ARTICLE

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

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Migraine is a common neurological 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 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 explored previously identified candidate genes in the meta-analysis data set. This revealed a modest gene-based significant association between migraine and the metadherin (*MTDH*) gene, previously identified in the first clinic-based GWA study (GWAS) for migraine (Bonferroni-corrected gene-based *P*-value=0.026). This finding is consistent with the involvement of the glutamate pathway in migraine. Additional research is necessary to further confirm the involvement of glutamate.

INTRODUCTION

Migraine is a common neurological disorder that is characterized by severe attacks of headache accompanied by symptoms such as nausea, vomiting and photophobia 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]

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] *ATP1A2*[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 Dutch and one of Australian origin were available. *De novo* genotyping was performed in the two Dutch replication cohorts (*N*=769 and 337

cases; 940 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

The five Dutch samples that were used for the meta-analysis came from the Erasmus Rucphen Family (ERF) study,[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 and included 357 migraine cases.[28] In addition to the 2446 migraine cases, 8534 non-migraine controls (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 (ie, AGES, NESDA, NTR1–2, Rotterdam), or a combination of questionnaires and telephone interview follow-up (ie, ERF).

Three additional independent samples were available for replication; two Dutch population-based samples (the GEM sample[29] 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 769 migraine cases and 940 non-migraine controls. The NTR replication sample consisted of 337 cases and 826 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[32] or IMPUTE[33] 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.

GWA 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: 10890) using the METAL program (http://www.sph.umich.edu/csg/abecasis/metal/). As 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_{study}/N_{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, 184350 SNPs that were available for <70% of all participants were excluded from the meta-analysis. This left a total of 2394913 autosomal SNPs for analysis. Annotation of meta-analysis results was performed with WGA viewer version 1.26E (Dongliang Ge and David B Goldstein; http://people.genome.duke.edu/~dg48/WGAViewer/).[34] *P*-values $<5\times10^{-8}$ were considered genome-wide significant.[35]

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 $<1\times10^{-5}$ 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 (Sequenom Inc., San Diego, CA, USA). Logistic regression was performed to test for association between these SNPs and migraine status. Third, all DICE SNPs with a *P*-value $<1\times10^{-4}$ 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 data sets. A more detailed description of the genotyping procedures and association analyses can be found in the Supplementary data.

Post hoc 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; http://www.biosemantics.org/anni).[36] 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 $<1\times10^{-4}$ coincided with a region containing a published migraine

Table 1 Sample descriptives

	Ages	ERF	NESDA	NTR1	NTR2	Rotterdam
Subjects						
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)
Genotyping and imputation						
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	2408991	2135034	2432125	2431993	2542087	2450030
Software for association analysis of imputed data	ProbABEL	ProbABEL	SNPTEST	SNPTEST	SNPTEST	ProbABEL

linkage peak. In addition, a selection of migraine candidate genes was made and inspected in our meta-analysis data set by calculating a gene-based *P*-value for each of the selected genes using the VEGAS program.[37] More details can be found in the Supplementary methods.

RESULTS

Meta-analysis

GWA 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 SNPs exceeded the threshold for genome-wide significance (Figure 2). A total of 10 SNPs

were located within genes; 8 in the metastasis associated in colon cancer 1 (*MACC1*) gene (7p21), 1 in the immunoglobulin lambda-like polypeptide 1 (*IGLL1*) gene (22q11) and 1 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 S1). 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=0.89, *P*=0.579; see Supplementary Table S2). 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 with the meta-analysis.
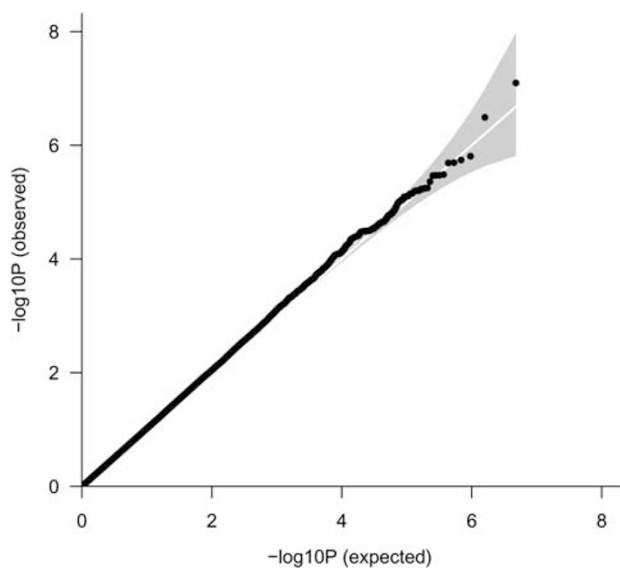

