

Abstract

Reviewer 3:
comment 1

Migraine is a common **neurologic** disorder with a genetically complex background. This paper describes a meta-analysis of genome-wide association (GWA) studies on migraine, performed by the Dutch-Icelandic migraine genetics (DICE) consortium, which brings together six population-based European migraine cohorts with a total sample size of 10,980 individuals (2446 cases and 8534 controls). A total of 32 SNPs showed marginal evidence for association at a p-value $< 10^{-5}$. The best result was obtained for SNP rs9908234, which had a p-value of 8.00×10^{-8} . This top SNP is located in the nerve growth factor receptor (*NGFR*) gene. However, **this SNP did not**

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replicate in three cohorts from the Netherlands and Australia. Of the other 31 SNPs, 18 SNPs were tested in two replication cohorts, but none replicated. In addition, we

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explored previously identified candidate genes in the meta-analysis data set. This revealed a modest gene-based significant association between migraine and the *MTDH*

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gene, previously identified in the first clinic-based GWAS for migraine (Bonferroni-corrected gene-based p-value = 0.026). This finding is consistent with the involvement

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of the glutamate pathway in migraine. Additional research is necessary to further confirm the involvement of glutamate.

Introduction

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Migraine is a common **neurologic** disorder that is characterized by severe attacks of headache, accompanied by symptoms such as nausea, vomiting, and photo- and phonophobia.¹ Two main types of migraine are distinguished based on the presence of an aura that can precede the headache: migraine with aura (MA) or without aura (MO). Although MA and MO have been considered distinct disease entities,^{2,3} it is now more and more accepted that they represent different manifestations of the same disease.⁴⁻⁶

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Genetic studies in familial hemiplegic migraine (FHM), a rare monogenic subtype of MA that is considered a suitable model for common migraine,⁷ revealed three genes (*CACNA1A*⁸, *ATPIA2*⁹ and *SCN1A*¹⁰) that are involved in ion and neurotransmitter transport in the brain. Despite considerable efforts, **linkage and candidate-gene association** studies in common migraine have had limited success, with only a few consistently replicated **linkage findings**.¹¹⁻¹⁶ A recent genome-wide association study (GWAS), using data from migraine patients who were recruited through headache clinics, found evidence for a role of the metadherin (*MTDH*) gene in common migraine.¹⁷ The associated SNP in that study affects *MTDH* gene expression and thereby indirectly regulates the expression of the glutamate transporter gene *SLC1A2* (also known as *EAAT2* or *GLT-1*), encoding a major glutamate transporter in the brain. This fits in well with the theory that increased glutamate release or reduced glutamate uptake increases the risk of migraine attacks.¹⁸⁻²²

Here, we present a GWA meta-analysis for common migraine by the Dutch Icelandic migraine genetics consortium (DICE). This is the first population-based GWAS for common migraine, including 2446 migraine cases and 8534 controls from six Dutch and Icelandic samples. **For replication, two population-based samples of**

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Dutch and one of Australian origin were available. *De novo* genotyping was performed in the two Dutch replication cohorts (N = 768 and 337 cases; 943 and 826 controls, respectively). In addition, an *in silico* replication study was performed in the Australian replication cohort (N = 1851 cases, 4008 controls).

Methods

Populations: subjects, phenotypes and genotyping

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The five Dutch samples that were used for the meta-analysis came from the Erasmus Rucphen Family study (ERF),^{23,24} the Netherlands Study of Depression and Anxiety (NESDA),²⁵ the Netherlands Twin Registry ('NTR1' and 'NTR2')²⁶ and the Rotterdam study,²⁷ and included 330, 756, 378, 276 and 349 migraine cases, respectively. The Icelandic sample came from the AGES-Reykjavik Study (AGES-RS) and included 357 migraine cases.²⁸ In addition to the 2446 migraine cases, 8534 non-migraine controls

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(2862 Icelandic and 5672 Dutch controls) from the respective cohorts were included (for details, see Table 1). All individuals came from population-based samples and were unrelated, with the exception of the ERF participants, who are part of a genetically isolated population in the Southwest of the Netherlands. Data on migraine symptomatology were collected by means of questionnaires (i.e. AGES, NESDA, NTR1-2, Rotterdam), or a combination of questionnaires and telephone interview follow-up (i.e. ERF).

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Three additional independent samples were available for replication; two Dutch population-based samples (the GEM sample²⁹ and a third sample from the NTR), and one Australian sample, the Australian Twin Migraine (ATM) GWA study.^{30,31} The Dutch GEM sample included 773 migraine cases and 942 non-migraine controls. The

NTR-replication sample consisted of 567 cases and 1602 non-migraine controls, and the Australian sample consisted of 5859 unrelated individuals (1851 migraine cases, 1631 non-migraine controls, and 2377 additional unselected controls).

Genotyping was performed using a variety of SNP genotyping platforms. To ensure sufficient overlap between studies, genotypes for ~2.5 million HapMap SNPs were imputed using MACH or IMPUTE software. An overview of the samples, including details on sample size, genotyping and imputations is provided in Table 1. More details on the background of the studies, phenotyping strategies and genotyping procedures can be found in the supplementary data.

Genome-wide association and meta-analysis

In each sample, a logistic regression association test was carried out. Next, a meta-analysis was performed combining the GWA results of the six samples (total number of individuals: 10,890) using the METAL program (<http://www.sph.umich.edu/csg/abecasis/metal/>). Since different phenotype definitions were used in the different samples, the effect sizes may not be directly comparable between studies. Therefore, a pooled Z-score approach was used. With the pooled Z-score method, an overall Z-score is calculated based on the summed Z-scores from the individual studies, weighted by each study's sample size. The weights are calculated as the square root of ($N_{\text{study}}/N_{\text{total}}$), and the squared weights sum to one. The direction of effect is indicated by the sign of the Z-score. To ensure that meta-analysis results were based on SNP data of a large enough number of individuals, 184,350 SNPs that were available for less than 70% of all participants were excluded from the meta-analysis. This left a total of 2,394,913 autosomal SNPs for analysis. Annotation of meta-analysis

results was performed with WGA viewer, version 1.26E. p-values $< 5 \times 10^{-8}$ were considered genome-wide significant.³²

Replication studies

A replication study was performed with direct genotyping in the GEM and the **NTR replication** samples. The top SNP was genotyped with a TaqMan assay in both GEM and the **NTR replication** sample. In addition, another 18 SNPs with a p-value $< 1 \times 10^{-5}$ were selected, based on informativeness given the LD structure. These SNPs were genotyped in the GEM sample using an in house Sequenom iPLEX Mass-ARRAY platform. Logistic regression was performed to test for association between these SNPs and migraine status. Third, all DICE SNPs with a p-value $< 1 \times 10^{-4}$ in the meta-analysis were selected, and for these SNPs, an *in silico* replication study was performed in the ATM GWA data set. Finally, the 19 SNPs that were genotyped in GEM and the **NTR replication** sample and measured or imputed in the ATM GWA replication sample were meta-analysed together with the discovery datasets. A more detailed description of the genotyping procedures and association analyses can be found in the supplementary data.

Posthoc analyses

Text mining

Relationships between genes (emerging from the meta-analysis) and migraine were studied using the Anni text-mining program (Anni version 2.1).³³ **For details see the supplementary data.**

Comparison of results with migraine genes and loci from previous studies

Genome-wide linkage studies and association studies for migraine were identified with a literature search in PubMed. We examined which SNPs with a p-value $< 1 \times 10^{-4}$

coincided with a region containing a published migraine linkage peak. In addition, a selection of migraine candidate genes was made and inspected in our meta-analysis dataset by calculating a gene-based p-value for each of the selected genes using the VEGAS program. More details can be found in the Supplementary methods.

Results

Meta-analysis

Genome-wide association analyses were performed in the six population-based samples and the results were meta-analyzed using a pooled Z-score approach. As shown in Figure 1, no systematic deviation from the expected distribution of p-values was observed in the Q-Q plot, which is reflected by a genomic inflation factor (λ) of 1.022. A total of 32 SNPs had a p-value $< 1 \times 10^{-5}$ (Table 2). None of these SNP exceeded the threshold for genome-wide significance (Figure 2). Ten SNPs were located within genes; eight in the metastasis associated in colon cancer 1 (*MACC1*) gene (7p21), one in the immunoglobulin lambda-like polypeptide 1 (*IGLL1*) gene (22q11), and one in the nerve growth factor receptor (*NGFR*) gene (17q21-q22). The most significant result was obtained for SNP rs9908234 ($p = 8.00 \times 10^{-8}$) in the *NGFR* gene, with the strongest evidence coming from the AGES and NESDA studies (Supplementary Table 1). Data for 17 additional SNPs in this gene were available, but none of these were associated with migraine (all p-values > 0.05). These SNPs were not in LD with rs9908234. Next, we performed text-mining with the Anni program. The concept ‘migraine’ was matched against the genes located within or close to our top SNPs (p-value $< 1 \times 10^{-4}$).

Remarkably, the *NGFR* gene surfaced as the best migraine candidate gene.