Figure 1 Q–Q plot showing the expected and observed distribution of *P*-values in the meta-analysis that included the five Dutch samples and the Icelandic sample. The genomic inflation factor (λ) for the meta-analysis was 1.022.
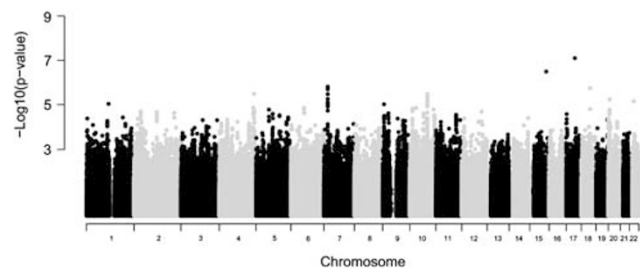

Figure 2 Manhattan plot showing the *P*-values by chromosome for the meta-analysis.

Table 2 Selected SNPs with *P*-values $< 1 \times 10^{-5}$ in the meta-analysis

SNP	Chr	P-value	Base-pair position	Type of SNP	Nearest gene	Distance to gene (kb)	A1	A2	Frequency A1	Direction of effect[a]	Number of SNPs in region (P $< 1 \times 10^{-5}$)
rs9908234	17	8.00×10^{-8}	44 932 347	Intronic	*NGFR*	0	A	G	0.93	−−−−−−	1
rs11636768	15	3.23×10^{-7}	85 496 515	Intergenic	*AGBL1*	164.2	A	G	0.15	++++?+	1
rs10275320	7	1.56×10^{-6}	20 148 579	Intronic	*MACC1*	0	A	G	0.15	−−−−−−	8
rs4939879	18	1.82×10^{-6}	45 399 981	Intergenic	*LIPG*	26.7	A	G	0.47	++++++	1
rs4861775	4	3.28×10^{-6}	180 553 645	Intergenic	*AGA*	1953.1	A	C	0.81	−−−−−−	1
rs986222	10	3.37×10^{-6}	91 920 867	Intergenic	*KIF20B*	396.2	A	G	0.46	++++++	16
rs6107848	20	5.90×10^{-6}	6 539 116	Intergenic	*BMP2*	157.6	A	G	0.37	+++++−	1
rs140174	22	6.98×10^{-6}	22 252 983	Intronic	*IGLL1*	0	A	G	0.75	−−−−−−	1
rs1146161	1	9.27×10^{-6}	115 460 299	Intergenic	*TSPAN2*	26.7	A	C	0.18	++++++	1
rs4742323	9	9.70×10^{-6}	7 276 743	Intergenic	*KDM4C*	111.1	C	G	0.61	−−−−−−	1

Abbreviations: Chr=chromosome; A1=effect allele in meta-analysis; A2=non-effect allele.
A total of 32 SNPs had a *P*-value $< 1 \times 10^{-5}$. 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).
[a]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.

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. A total of 11 SNPs had P-values between 0.01 and 0.05, but for only 3 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 19 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 with the first meta-analysis (Supplementary Table S2).

Comparison of meta-analysis results with previous 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 S3). 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=6.02×10^{-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 an SNP (rs1835740) that was located between two interesting candidate genes: the *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 *SLC1A2*, a gene that encodes an important glutamate transporter in the brain. Therefore, we selected *MTHD*, *PGCP* and *SLC1A2* as candidate genes, and also inspected SNP rs1835740 and two nearby correlated SNPs (rs982502 and rs2436046). Finally, the three FHM genes (*CACNA1A*, *ATP1A2* 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 13 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 was significantly associated with migraine in the meta-analysis. Although there were nominally significant SNPs in the *LTA*, *ESR1* and *INSR* genes, results were not significant after correction for the number of SNPs tested within the respective genes. The *PGCP* and *SLC1A2* 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 S4). 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