Replication analysis of the top SNPs

From the 32 top SNPs with p-values $< 1 \times 10^{-5}$, we selected 19 SNPs for genotyping in the GEM sample. The selection was made such that the genotyped SNPs were maximally informative given the LD between them. The top SNP rs9908234, located in the *NGFR* gene, was genotyped in one additional replication sample from the NTR. The association observed in the discovery samples could not be replicated for rs9908234 (GEM: OR = 0.86, p = 0.31; NTR replication sample: OR = 0.96, p = 0.778; see Supplementary Table 2). The findings for the other 18 SNPs were not replicated either. None showed a p-value < 0.05 in the GEM sample: the smallest p-value observed was 0.10, but this effect was in the opposite direction compared to the meta-analysis.

An *in silico* replication study was performed in the ATM GWA sample. This analysis included all SNPs with p-values $< 1 \times 10^{-4}$ in the DICE meta-analysis. In the ATM GWA sample, there were data for 327 out of 340 SNPs with p-values $< 1 \times 10^{-4}$. None of these SNPs had a p-value < 0.01 in the ATM GWA data set. Eleven SNPs had p-values between 0.01 and 0.05, but for only three SNPs the effect was in the same direction as in the DICE cohorts (rs6919479, p = 0.045; rs9363693, p = 0.045, and rs9294736, p = 0.037; all on chromosome 6). These results were not significant after correction for multiple testing.

Finally, a new meta-analysis was carried out in the DICE discovery samples, the GEM sample and the ATM GWA sample, for the 18 SNPs genotyped in GEM. For rs9908234, the meta-analysis also included the NTR replication cohort. The p-values for these SNPs did not decrease compared to the first meta-analysis (Supplementary Table 2).

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Comparison of meta-analysis results with prior genetic findings in migraine

The large sample size of the present study provided a unique opportunity to further investigate previous findings from linkage and candidate gene studies on a larger scale, and to try and replicate the findings recently reported in a large clinic-based GWAS for migraine.¹⁷

First, we investigated whether there were any SNPs with p-values $<10^{-4}$ that were located in previously identified migraine linkage regions (Supplementary Table 3). Five SNPs were located on chromosome 10q22-q23, a locus that has been reported for migraine several times.^{11,15,16} However, none were located in or near a gene that could easily be linked to migraine pathophysiology. Interestingly, one SNP (rs1972860, $p = 6.02 \times 10^{-5}$) was located in the glutamate receptor, ionotropic, delta 2 (*GRID2*) gene on chromosome 4q22, a region reported in several different migraine linkage studies.¹¹⁻¹⁴

In addition, we performed a gene-based association test for selected candidate genes for migraine (Table 3). Seven candidate genes were selected based on the results of previous candidate gene association studies for common migraine. Furthermore, a recently published GWAS of clinic-based migraine identified a SNP (rs1835740) that was located between two interesting candidate genes: the metadherin (*MTHD*) gene and the plasma glutamate carboxypeptidase (*PGCP*) gene.¹⁷ An eQTL analysis revealed that rs1835740 most likely affects migraine through *cis*-regulation of *MTHD*, which in turn downregulates *SLCIA2*, a gene that encodes an important glutamate transporter in the brain. Therefore, we selected *MTHD*, *PGCP* and *SLCIA2* as candidate genes, and also inspected SNP rs1835740 and two nearby correlated SNPs (rs982502 and rs2436046). Finally, the three FHM genes (*CACNA1A*, *ATPIA2* and *SCN1A*) were included in the analysis.

Gene-based tests were performed for each of the selected candidate genes, using the meta-analysis results of all SNPs tested in the respective genes (Table 3). A gene-based test result was considered significant at an alpha level of 0.05, with Bonferroni correction for thirteen tests, which corresponds to a gene-based p-value of $0.05/13 = 0.0038$. None of the genes identified through candidate gene association studies were significantly associated with migraine in the meta-analysis. Although there were nominally significant SNPs in the *LTA*, *ESRI*, and *INSR* genes, results were not significant after correction for the number of SNPs tested within the respective genes. The *PGCP* and *SLCIA2* genes also had several nominally significant SNPs, but again were not significant in the gene-based test. However, in the *MTHD* gene, 19 of the 28 tested SNPs had a p-value < 0.01 in the meta-analysis (Supplementary Table 4). The gene-based p-value for *MTDH* was 0.002, which remained significant after Bonferroni correction. The SNP that showed association in the clinic-based GWA study (rs1835740)¹⁷ did not show significant association with migraine in the meta-analysis (p = 0.64). Two nearby SNPs (rs982502 and rs2436046) reported in the same GWAS, were also not associated with migraine in the present study.

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Finally, we tested the three FHM genes, and found several nominally significant SNPs within *CACNA1A* and *ATPIA2*. The gene-based test for *CACNA1A* (best SNP rs3764615, p = 0.004) was not significant (p = 0.30). The gene-based p-value for *ATPIA2* (best SNP rs2854248, p = 3.62×10^{-4}) was 0.006.

Discussion

This study describes the first meta-analysis of genome-wide association studies for population-based migraine, and contains a total of 2446 migraine cases and 8534

controls. The best p-value was obtained for SNP rs9908234, which is located in the nerve growth factor receptor (*NGFR*) gene. A replication study was performed in two Dutch replication cohorts that were available for wet replication; the GEM cohort (773 cases, 942 controls) and the NTR replication cohort (567 cases, 1602 controls). In addition, the ATM GWA cohort (1851 cases, 1631 controls) was available for *in silico* replication. Although the *NGFR* gene is an interesting candidate gene for migraine, the association of *NGFR* with migraine could not be replicated in these cohorts. Eighteen additional top SNPs (p-value < 10⁻⁵) from the meta-analysis were tested in the GEM cohort and the ATM GWA cohort, but none could be replicated successfully.

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There are several possible explanations for the lack of replication. First, several different genotyping platforms were used, which made imputation necessary to ensure sufficient overlap between the studies. Also, two different programs (MACH and IMPUTE) were used for imputation. However, given that MACH and IMPUTE use very similar imputation algorithms, and have been reported to be very similar in imputation accuracy,³⁵ we do not expect this to have a major effect on our results. Second, there were some differences between the samples in the precision of the migraine diagnoses, and in most samples a clinical migraine diagnosis was not available. This is often the case in population-based studies because, for reasons of efficiency, diagnoses are commonly made with (short) headache questionnaires. Unlike in clinic-based studies, they are not usually further evaluated with more extensive questionnaires or interviews by specialized physicians. Less accuracy of diagnosis may result in reduced power to detect association. The phenotypic differences also extend to the control groups, since all non-migraine individuals were included as controls. These differences between studies mean that effect sizes may not be directly comparable. To

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address this, a pooled Z-score meta-analysis was performed. This type of analysis does not require a direct comparison of effect sizes.³⁶ Third, population-based cohorts also include many patients that have less severe migraine and a lower attack frequency. This means they may be a genetically more heterogeneous group than patients from clinic-based cohorts. In addition, they are likely to have a lower genetic risk of migraine than the more severely affected patients in clinic-based cohorts. As a consequence, population-based studies may require a larger number of patients for sufficient power. Given that this study replicates previous findings, but does not produce genome-wide significant results, insufficient power (possibly due to the reasons above) seems the most likely explanation for the lack of replication of our top results. A lack of power

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makes it difficult to distinguish between true associations and false positive findings in the original meta-analysis. Therefore, when the discovery samples have insufficient

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power, SNPs selected for replication based on small p-values may not replicate (even in sufficiently large replication samples) because they are false positives. Finally, it should

be mentioned that the NESDA sample differed from the other samples because the majority of NESDA participants were selected for major depression. Due to the comorbidity of migraine and major depression, there is a higher prevalence of migraine

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in this sample than in the other samples. However, given that the percentage of MDD was similar in the migraine cases and the controls (94.3% vs. 86.6%), any associations detected in this sample will be related to migraine and not MDD.

In the present study we also investigated SNP rs1835740 that was found to be significantly associated with migraine with aura in the first GWA study of clinic-based populations.¹⁷ This SNP is located on 8q21 between the *MTDH* and *PGCP* genes. The

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SNP itself was not associated with migraine in our study, but our gene-based analyses provided modest support for an association of *MTDH* with migraine.

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In summary, although this study does not provide genome-wide significant association of a SNP with migraine, it provides suggestive evidence for an association with the *MTDH* gene, which is involved in the glutamate pathway, previously hypothesized to play a role in migraine based on findings in familial hemiplegic migraine.³⁷ Clearly, even though a large number of patients and controls were included, the present study suffered from a lack of power. In addition to simply increasing the sample size, additional strategies may be necessary aimed at minimizing phenotypic and genetic heterogeneity. Strategies to achieve this can include the identification of reliable biomarkers or stratification of samples based on phenotypic similarity (e.g. by looking at trait components,¹¹ specific symptoms,^{16,38} and/or comorbid pathology). In addition, in future studies it may be worth focusing specifically on the glutamate pathway to assess whether genetic variants affecting glutamate levels are systematically associated with migraine.

Supplementary information is available at European Journal of Human Genetics website (<http://www.nature.com/ejhg>)

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Titles and legends to figures

Figure 1

Q-Q plot showing the expected and observed distribution of p-values in the meta-analysis that included the five Dutch samples and the Icelandic sample. The genomic inflation factor (λ) for the meta-analysis was 1.022.

Figure 2

Manhattan plot showing the p-values by chromosome for the meta-analysis.

Table 1: Sample descriptives.

	AGES	ERF	NESDA	NTR1	NTR2	Rotterdam
<i>Subjects</i>						
Total N	3219	1546	1530	1593	1094	1998
N cases (♂, ♀)	357 (71, 286)	330 (81, 249)	756 (165, 591)	378 (69, 309)	276 (59, 217)	349 (79,270)
N controls (♂, ♀)	2862 (1281,1581)	1216 (615, 601)	774 (322, 452)	1215 (509, 706)	818 (396, 422)	1649 (805,844)
Mean age (SD)	51.22 (± 6.33)	48.4 (± 14.6)	42.9 (± 12.5)	44.8 (± 15.0)	48.6 (± 14.4)	55.37 (± 4.51)
<i>Genotyping & Imputation</i>						
Platform	Illumina 370CNV	Illumina HumanHap300 HumanHap370 Affymetrix 250K Nsp array	Perlegen/Affymetrix 600K	Perlegen/Affymetrix 600K	Illumina Human660W- Quad BeadChip	Illumina Infinium II HumanHap550 version 3.0
Software used for imputation	MACH 1.0.16	MACH	IMPUTE	IMPUTE	IMPUTE	MACH 1.0.15
Reference set	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU
NCBI build	36	36	36	36	36	36
Hapmap release	22	22	22	22	24	22
Number of SNPs analyzed	2,408,991	2,135,034	2,432,125	2,431,993	2,542,087	2,450,030
Software for association analysis of imputed data	ProbABEL	ProbABEL	SNPTEST	SNPTEST	SNPTEST	ProbABEL

Table 2: Selected SNPs with p-values < 1x10⁻⁵ in the meta-analysis.

SNP	Chr	P-value	Base pair position	Type of SNP	Nearest gene	Distance to gene (Kb)	Distance		Frequency		Direction of effect*	Number of SNPs in region (p < 1 x 10 ⁻⁵)
							A1	A2	A1	A2		
rs9908234	17	8.00 x 10 ⁻⁸	44932347	intronic	<i>NGFR</i>	0	A	G	0.93	-----	1	
rs11636768	15	3.23 x 10 ⁻⁷	85496515	intergenic	<i>AGBL1</i>	164.2	A	G	0.15	++++?+	1	
rs10275320	7	1.56 x 10 ⁻⁶	20148579	intronic	<i>MACC1</i>	0	A	G	0.15	-----	8	
rs4939879	18	1.82 x 10 ⁻⁶	45399981	intergenic	<i>LIPG</i>	26.7	A	G	0.47	++++++	1	
rs4861775	4	3.28 x 10 ⁻⁶	180553645	intergenic	<i>AGA</i>	1953.1	A	C	0.81	-----	1	
rs986222	10	3.37 x 10 ⁻⁶	91920867	intergenic	<i>KIF20B</i>	396.2	A	G	0.46	++++++	16	
rs6107848	20	5.90 x 10 ⁻⁶	6539116	intergenic	<i>BMP2</i>	157.6	A	G	0.37	+++++-	1	
rs140174	22	6.98 x 10 ⁻⁶	22252983	intronic	<i>IGLL1</i>	0	A	G	0.75	-----	1	
rs1146161	1	9.27 x 10 ⁻⁶	115460299	intergenic	<i>TSPAN2</i>	26.7	A	C	0.18	++++++	1	
rs4742323	9	9.70 x 10 ⁻⁶	7276743	intergenic	<i>KDM4C</i>	111.1	C	G	0.61	-----	1	

Chr = chromosome; A1 = effect allele in meta-analysis; A2 = non-effect allele.

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A total of 32 SNPs had a p-value < 1 x 10⁻⁵. In case multiple SNPs were located close together in the same region, the most significant SNP is reported. In the last column the number of neighboring SNPs that exceeded the threshold is shown (chromosome 7; 8 SNPs within a 43.7 Kb region; chromosome 10, 16 SNPs in a 104.3 Kb region). *The direction of effect of the respective SNP is given for each of the six samples, in the following order: AGES, ERF, NESDA, NTR1, NTR2, Rotterdam. A question mark indicates that a SNP was not tested in a particular sample (because it was removed during quality control). Positions are based on NCBI Build 36. The frequency of A1 was calculated as a weighted average across all samples.

Table 3: Results of the meta-analysis in previously identified candidate genes and genes previously implicated in familial hemiplegic migraine.

Gene Symbol	Reference	Location	Best SNP in meta-analysis		Pooled Z-score	Pooled P-value of effect	Direction	N SNPs with		Total N SNPs tested	Gene-based p-value		
			Base pair position					P < 0.05	P < 0.01				
<i>Genes identified through candidate gene association studies</i>													
<i>MTHFR</i>	39-44	1p36.3	rs4846049	11772952	-1.802	0.0715	-----	T	G	0	0	35	0.303
<i>LTA</i>	45,46	6p21.3	rs3093542	31648672	2.553	0.0107	++++++	C	G	1	0	4	0.168
<i>TNF</i>	47	6p21.3	rs3093662	31652168	-1.405	0.1600	-----	A	G	0	0	3	0.163
<i>ESR1</i>	48-50	6q25.1	rs9322336	152242123	-3.186	0.0014	-----	T	C	23	9	425	0.608
<i>DBH</i>	51-53	9q34	rs129882	135513490	1.841	0.0656	++-+++	T	C	0	0	52	0.415
<i>ACE</i>	39,54	17q23	rs4305	58911961	-1.095	0.2733	-----	A	G	0	0	15	0.209
<i>INSR</i>	55	19p13.3-13.2	rs8103483	7096374	1.995	0.0460	++++++	T	C	1	0	144	0.289
<i>Genes identified through GWAS</i>													
<i>MTDH¹</i>	17	8q22-q23	rs2438224	98760646	3.237	0.0012	++++++	A	G	19	19	28	0.002*
<i>PGCP</i>	17	8q22-q23	rs2455044	97767418	2.696	0.0070	++++++	A	G	24	3	250	0.290
<i>SLC1A2</i>	17	8q22-q23	rs1570226	35371222	-2.683	0.0073	---+--	T	G	19	4	209	0.319
<i>Genes for familial hemiplegic migraine</i>													
<i>ATPIA2</i>	9	1q21-q23	rs2854248	158360551	3.566	0.0004	++++++	A	T	5	4	20	0.006
<i>SCN1A</i>	10	2q24.3	rs12151636	166630459	2.142	0.0322	+?+--+	T	C	1	0	99	0.806
<i>CACNA1A</i>	8	19p13	rs3764615	13424952	2.903	0.0037	-----	A	C	17	9	241	0.305

The best results in the meta-analysis, located within genes previously reported to be associated with migraine in a candidate-gene study, and genes involved in familial hemiplegic migraine. The direction of effect for the best SNP is indicated per sample, in the following order: AGES, ERF, NESDA, NTR1, NTR2, Rotterdam. A question mark indicates the SNP has not been tested for a particular sample, because it was removed during quality control. A1 is the effect allele, A2 is the non-effect allele. ¹ Not the gene itself, but a nearby SNP regulating its expression (rs1835740) was associated with migraine in the original study. * Significant at $\alpha = 0.05$ level after Bonferroni correction for 13 tests.

Supplementary data

Populations: subjects and phenotypes

AGES-RS

The Reykjavik Study is a population-based cohort study established in 1967 to prospectively study cardiovascular disease in Iceland. The cohort included a random sample of men and women born between 1907 and 1935 and living in Reykjavik at baseline. In 2002, the Reykjavik Study continued as the AGES-Reykjavik Study to examine risk factors, genetic susceptibility, and gene-environment interactions in relation to disease and disability in old age. Headache data were collected as part of the Reykjavik study. The Reykjavik Study and AGES-Reykjavik Study have been described in detail elsewhere.¹⁻⁴ The AGES-Reykjavik study included 357 migraine cases (71 male, 286 female). The control group included 1281 males and 1581 female, a total of 2862 controls. The mean age was 51.03 years (SD = 6.37). All subjects were unrelated.

ERF

The ERF study is a family-based study in a genetically isolated population in the southwest of the Netherlands. This young genetic isolate was founded in the mid 18th century and minimal immigration and/or marriages occurred between surrounding settlements due to social and religious reasons. The ERF population includes 3,465 individuals that are living descendants of 22 couples with at least six children baptized in the community church around 1850–1900. The subjects were unselected with respect to phenotypes. Details about the extensive genealogy and pedigree of the population are described elsewhere.⁵

The present study includes data from 1546 ERF participants; 330 migraineurs and 1216 controls. Of the cases, 81 (25%) were male and 249 (75%) were female; of the controls, 615 (51%) were male and 601 (49%) were female. The mean age was 48.4 years (SD = 14.6).

NESDA

The NESDA sample⁶ consisted of 1530 unrelated individuals from the Netherlands (mostly patients with major depressive disorder [MDD]) who were genotyped in the context of the NIH GAIN project, for a GWAS study originally designed to find genes for major depressive disorder (MDD).⁷ In the NESDA sample, 1383 subjects had MDD, and 147 were selected for low risk of MDD. In this sample, there were 756 individuals with migraine (713 with MDD and 43 without MDD) and 774 controls (670 with MDD and 104 without MDD). In the case group, 165 individuals (22%) were male and 591 (78%) were female. In the control group, 322 (42%) were male and 452 (58%) were female. The mean age was 42.9 years (SD = 12.5).