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	Base pair position	Pooled Z-score	Pooled P-value	Direction of effect	A1	A2	No. of SNPs with P<0.05	No. of SNPs with P<0.01	Total No. of SNPs tested	Gene-based P-value
Genes identified through candidate gene association studies													
MTHFR	42–47	1p36.3	rs4846049	11772952	−1.802	0.0715	−+−−−−	T	G	0	0	35	0.303
LTA	48,49	6p21.3	rs3093542	31648672	2.553	0.0107	+++++	C	G	1	0	4	0.168
TNF	50	6p21.3	rs3093662	31652168	−1.405	0.1600	−−−−−	A	G	0	0	3	0.163
ESR1	51–53	6q25.1	rs9322336	152242123	−3.186	0.0014	−−−−−	T	C	23	9	425	0.608
DBH	54–56	9q34	rs129882	135513490	1.841	0.0656	++−+++	T	C	0	0	52	0.415
ACE	42,57	17q23	rs4305	58911961	−1.095	0.2733	−++−−	A	G	0	0	15	0.209
INSR	58	19p13.3–13.2	rs8103483	7096374	1.995	0.0460	+++−+	T	C	1	0	144	0.289
Genes identified through GWAS													
MTDH[a]	17	8q22–q23	rs2438224	98760646	3.237	0.0012	+++++	A	G	19	19	28	0.002[b]
PGCP	17	8q22–q23	rs2455044	97767418	2.696	0.0070	+++++	A	G	24	3	250	0.290
SLC1A2	17	8q22–q23	rs1570226	35371222	−2.683	0.0073	−−+−−	T	G	19	4	209	0.319
Genes for familial hemiplegic migraine													
ATP1A2	9	1q21–q23	rs2854248	158360551	3.566	0.0004	+++++	A	T	5	4	20	0.006
SCN1A	10	2q24.3	rs12151636	166630459	2.142	0.0322	+?+−++	T	C	1	0	99	0.806
CACNA1A	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 that 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.
aNot the gene itself, but a nearby SNP regulating its expression (rs1835740) was associated with migraine in the original study.
bSignificant at α=0.05 level after Bonferroni correction for 13 tests.

(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.

Finally, we tested the three FHM genes, and found several nominally significant SNPs within *CACNA1A* and *ATP1A2*. The gene-based test for *CACNA1A* (best SNP rs3764615, $P=0.004$) was not significant ($P=0.30$). The gene-based *P*-value for *ATP1A2* (best SNP rs2854248, $P=3.62\times10^{-4}$) was 0.006.

DISCUSSION
This study describes the first meta-analysis of GWAS 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 *NGFR* gene. A replication study was performed in two Dutch replication cohorts that were available for wet replication; the GEM cohort (769 cases, 940 controls) and the NTR replication cohort (337 cases, 826 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. A total of 18 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.

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,[38] 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, as all non-migraine individuals were included as controls. These differences between studies mean that effect sizes may not be directly comparable. To address this, a pooled *Z*-score meta-analysis was performed. This type of analysis does not require a direct comparison of effect sizes.[39] Third, population-based cohorts also include many patients who have less severe migraine and a lower attack frequency. This means that they might 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 because of the reasons above) seems the most likely explanation for the lack of replication of our top results. A lack of power makes it difficult to distinguish between true associations and false-positive findings in the original meta-analysis. Therefore, when the discovery samples have insufficient 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. Because of the comorbidity of migraine and major depression, there is a higher prevalence of migraine 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 to MDD.

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

In summary, although this study does not provide genome-wide significant association of an SNP with migraine, it provides suggestive evidence for an association with the *MTDH* gene, which is involved in the glutamate pathway, previously hypothesized to have a role in migraine based on findings in FHM.[40] 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 aimed at minimizing phenotypic and genetic heterogeneity may be necessary. Strategies to achieve this can include the identification of reliable biomarkers or stratification of samples based on phenotypic similarity (eg, by looking at trait components,[11] specific symptoms[16,41] 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.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

1 Headache classification subcommittee of the International Headache Society: The International Classification of Headache Disorders, 2nd edn. Cephalalgia, 2004, Vol 24 (Suppl 1), pp 9–160.

2 Russell MB, Rasmussen BK, Fenger K, Olesen J: Migraine without aura and migraine with aura are distinct clinical entities: a study of four hundred and eighty-four male and female migraineurs from the general population. Cephalalgia 1996; 16: 239–245.

3 Russell MB, Ulrich V, Gervil M, Olesen J: Migraine without aura and migraine with aura are distinct disorders. A population-based twin survey. Headache 2002; 42: 332–336.

4 Kallela M, Wessman M, Havanka H, Palotie A, Farkkila M: Familial migraine with and without aura: clinical characteristics and co-occurrence. Eur J Neurol 2001; 8: 441–449.

5 Ligthart L, Boomsma DI, Martin NG, Stubbe JH, Nyholt DR: Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. Twin Res Hum Genet 2006; 9: 54–63.

6 Nyholt DR, Gillespie NG, Heath AC, Merikangas KR, Duffy DL, Martin NG: Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol 2004; 26: 231–244.

7 Ferrari MD, Van den Maagdenberg AM, Frants RR, Goadsby PJ: Migraine as a cerebral ionopathy with impaired central sensory processing; in Waxman SG (ed): Molecular Neurology. Amsterdam: Elsevier, 2007, pp 439–461.