NTR1

The Netherlands Twin Registry collects data in Dutch twins, their parents, siblings and partners. The migraine data were collected in the context of a longitudinal study on health, lifestyle and personality. As the NESDA sample, the first NTR cohort was genotyped as part of the GAIN MDD study.⁷ The majority of subjects (N = 1481) were selected for low risk of MDD, 112 subjects were MDD patients. Migraine data were available for 1593 individuals: 378 cases [56 with MDD and 322 without MDD], and 1215 controls [56 with MDD and 1159 without MDD]. In the case group, 69 individuals (18%) were male and 309 (82%) were female. In the control group, 509 (42%) were male and 706 (58%) were female. The mean age was 44.8 years (SD = 15.0). All subjects were unrelated.

NTR2

The second cohort from the Netherlands Twin Registry was an unselected sample. All subjects were unrelated. For 1094 individuals, migraine data were available. There were 276 migraine cases, including 59 (21%) males and 217 (79%) females. The control group consisted of 818 individuals, including 396 (48%) males and 422 (52%) females. The mean age in this cohort was 48.6 years (SD = 14.4).

Rotterdam Study

This sample included participants of the Rotterdam Study, a prospective population based cohort study among persons 55 years or older who were living in Ommoord, a well-defined district of Rotterdam, the Netherlands.⁸ The aim of this study was to investigate causes of frequent chronic diseases, with a focus on cardiovascular, neurologic, psychiatric, and ophthalmic diseases. The Medical Ethics Committee of Erasmus Medical Center approved of the study. The original cohort of the Rotterdam Study (7,983 participants) was expanded in 2000 (N = 3,011) and again in 2006 to include 3,919 persons who were 45 years of age or older. At study entry all participants underwent a structural interview and a physical examination, which was repeated every 3-4 years. The migraine questionnaire was introduced into the core study protocol in 2006 (response rate of 64.8%). For the current report, we used data from persons from the second cohort expansion (2006 to 2008) who completed the migraine questionnaire. Migraine data were available for 1,998 unrelated individuals, including 349 cases (79 male, 270 female) and 1,649 controls (805 male, 844 female). The mean age of the sample was 55.37 years (SD=4.51).

GEM

The Genetic Epidemiology of Migraine (GEM) cohort is well characterized population-based migraine cohort from the Netherlands.⁹ The GEM cohort is embedded in the MORGEN project, a population-based study designed to monitor risk factors for and the prevalence of chronic diseases of public health importance in Dutch adults from 20 to 60 years old. DNA was available from 773 unrelated migraine cases and 942 unrelated non-migraine controls.

Australian Twin Migraine GWA Study

The Australian Twin Migraine (ATM) GWA study includes data from Australian twins and their families. All cases and controls included in this study were unrelated individuals; one individual was selected from each family. The cases (N = 1851; 389 [21%] male, 1462 [79% female]) were preferentially selected from each family based on migraine severity. The non-migraine controls (N = 1631; 772 [47%] male, 859 [53%] female) were selected from families containing no migraine cases. The

unselected controls (N = 2377; 1128 [47%] male, 1249 [53%] female) came from families containing no individuals with migraine information. The mean age at interview was 37.5 years (SD = 11.3). All subjects gave informed consent and approval to conduct the research was obtained from the QIMR Human Research Ethics Committee.

NTR replication sample

The third sample from Netherlands Twin Registry consisted of unrelated individuals, unselected with respect to migraine. Migraine data were available for 1163 individuals. There were 337 migraine cases, including 72(21%) males and 265 (79%) females. The control group consisted of 826 individuals, including 347 (42%) males and 479 (58%) females. The mean age in this cohort was 40.5 years (SD = 14.3).

Phenotypes

AGES-RS

Subjects reporting headache at least once a month were asked whether the headaches were accompanied by any of the following migraine features: nausea/vomiting, unilateral location, photophobia, visual disturbance during or preceding headache, and unilateral numbness preceding headache. Individuals were defined as having migraine with aura if they had visual or sensory aura, or both. Subjects with at least 2 of the non-aura symptoms were classified as having migraine without aura. In this study, both migraine with and without aura were included as cases. The remaining individuals were included as controls.¹⁰

ERF

Migraine was diagnosed according to ICHD-II criteria.¹¹ Migraineurs were identified using a three-stage screening procedure which has been validated in a population based study⁹. The screening procedure is described in detail by Stam and colleagues.¹² In brief, all participants filled out a concise screening questionnaire on headache and aura symptoms, and those who screened positive also completed a detailed extended questionnaire. The screening questions select primarily on moderate to severe headache.

Therefore, patients with aura symptoms without headache will not pass the screening questions positively. Based on the characteristics of this questionnaire, we assumed this screening instrument to have a very high positive predictive value in the ERF population. The extended questionnaire was based on the ICHD-II¹¹ and included multiple items on primary migraine headache and aura characteristics, premonitory symptoms, trigger factors, and medication use. The questions were to be answered by choosing from categorical alternatives.

All participants who screened positive were telephone-interviewed to clarify their clinical symptoms by trained physicians who are experienced in diagnosing migraine patients. Final diagnosis was always made after this telephone interview and in consultation with a neurologist specialized in headache (GMT). The control group consisted of ERF participants negative for migraine based on the written three-stage screening procedure.

NESDA, NTRI-3

Migraine was assessed with a questionnaire that provided information on the symptoms listed in the ICHD-II criteria. For the NTR participants, the headache questions were embedded in surveys that were held in the context of a longitudinal study on health, lifestyle and personality. The data used in this study were collected in two waves that took place in 2002 and 2004. Both surveys included the same set of headache items. Data collection procedures are described in detail elsewhere.^{13,14} When a participant answered the headache section in both surveys, the most recent (2004) survey was used.

The NESDA participants underwent a 4-hour baseline assessment at one of seven clinic sites at the beginning of the study. This assessment included an interview on somatic health, functioning and health care use, and the administration of several written questionnaires. Headache data were collected using the same questionnaire that was included in the NTR survey. Further details on the NESDA data collection procedures can be found elsewhere.⁹

The NTR/NESDA migraine questionnaire was preceded by a screening question (“do you ever experience headache attacks, for instance migraine?”). Individuals screening positive subsequently answered a set of more detailed questions about their headaches. This information was used to determine the presence of eight of the

symptoms present in the ICHD-II criteria: moderate/severe pain intensity, aggravation by physical activity, pulsating quality, nausea or vomiting, and photo- or phonophobia. The IHS migraine symptom variables were analysed with Latent Class Analysis to determine each participant's affection status for migrainous headache. This method has been described extensively in previous work.¹⁵ The LCA was performed based on headache data from all available NESDA and NTR participants, using the program Latent Gold 4.0 (Statistical Innovations, Inc., Belmont, MA). As in previous studies, four classes of headache sufferers were identified. Participants in the two severest classes (who were positive, on average, for at least half of the symptoms), were classified as affected. The remaining individuals (mild non-migrainous headache and individuals without headaches) were classified as unaffected.

Rotterdam Study

The migraine questionnaire used in the Rotterdam Study was based on the ICHD-II criteria and was a modified questionnaire according to the GEM study of Leiden.¹⁶ The first question was "Have you ever experienced a severe headache that affected your daily activities?" If the answer was negative or if it was clearly indicated that the participants experienced a severe headache due to other causes, such as a tumor, sinusitis, stroke, trauma or meningitis, no further questions on headaches were asked. If the answer to the first question was positive, headache duration and headache frequency were asked. Next, if a person experienced headaches of which 1) the duration was between four and seventy-two hours (untreated) or the participant did not know the answer to this question, because they always treated their headache attack and 2) the attack frequency was two or more attacks in a lifetime, details on the characteristics and symptoms of the headaches were asked. These included age of onset, unilateral location, pulsating quality, aggravation by daily activities, sensitivity to light and sound, nausea or vomiting. The frequency of the symptoms accompanying the headaches was assessed and defined as never, sometimes, half of the time and more than half of the time. In this group of participants, questions on medication use were assessed. Furthermore, every participant was asked about aura symptoms and physician diagnosis, if they ever had a severe headache. If the participant experienced an aura or the physician had diagnosed migraine, questions on medication use were assessed. Participants whose duration of

headache was unknown, because they always used medication to prevent or treat the attack, were considered migraineurs if they fulfilled the remaining IHS criteria. Individuals who were not classified as migraineurs were included as controls.

GEM

Participants for the Genetic Epidemiology of Migraine (GEM) study were identified through the population-based Monitoring Risk Factors for Health in the Netherlands (MORGEN) study⁹ - a population-based study designed to monitor risk factors for and the prevalence of chronic diseases of public health importance in Dutch adults 20–65 years of age. The study was approved by the local ethics committee. Respondents signed a general informed consent for the MORGEN project, and a specific informed consent for the GEM Study. For case-finding, MORGEN participants were mailed an extensive self-administered questionnaire that included questions about sociodemographic characteristics, medical history, psychosocial functioning and five migraine screening questions (adapted from Stewart et al.¹⁷). Screen-positive was defined as those who ever had or had in the last 12 months, a severe headache (excluding those due to hangover or sinus infection) and the pain was 5 or higher on a 10-point pain scale or the participant was diagnosed with migraine by a doctor or used antimigraine medication (including sumatripten and ergotamine compounds). A respondent was also classified as screen-positive if there was a history of severe headache in the last 12 months and the pain was rated between 1 and 4 and one of 10 visual aura symptoms was experienced. Trained field workers at the study center reviewed the screening questions and identified screen-positive participants. Additional questions regarding medical history were asked in a personal interview, a simple clinical examination was performed, and a blood sample drawn.

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Participants that were positive on the screening questionnaire completed a more detailed questionnaire that focused on signs and symptoms of migraine headache and aura as outlined in the IHS criteria.¹¹ Very special care was given to diagnose aura and those reporting visual aura symptoms were also asked to draw what they saw. The extended questionnaire was based on the IHS criteria¹¹ and included multiple items on primary migraine headache and aura characteristics, premonitory symptoms, trigger factors, and medication use. The questions were to be answered by choosing from

categorical alternatives. A semi-structured telephone interview to validate the questionnaires was obtained in a random sample of screen positive (83%) and screen negative (5%) participants to clarify their clinical symptoms. This interview was done by trained physicians or medical students who are experienced in diagnosing migraine patients. Final diagnosis was always made after this telephone interview and in consultation with a neurologist specialized in headache.

The control sample was drawn from the participants that were screen-negative on the five migraine screening questions and matched the cases for age and gender.

Australian Twin Migraine GWA Study

In the Australian sample, migraine data were collected by means of a semi-structured telephone interview. The questionnaire was based on the symptoms from the ICHD-II diagnostic criteria.¹¹ Participants were classified as affected or unaffected with latent class analysis, as described for the NTR and NESDA participants. In addition, a diagnosis was made based on the full IHS criteria. The sample was divided into two risk strata: IHS migraine vs. non-migraine controls (narrowly defined) and LCA-migraine vs. unselected controls (broadly defined). This was done to allow for expected differences in migraine risk between these groups. More details on the phenotyping procedures can be found elsewhere.^{18,19}

Genotyping and imputation

AGES-RS

Genotyping was performed using the Illumina 370CNV platform. Genotypes for ~ 2.5 million SNPs were imputed using the MACH 1.0.16 program, using HapMap CEU as the reference set (NCBI build 36, HapMap phase II, release 22). SNPs were excluded in case of a minor allele frequency (MAF) smaller than 1%, call rate < 97%, low imputation quality ($R^2 < 0.3$) or HWE p-value < 1×10^{-6} , leaving 2,408,991 SNPs for analysis.

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ERF

Genotyping was performed on several different platforms (Illumina HumanHap300, HumanHap370, Affymetrix 250K Nsp array). These sets were merged and genotypes

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for ~2.5 million SNPs were imputed to (HapMap CEU, **phase II**, release 22, NCBI build 36) using the MACH program. Data were filtered for rare variants and **imputation**

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quality (SNPs with call rate > 95%, MAF \geq 0.05 and R² \geq 0.3 were included), leaving 2,135,034 SNPs for analysis.

NTR1 and NESDA

Individual genotyping for the GAIN sample was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays. The SNPs on these arrays were selected to tag common variation in the HapMap European and Asian panels. Of the 3,820 Dutch samples sent to Perlegen, genotypes were delivered for 3,761 samples. After quality control, there were 3,540 subjects in the final analysis dataset (1,738 MDD cases and 1,802 controls).

The unfiltered dataset obtained from dbGaP contained 599,156 unique SNPs. To be included in the final analysis dataset, SNPs were required not to have any of the following features: gross mapping problem, ≥ 2 genotype disagreements in 40 duplicated samples, ≥ 2 Mendelian inheritance errors in 38 complete trio samples, minor allele frequency < 0.01 , or > 0.05 missing genotypes in either cases or controls. A total of 427,049 autosomal SNPs met these criteria and were included in the analyses.

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Genotypes for ~2.5 million SNPs were imputed using the IMPUTE software, using the HapMap CEU data (**phase II**, release 22, NCBI build 36), available from the IMPUTE website (<https://mathgen.stats.ox.ac.uk/impute/impute.html>), as reference. For each SNP an R² value was calculated using the QUICKTEST program

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(<http://toby.freeshell.org/software/quicktest.shtml>). SNPs were excluded **in case of a MAF < 1%, per SNP call rate < 95% or low imputation quality (R² < 0.3)**, leaving 2,432,125 SNPs for analysis in the NESDA sample and 2,431,993 in the NTR sample.

NTR2

Genotyping for 657,366 SNPs was performed on the Human660W-Quad BeadChip. SNPs were excluded based on MAF < 0.01 , missing genotype rate > 0.05 or a p-value $< 1 \times 10^{-5}$ in a test of Hardy-Weinberg equilibrium. After quality control, 515,781 SNPs were left. Genotypes of ~3.8 million SNPs were imputed with the IMPUTE program,¹⁷ using the HapMap CEU data (**phase II**, release 24, NCBI build 36), available from the

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IMPUTE website, as reference. Imputed SNPs were excluded if they had a MAF < 0.01, per SNP call rate < 95% or a low imputation quality ($R^2 < 0.3$), leaving 2,542,087 SNPs for analysis.

Rotterdam Study

Genotyping was performed using the Illumina Infinium II HumanHap550 chip, version 3.0. A total of 572,129 SNPs were genotyped. SNPs were excluded based on the following criteria: HWE p-value < 10^{-6} , call rate < 98% and a minor allele frequency < 0.01. The number of SNPs that survived quality control was 514,139. Genotypes were imputed for 2,543,888 SNPs, using the Hapmap CEU (build 36, phase II, rel. 22) as reference. Imputations were performed in MACH.²⁰ SNPs were excluded if they had a

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comment 3

minor allele frequency < 0.01, per SNP call rate < 98%, HWE p-value < 1×10^{-6} or low imputation quality ($R^2 < 0.3$), leaving a total of 2,450,030 SNPs for analysis.

Reviewer 1:
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9/10

GEM & NTR replication samples

The top SNP was genotyped in two samples (GEM and NTR replication sample) using TaqMan technology. Probes and primers were designed by Applied Biosystems. A standard PCR reaction was carried out using the TaqMan Universal PCR Master Mix reagent. Genotyping clusters were analysed using the LightScanner480 machine and LightCycler®480 SW 1.5 software (Roche Applied Science). SNPs deviating from Hardy-Weinberg equilibrium ($p < 0.01$) were excluded from further analysis.

GEM

An additional selection of top SNPs was genotyped by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAY™ methodology (Sequenom Inc, San Diego, CA, USA). Amplification reactions and parameters were based on the manufacturer's instructions. Each 384-wells plate contained at least 4 positive (CEPH DNA) and 6 negative controls, to check for assay performance and contaminations, respectively. Spectrocaller software supplied by the manufacturer was used to automatically call the genotypes. Clusters were checked manually and all doubtful calls were evaluated. SNPs deviating from Hardy-Weinberg equilibrium ($p < 0.01$) were excluded from the analyses.

Australian Twin Migraine GWA Study

The ATM GWA cohort was drawn from 9 different projects utilising a variety of Illumina GWA arrays. After stringent quality control (QC) a consensus set of 280,168 SNPs were available for all individuals; which were then utilised to impute up to the August 2009 release of phased data from the 1000 Genomes Project [112 haplotypes from 56 Caucasians, downloaded from <http://www.sph.umich.edu/csg/yli/mach/download/1000G-Sanger-0908.html>] using the MACH program.²⁰ A total of 7,365,026 SNPs satisfied the recommended imputation QC threshold of $R^2 \geq 0.3$. SNPs with $MAF \geq 0.05$ ($N = 6,085,112$) were included in the GWA analyses.

umich.edu/csg/yli/mach/download/1000G-Sanger-0908.html] using the MACH program.²⁰ A total of 7,365,026 SNPs satisfied the recommended imputation QC threshold of $R^2 \geq 0.3$. SNPs with $MAF \geq 0.05$ ($N = 6,085,112$) were included in the GWA analyses.

Statistical analyses in the individual samples

AGES-RS, Rotterdam

A logistic regression test was performed with sex, age and age² included as covariates. The analyses were performed in ProbABEL.²¹ Uncertainty of imputation was taken into account in the analyses. The genomic inflation factor was 1.002 in AGES-RS and 1.021 in the Rotterdam Study.

ERF

The study-specific genomic inflation factor (λ) in the ERF study was 1.166, reflecting relatedness between study participants. This was corrected for by applying genomic control. Genome-wide association analyses were carried out in ProbABEL,²¹ using a logistic regression test with sex and age included as covariates, assuming an additive model, and accounting for uncertainty of imputation.

NESDA and NTR (all cohorts)

Genome-wide association testing was performed using SNPTEST.¹⁶ A logistic regression test was used, sex, age, and age² were included as covariates and an additive model was assumed. As in the other samples, uncertainty of imputation was taken into account in the analyses. The genomic inflation factors were 1.006, 1.013 and 1.000 in NESDA, NTR1 and NTR2, respectively.