8 Ophoff RA, Terwindt GM, Vergouwe MN et al: Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell 1996; 87: 543–552.

9 De Fusco M, Marconi R, Silvestri L et al: Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 2003; 33: 192–196.

10 Dichgans M, Freilinger T, Eckstein G et al: Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet 2005; 366: 371–377.

11 Anttila V, Kallela M, Oswell G et al: Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet 2006; 79: 85–99.

12 Oedegaard KJ, Greenwood TA, Lunde A, Fasmer OB, Akiskal HS, Kelsoe JR: A genome-wide linkage study of bipolar disorder and co-morbid migraine: replication of migraine linkage on chromosome 4q24, and suggestion of an overlapping susceptibility region for both disorders on chromosome 20p11. J Affect Disord 2010; 122: 14–26.

13 Wessman M, Kallela M, Kaunisto MA et al: A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet 2002; 70: 652–662.

14 Bjornsson A, Gudmundsson G, Gudfinnsson E et al: Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet 2003; 73: 986–993.

15 Anttila V, Nyholt DR, Kallela M et al: Consistently replicating locus linked to migraine on 10q22-q23. Am J Hum Genet 2008; 82: 1051–1063.

16 Nyholt DR, Morley KI, Ferreira MA et al: Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet 2005; 77: 500–512.

17 Anttila V, Stefansson H, Kallela M et al: Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. Nat Genet 2010; 42: 869–873.

18 Andreou AP, Goadsby PJ: Therapeutic potential of novel glutamate receptor antagonists in migraine. Expert Opin Investig Drugs 2009; 18: 789–803.

19 Ferrari MD: Migraine. Lancet 1998; 351: 1043–1051.

20 Goadsby PJ, Classey JD: Glutamatergic transmission in the trigeminal nucleus assessed with local blood flow. Brain Res 2000; 875: 119–124.

21 van den Maagdenberg AM, Haan J, Terwindt GM, Ferrari MD: Migraine: gene mutations and functional consequences. Curr Opin Neurol 2007; 20: 299–305.

22 Martinez F, Castillo J, Rodriguez JR, Leira R, Noya M: Neuroexcitatory amino acid levels in plasma and cerebrospinal fluid during migraine attacks. Cephalalgia 1993; 13: 89–93.

23 Sleegers K, de Koning I, Aulchenko YS et al: Cerebrovascular risk factors do not contribute to genetic variance of cognitive function: the ERF study. Neurobiol Aging 2007; 28: 735–741.

24 Stam AH, de Vries B, Janssens AC et al: Shared genetic factors in migraine and depression. Evidence from a genetic isolate. Neurology 2010; 74: 288–294.

25 Penninx BW, Beekman AT, Smit JH et al: The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res 2008; 17: 121–140.

26 Boomsma DI, de Geus EJ, Vink JM et al: Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet 2006; 9: 849–857.

27 Hofman A, Breteler MM, van Duijn CM et al: The Rotterdam Study: objectives and design update. Eur J Epidemiol 2007; 22: 819–829.

28 Scher AI, Gudmundsson LS, Sigurdsson S et al: Migraine headache in middle age and late-life brain infarcts. JAMA 2009; 301: 2563–2570.

29 Launer LJ, Terwindt GM, Ferrari MD: The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology 1999; 53: 537–542.

30 Medland SE, Zayats T, Glaser B et al: A variant in LIN28B is associated with 2D:4D finger-length ratio, a putative retrospective biomarker of prenatal testosterone exposure. Am J Hum Genet 2010; 86: 519–525.

31 Nyholt DR, LaForge KS, Kallela M et al: A high-density association screen of 155 ion transport genes for involvement with common migraine. Hum Mol Genet 2008; 17: 3318–3331.

32 Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR: MaCH: Using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 2006; 34: 816–834.

33 Marchini J, Howie B, Myers S, McVean G, Donnelly P: A new multipoint method for genome-wide association studies by imputation of genotypes. Nature genetics 2007; 39: 906–913.

34 Ge D, Zhang D, Need AC et al: WGAViewer: Software for Genomic Annotation of Whole Genome Association Studies. Genome Res 2008; 18: 640–643.

35 Dudbridge F, Gusnanto A: Estimation of significance thresholds for genomewide association scans. Genet Epidemiol 2008; 32: 227–234.

36 van Haagen HH, t Hoen PA, Botelho Bovo A et al: Novel protein-protein interactions inferred from literature context. PLoS One 2009; 4: e7894.