Replication cohorts

In the GEM and NTR replication samples, association tests were performed using Plink.²² For each SNP, a logistic regression test was performed, under an additive model, with sex, age and age² as covariates. For the ATM GWA sample, association testing was performed with the mach2dat program (<http://www.sph.umich.edu/csg/yli/mach/download/>), accounting for genotype uncertainty, and adjusting for sex and the risk strata described above.

Power of the replicaton study

The power to replicate the effect of SNP rs9908234 in the GEM sample was calculated using the genetic power calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/>). Assuming a migraine prevalence of 12%, an OR of 1.35 and a risk allele frequency of .07 (the average allele frequency in the original samples) the power to detect the effect at an alpha level of .05 was 66%, based on 768 cases and 943 controls. The number of cases required for 80% power was 1072. In the NTR replication sample, based on the same parameters and 337 cases and 826 controls, the power was 44% at an alpha level of .05. For a power of 80%, 798 cases would have been needed. However, this is most likely a conservative estimate, given the more liberal phenotype definition, and therefore higher disease prevalence in the NTR samples. Taken together (1105 cases, 1769 controls) the samples were estimated to have a power of 86%.

Reviewer 1:
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Text mining

Relationships between genes (emerging from the meta-analysis) and migraine were studied using the Anni text-mining program (Anni version 2.1).³³ The program generated a concept profile for each gene and for migraine. A concept profile is a summary of all concepts directly co-mentioned with the disease or gene concept (i.e. the main concept) in PubMed abstracts. The strength of association for each concept with the main concept is calculated using 2x2 contingency tables and an uncertainty coefficient. The association between two concept profiles is calculated using vector based matching (e.g. inner product score) over the concepts that the two profiles have in common.

Comparison of results with migraine genes and loci from previous studies

Genome-wide linkage studies for migraine were identified with a literature search in PubMed. Since not all studies clearly specified the confidence intervals of their linkage peaks, the region within 15 Mb of the best linkage marker was inspected. Next, we examined which SNPs with a p-value $< 1 \times 10^{-4}$ coincided with a region containing a published linkage peak. If multiple SNPs had small p-values due to high LD, the SNP with the smallest p-value was retained.

In addition, a selection of candidate genes was made, based on the results of previous association studies (including candidate gene and GWA studies). Genes from candidate gene studies were selected if there was evidence for association with migraine based on one or more studies that included at least 275 migraine cases. Genes were selected only if a study-wide significant result had been obtained in this gene at least once (for MO, MA or MO/MA combined), or if nominal significance was reported in multiple independent studies. We also selected the FHM genes (i.e. *CACNA1A*, *ATPIA2*, and *SCN1A*) and the genes which surfaced as candidates in the first clinic-based GWA study of migraine (*MTHD*, *PGCP* and *SLC1A2*).¹⁷ A gene-based p-value was calculated for each of the selected genes, based on the meta-analysis results of all SNPs tested within the gene. This was done using the VEGAS program (version 0.6.28).³⁴ This gene-based p-value was used to determine whether the results in a gene were significant after correcting for the number of SNPs tested in a gene, taking the LD structure into account. **A Bonferroni correction was applied to the gene-based p-values to account for the fact that thirteen genes were tested.**

Reviewer 1:
comment 3

Finally, we specifically inspected three individual SNPs near the *MTHD* gene that showed a strong association with migraine in the clinic-based migraine GWAS described by Anttila and colleagues.¹⁷

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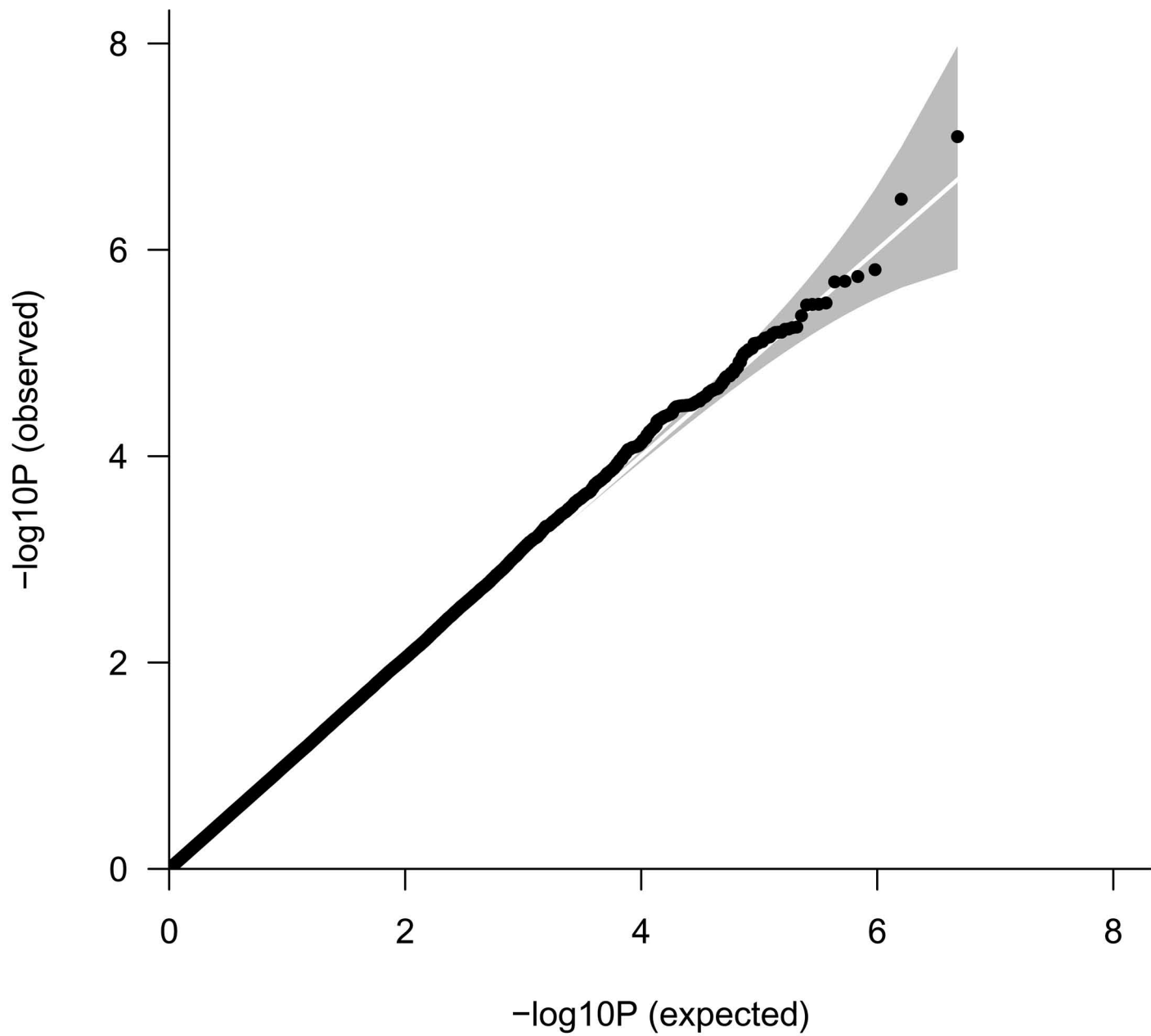
Acknowledgments

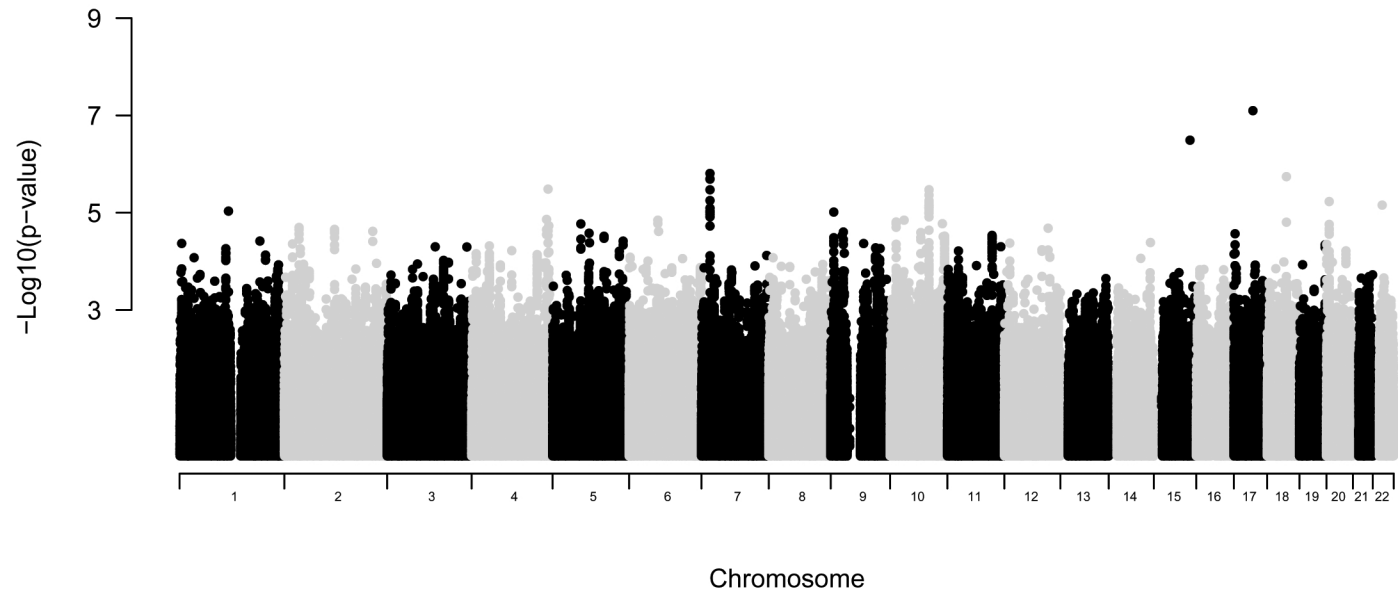
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Supplementary Table 1: Details on top SNP rs9908234 for the individual samples.

Cohort	Beta	SE	OR	P-value	Imputed	R²	MAF
AGES	0.763	0.185	2.145 (1.493 - 3.081)	3.6×10^{-5}	Yes	0.55	0.06
ERF	0.306	0.134	1.357 (1.043 - 1.767)	0.023	Yes	0.61	0.10
NESDA	0.423	0.149	1.527 (1.140 - 2.045)	4.5×10^{-3}	No	-	0.07
NTR1	0.256	0.186	1.292 (0.896 - 1.861)	0.170	No	-	0.06
NTR2	0.215	0.270	1.240 (0.730 - 2.105)	0.426	Yes	0.63	0.05
Rotterdam	0.229	0.217	1.257 (0.822 - 1.922)	0.297	Yes	0.59	0.07

SE = standard error; OR = odds ratio, MAF = minor allele frequency, R² = quality of imputation.
The OR is given for the effect of the minor (risk increasing) allele G, compared to allele A.

Supplementary Table 2: replication study of 19 top SNPs with p-values < 1 x 10⁻⁴

Meta-analysis of the original 6 samples											
SNP	Chr	Base pair position	Type	Nearest gene	Distance to gene (Kb)	alleles	Minor allele (= effect allele)	P-value	Z-score	Direction of effect*	Overall MAF in meta-analysis
rs1146161	1	115460299	intergenic	TSPAN2	26.7	A/C	A	9.27E-06	4.433	++++++	0.181
rs4861775	4	180553645	Intergenic	AGA	1953.1	C/A	C	3.28E-06	4.653	++++++	0.186
rs10275320	7	20148579	intronic	MACC1	0	A/G	A	1.56E-06	-4.804	-----	0.145
rs10252448	7	20173882	intronic	MACC1	0	T/C	C	5.62E-06	-4.540	-----	0.152
rs17142558	7	20192272	intronic	MACC1	0	T/C	C	9.18E-06	-4.436	-----	0.145
rs4742323	9	7276743	intergenic	KDM4C	111.1	C/G	G	9.70E-06	4.424	++++++	0.393
rs2182239	10	91825270	intergenic	KIF20B	300.6	T/C	T	7.84E-06	4.469	++++++	0.392
rs4933548	10	91836719	intergenic	KIF20B	312.0	A/G	A	7.19E-06	4.488	++++++	0.397
rs10881750	10	91859823	intergenic	KIF20B	335.1	A/G	A	9.00E-06	4.440	++++++	0.374
rs750312	10	91896272	intergenic	KIF20B	371.6	T/C	T	5.67E-06	-4.538	-----	0.312
rs7085577	10	91898654	intergenic	KIF20B	374.0	T/C	T	4.35E-06	4.594	++++++	0.423
rs999903	10	91918021	upstream	KIF20B	393.3	A/G	A	3.42E-06	4.644	++++++	0.462
rs986222	10	91920867	intergenic	KIF20B	396.2	A/G	A	3.37E-06	4.647	++++++	0.462
rs1329179	10	91924433	intergenic	KIF20B	399.8	C/G	C	7.09E-06	-4.491	-----	0.319
rs11636768	15	85496515	intergenic	AGBL1	164.2	A/G	A	3.23E-07	5.109	++++?+	0.153
rs9908234	17	44932347	intronic	NGFR	0	A/G	G	8.00E-08	5.367	++++++	0.067
rs4939879	18	45399981	intergenic	LIPG	26.7	A/G	A	1.82E-06	4.773	++++++	0.472
rs6107848	20	6539116	intergenic	BMP2	157.6	A/G	A	5.90E-06	4.530	+++++-	0.367
rs140174	22	22252983	intronic	IGLL1	0	A/G	G	6.98E-06	4.494	++++++	0.255

Chr = chromosome; MAF = minor allele frequency; OR = odds ratio. The OR refers to the effect of the minor allele.

*Direction of effect is indicated for each sample individually, in the following order:

AGES-RS, ERF, NESDA, NTR1, NTR2, Rotterdam

**Direction of effect is indicated for each sample individually, in the following order:

AGES-RS, ERF, NESDA, NTR1, NTR2, Rotterdam, GEM, Australia, NTR3 (the latter for rs9908234 only)

***The column "improvement" indicates whether or not the p-value decreased after adding the replication samples.

A question mark indicates that a genotype for this SNP was not available in a particular sample.

GEM replication sample			
OR	Direction of effect	P-value	MAF
1.09	+	0.373	0.18
1.06	+	0.550	0.21
1.08	+	0.450	0.14
1.04	+	0.712	0.15
1.04	+	0.749	0.14
1.01	+	0.877	0.40
0.97	-	0.667	0.37
0.97	-	0.684	0.38
0.98	-	0.798	0.36
1.14	+	0.104	0.32
0.93	-	0.319	0.41
0.93	-	0.304	0.45
0.93	-	0.341	0.45
1.14	+	0.100	0.32
1.13	+	0.242	0.15
0.86	-	0.305	0.07
0.89	-	0.110	0.47
0.99	-	0.875	0.38
1.01	+	0.879	0.28

Australian replication sample			
AUS OR	Direction of effect	P-value	MAF
1.036	+	0.511	0.17
0.951	-	0.338	0.19
1.045	+	0.522	0.12
1.014	+	0.822	0.14
1.014	+	0.820	0.13
0.927	-	0.075	0.46
0.974	-	0.542	0.37
0.979	-	0.639	0.38
0.978	-	0.617	0.35
1.059	+	0.194	0.32
1.007	+	0.871	0.32
1.008	+	0.851	0.43
1.008	+	0.846	0.44
1.056	+	0.253	0.34
1.248	+	0.151	0.20
0.939	-	0.599	0.07
1.027	+	0.541	0.45
1.007	+	0.870	0.40
1.080	+	0.091	0.28

NTR3 replication sample			
OR	Direction of effect	P-value	MAF
<i>(only genotyped at rs9908234)</i>			
0.89	-	0.579	0.06

Meta-analysis of c
P-value
5.07E-05
1.26E-03
1.86E-03
1.12E-03
1.42E-03
1.42E-02
2.99E-03
2.15E-03
2.22E-03
2.29E-02
8.71E-04
7.46E-04
6.74E-04
9.29E-04
4.52E-07
1.01E-03
4.06E-04
4.12E-04
8.30E-06

original and replication samples	
Direction of effect**	improvement?***
+++++++	no
+++++++	no
-----+	no
-----+	no
-----+	no
+++++++	no
+++++++	no
+++++++	no
+++++++	no
-----+	no
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+++++++	no
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-----+	no
+++++?+++	no
+++++++	no
+++++++	no
+++++++	no
+++++++	no

Supplementary Table 3: comparison of meta-analysis with regions reported in genome-wide linkage studies of migraine