37 Liu JZ, McRae AF, Nyholt DR et al: A versatile gene-based test for genome-wide association studies. Am J Hum Genet 2010; 87: 139–145.

38 Nothnagel M, Ellinghaus D, Schreiber S, Krawczak M, Franke A: A comprehensive evaluation of SNP genotype imputation. Hum Genet 2009; 125: 163–171.

39 De Bakker PI, Ferreira MA, Jia X, Neale BM, Raychaudhuri S, Voight BF: Practical aspects of imputation-driven meta-analysis of genome-wide association studies. Hum Mol Genet 2008; 17: R122–R128.

40 De Vries B, Frants RR, Ferrari MD, van den Maagdenberg AM: Molecular genetics of migraine. *Hum Genet* 2009; **126**: 115–132.

41 Ligthart L, Nyholt DR, Hottenga JJ, Distel MA, Willemsen G, Boomsma DI: A genome-wide linkage scan provides evidence for both new and previously reported loci influencing common migraine. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1186–1195.

42 Schurks M, Rist PM, Kurth T: MTHFR 677C>T and ACE D/I polymorphisms in migraine: a systematic review and meta-analysis. *Headache* 2009; **50**: 588–599.

43 Schurks M, Zee RY, Buring JE, Kurth T: Interrelationships among the MTHFR 677C>T polymorphism, migraine, and cardiovascular disease. *Neurology* 2008; **71**: 505–513.

44 Schurks M, Zee RY, Buring JE, Kurth T: MTHFR 677C→T and ACE D/I polymorphisms and migraine attack frequency in women. *Cephalalgia* 2010; **30**: 447–456.

45 Rubino E, Ferrero M, Rainero I, Binello E, Vaula G, Pinessi L: Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. *Cephalalgia* 2009; **29**: 818–825.

46 Scher AI, Terwindt GM, Verschuren WM *et al*: Migraine and MTHFR C677T genotype in a population-based sample. *Ann Neurol* 2006; **59**: 372–375.

47 Oterino A, Valle N, Pascual J *et al*: Thymidylate synthase promoter tandem repeat and MTHFD1 R653Q polymorphisms modulate the risk for migraine conferred by the MTHFR T677 allele. *Brain Res* 2005; **139**: 163–168.

48 Lee KA, Jang SY, Sohn KM *et al*: Association between a polymorphism in the lymphotoxin-a promoter region and migraine. *Headache* 2007; **47**: 1056–1062.

49 Asuni C, Stochino ME, Cherchi A *et al*: Migraine and tumour necrosis factor gene polymorphism. An association study in a Sardinian sample. *J Neurol* 2009; **256**: 194–197.

50 Rainero I, Grimaldi LM, Salani G *et al*: Association between the tumor necrosis factor-alpha -308 G/A gene polymorphism and migraine. *Neurology* 2004; **62**: 141–143.

51 Oterino A, Toriello M, Cayon A *et al*: Multilocus analyses reveal involvement of the ESR1, ESR2, and FSHR genes in migraine. *Headache* 2008; **48**: 1438–1450.

52 Oterino A, Pascual J, Ruiz de Alegria C *et al*: Association of migraine and ESR1 G325C polymorphism. *Neuroreport* 2006; **17**: 61–64.

53 Kaunisto MA, Kallela M, Hamalainen E *et al*: Testing of variants of the MTHFR and ESR1 genes in 1798 Finnish individuals fails to confirm the association with migraine with aura. *Cephalalgia* 2006; **26**: 1462–1472.

54 Lea RA, Dohy A, Jordan K, Quinlan S, Brimage PJ, Griffiths LR: Evidence for allelic association of the dopamine beta-hydroxylase gene (DBH) with susceptibility to typical migraine. *Neurogenetics* 2000; **3**: 35–40.

55 Fernandez F, Colson N, Quinlan S, MacMillan J, Lea RA, Griffiths LR: Association between migraine and a functional polymorphism at the dopamine beta-hydroxylase locus. *Neurogenetics* 2009; **10**: 199–208.

56 Fernandez F, Lea RA, Colson NJ, Bellis C, Quinlan S, Griffiths LR: Association between a 19 bp deletion polymorphism at the dopamine beta-hydroxylase (DBH) locus and migraine with aura. *J Neurol Sci* 2006; **251**: 118–123.

57 Paterna S, Di Pasquale P, D'Angelo A *et al*: Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura. *Eur Neurol* 2000; **43**: 133–136.

58 McCarthy LC, Hosford DA, Riley JH *et al*: Single-nucleotide polymorphism alleles in the insulin receptor gene are associated with typical migraine. *Genomics* 2001; **78**: 135–149.

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