Closest SNP	Location	P-value SNP	Nearest gene	Type	Reference Linkage study	Phenotype	Best marker	Location marker	Dist	LOD score
rs1972860	4q22.2	6.02 x 10 ⁻⁰⁵	<i>GRID2</i>	intronic	1	age at onset	D4S2380	4q22	1.30	HLOD 1.96 ^c
rs1972860	4q22.2	6.02 x 10 ⁻⁰⁵	<i>GRID2</i>	intronic	2	MO	D4S1534	4q21.23	8.27	2.05 ^b
rs1972860	4q22.2	6.02 x 10 ⁻⁰⁵	<i>GRID2</i>	intronic	1, 3, 4	MA, various, migraine broad	D4S1647	4q23	4.86	4.20 ^c , HLOD 4.52 ^{*c} , 2.26 ^b
rs2303655	5q23.2	3.05 x 10 ⁻⁰⁵	<i>ZNF474</i>	intergenic	5,6	LCA, photo/phono	D5S2501	5q22.1	11.48	3.70 ^{b*} , 1.97 ^b
rs6919479	6p21.1	9.86 x 10 ⁻⁰⁵	<i>CDC5L</i>	intergenic	7	MO and MA	D6S452	6p12.2-21.1	2.46	5.41 ^c
rs10999688	10q22.1	2.55 x 10 ⁻⁰⁵	<i>UNC5B</i>	intergenic	5	LCA migraine	D10S2327	10q22.3	7.83	2.32 ^b
rs10999688	10q22.1	2.55 x 10 ⁻⁰⁵	<i>UNC5B</i>	intergenic	1	Various	D10S1786	10q23.1	11.37	7.68 ^{*b}
rs11200686	10q23.1	5.76 x 10 ⁻⁰⁵	<i>GHITM</i>	intergenic	5	LCA migraine	D10S2327	10q22.3	5.21	2.32 ^b
rs11200686	10q23.1	5.76 x 10 ⁻⁰⁵	<i>GHITM</i>	intergenic	8	Various	D10S1786	10q23.1	1.67	7.68 ^{*b}
rs3862561	10q23.1	5.65 x 10 ⁻⁰⁵	<i>GHITM</i>	intergenic	5	LCA migraine	D10S2327	10q22.3	5.19	2.32 ^b
rs3862561	10q23.1	5.65 x 10 ⁻⁰⁵	<i>GHITM</i>	intergenic	8	Various	D10S1786	10q23.1	1.65	7.68 ^{*b}
rs4933526	10q23.31	1.02 x 10 ⁻⁰⁵	<i>KIF20B</i>	intergenic	5	LCA migraine	D10S2327	10q22.3	11.39	2.32 ^b
rs4933526	10q23.31	1.02 x 10 ⁻⁰⁵	<i>KIF20B</i>	intergenic	8	Various	D10S1786	10q23.1	7.85	7.68 ^{*b}
rs986222	10q23.31	3.37 x 10 ⁻⁰⁵	<i>KIF20B</i>	intergenic	5	LCA migraine	D10S2327	10q22.3	11.54	2.32 ^b
rs986222	10q23.31	3.37 x 10 ⁻⁰⁵	<i>KIF20B</i>	intergenic	8	Various	D10S1786	10q23.1	8.00	7.68 ^{*b}
rs1946047	11q24.2	5.00 x 10 ⁻⁰⁵	<i>PRR10</i>	intergenic	9	MA	D11S4464	11q24.1	3.05	5.6 ^a
rs2722223	12q21.33	5.78 x 10 ⁻⁰⁵	<i>ATP2B1</i>	intergenic	1	aggravation	D12S1064	12q21.33	0.59	HLOD 2.17 ^c
rs5028961	12q23.3	5.85 x 10 ⁻⁰⁵	<i>TXNRD1</i>	intergenic	1	aggravation	D12S1064	12q21.33	13.97	HLOD 2.17 ^c
rs6539150	12q23.3	2.08 x 10 ⁻⁰⁵	<i>CHST11</i>	intergenic	1	aggravation	D12S1064	12q21.33	13.98	HLOD 2.17 ^c
rs11851709	14q32.2	4.10 x 10 ⁻⁰⁵	<i>C14orf177</i>	intergenic	10	FHM/MO/MA	rs1054195	14q32.13	3.73	3.83 ^c
rs11247555	17p13.3	7.08 x 10 ⁻⁰⁵	<i>VPS53</i>	intronic	1	Pulsation	D17S945	17p13.1	9.30	HLOD 4.65 ^c
rs9913267	17p13.3	2.71 x 10 ⁻⁰⁵	<i>GARNL4</i>	intronic	1	Pulsation	D17S945	17p13.1	6.97	HLOD 4.65 ^c
rs6066559	20q13.13	6.08 x 10 ⁻⁰⁵	<i>SULF2</i>	intergenic	2	MO	D20S96	20q13.11	4.48	1.6 ^b

rs6107848	20p12.3	5.90 x 10 ⁻⁰⁵	<i>BMP2</i>	intergenic	6	LCA migraine	D20S112	20p12.1	10.72	1.85 ^b
rs6107848	20p12.3	5.90 x 10 ⁻⁰⁵	<i>BMP2</i>	intergenic	4	migraine/bipolar	D20S470	20p12.1	10.78	1.95 ^b
rs979012	20p12.3	9.74 x 10 ⁻⁰⁵	<i>BMP2</i>	intergenic	6	LCA migraine	D20S112	20p12.1	10.69	1.85 ^b
rs979012	20p12.3	9.74 x 10 ⁻⁰⁵	<i>BMP2</i>	intergenic	4	migraine/bipolar	D20S470	20p12.1	10.75	1.95 ^b

Overview of meta-analysis results overlapping with previously reported linkage regions.

^a Multipoint parametric

^b Multipoint nonparametric

^c Two-point parametric

* Highest LOD score detected using multiple phenotypes

MO = migraine without aura; MA = migraine with aura; FHM = familial hemiplegic migraine; HLOD = LOD score under locus heterogeneity; Dist = distance to SNP (Mb).

Positions are based on NCBI build 36.

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Supplementary table 4: meta-analysis P-values in the MTHD gene.

SNP	Chr	Base pair		Frequency		Z-score	P	Direction of effect**	AGES	ERF	NESDA	NTR1	NTR2	Rotterdam
		position	A1	A2	A1									
rs17824629	8	98727268	A	G	0.06	-0.796	0.426	-+-----	0.42	0.06	0.89	0.22	0.73	0.21
rs2516278	8	98728667	A	G	0.29	-2.699	0.007*	-----	0.39	0.85	0.88	0.14	0.14	0.01
rs2513373	8	98729703	T	C	0.29	-2.706	0.007*	-----	0.39	0.85	0.88	0.14	0.14	0.01
rs2468025	8	98732983	A	G	0.30	-2.701	0.007*	-----	0.38	0.88	0.88	0.14	0.14	0.01
rs10955127	8	98733401	A	G	0.22	-0.736	0.462	++-----	0.80	0.51	0.94	0.65	0.37	0.14
rs4448244	8	98733704	T	C	0.16	-0.569	0.569	+----+	0.49	0.57	0.63	0.84	0.15	0.22
rs16896060	8	98736953	T	C	0.01	-1.774	0.076	-?----+	0.05	n/a	0.60	0.47	0.72	0.06
rs2438222	8	98738463	A	C	0.30	-2.695	0.007*	-----	0.38	0.89	0.88	0.14	0.13	0.01
rs16896067	8	98742426	T	C	0.22	-0.913	0.361	+++----	0.91	0.65	0.90	0.60	0.35	0.10
rs2438215	8	98746023	A	G	0.30	-2.692	0.007*	-----	0.38	0.89	0.88	0.14	0.13	0.01
rs2468020	8	98746836	A	G	0.30	-2.692	0.007*	-----	0.38	0.89	0.88	0.14	0.13	0.01
rs7843569	8	98748411	A	G	0.22	-0.936	0.349	+++----	0.91	0.65	0.94	0.59	0.35	0.10
rs2468019	8	98751386	A	T	0.30	-2.695	0.007*	-----	0.38	0.89	0.88	0.14	0.13	0.01
rs2513371	8	98759210	A	G	0.30	-2.697	0.007*	-----	0.38	0.89	0.88	0.14	0.13	0.01
rs2438224	8	98760646	A	G	0.07	-3.237	0.001*	-----	0.08	0.42	0.57	0.13	0.20	0.06
rs2468017	8	98760803	A	G	0.07	-3.228	0.001*	-----	0.08	0.42	0.53	0.15	0.20	0.06
rs2438223	8	98760894	A	G	0.30	-2.700	0.007*	-----	0.38	0.88	0.88	0.14	0.13	0.01
rs10504977	8	98761399	A	G	0.22	-0.945	0.345	+++----	0.92	0.66	0.94	0.60	0.35	0.10
rs2438206	8	98777591	C	G	0.07	-3.218	0.001*	-----	0.08	0.35	0.72	0.08	0.23	0.08
rs2468012	8	98778197	A	G	0.06	-3.066	0.002*	-----	0.08	0.47	0.27	0.25	0.30	0.11
rs2513973	8	98779719	T	C	0.07	-3.215	0.001*	-----	0.09	0.42	0.53	0.15	0.20	0.06
rs17752436	8	98782364	T	C	0.05	-0.940	0.347	-+-----	0.42	0.11	0.98	0.22	0.74	0.21
rs2449554	8	98783174	A	G	0.30	-2.669	0.008*	-----	0.37	0.88	0.95	0.15	0.13	0.01
rs16896121	8	98797002	A	G	0.22	-0.960	0.337	+++----	0.93	0.67	0.94	0.60	0.35	0.10
rs2468004	8	98805852	T	C	0.07	-3.198	0.001*	-----	0.09	0.42	0.53	0.15	0.20	0.07
rs2438211	8	98806925	T	C	0.07	-3.189	0.001*	-----	0.09	0.42	0.53	0.15	0.20	0.07
rs2449512	8	98807085	A	G	0.07	-3.187	0.001*	-----	0.09	0.42	0.53	0.15	0.20	0.07
rs1311	8	98807537	T	C	0.07	-3.187	0.001*	-----	0.09	0.42	0.53	0.15	0.20	0.07

Meta-analysis results for all SNPs in the MTHD gene, and p-values in the individual samples.

Chr = chromosome; A1 = effect allele; A2 = non-effect allele

* Significant at $\alpha = .01$ level

** Direction of effect is indicated for each sample individually, in the following order: AGES-RS, ERF, NESDA, NTR1, NTR2, Rotterdam

The frequency of allele 1 was calculated as the weighted average across all samples.