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2016-06

de Vries , B , Anttila , V , Freilinger , T , Wessman , M , Kaunisto , M A , Kallela , K M , Artto , V , Vijfhuizen , L S , Goebel , H , Dichgans , M , Kubisch , C , Ferrari , M D , Palotie , A , Terwindt , G M , van den Maagdenberg , A M J M & Int Headache Genetics Consortium
2016 , ' Systematic re-evaluation of genes from candidate gene association studies in migraine using a large genome-wide association data set ' , Cephalalgia , vol. 36 , no. 7 , pp. 604-614 . <https://doi.org/10.1177/0333102414566820>

<http://hdl.handle.net/10138/224082>

<https://doi.org/10.1177/0333102414566820>

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Cephalalgia

2016, Vol. 36(7) 604–614

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DOI: 10.1177/0333102414566820

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Abstract

Background: Before the genome-wide association (GWA) era, many hypothesis-driven candidate gene association studies were performed that tested whether DNA variants in genes that had been selected based on prior knowledge about migraine pathophysiology were associated with migraine. Most studies involved small sample sets without robust replication, thereby making the risk of false-positive findings high. Genome-wide marker data of thousands of migraine patients and controls from the International Headache Genetics Consortium provide a unique opportunity to re-evaluate key findings from candidate gene association studies (and other non-GWA genetic studies) in a much larger data set.

Methods: We selected 21 genes from published candidate gene association studies and six additional genes from other non-GWA genetic studies in migraine. Single nucleotide polymorphisms (SNPs) in these genes, as well as in the regions 500 kb up- and downstream, were inspected in IHGC GWAS data from 5175 clinic-based migraine patients with and without aura and 13,972 controls.

Results: None of the SNPs in or near the 27 genes, including the SNPs that were previously found to be associated with migraine, reached the Bonferroni-corrected significance threshold; neither when analyzing all migraine patients together, nor when analyzing the migraine with and without aura patients or males and females separately.

Conclusion: The available migraine GWAS data provide no clear evidence for involvement of the previously reported most promising candidate genes in migraine.

Keywords

Migraine, candidate genes, SNP, GWAS data

Date received: 15 December 2013; revised: 22 September 2014; accepted: 6 December 2014

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Introduction

Disease susceptibility for common disorders, including migraine, is thought to be conferred by a combination of environmental factors and genetic factors that are either common (i.e. with a minor allele frequency (MAF) larger than 5% in the population) or rare. In the past decades, many genetic association studies have been performed by testing DNA variants in dozens of candidate genes in order to identify genetic factors for migraine (1,2). Genes were selected based on the hypothesis that the respective pathway was implicated in migraine pathophysiology; e.g. genes that play a role in serotonin and dopamine pathways (3). The majority of the studies investigated only a single or a limited number of DNA variants per gene and therefore had a low *a priori* likelihood of targeting the correct variant that confers disease susceptibility. Moreover, rather low numbers of cases and controls (rarely more than 300 per group) were studied, resulting in limited statistical power to evaluate their association. For the majority of the associations no replication of the findings in independent cohorts was provided (for review, see de Vries et al. (1)). Consequently, many of the associations may in fact represent false-positive findings. Similar experiences have been observed in other common diseases (4–6).

Over the last few years, genome-wide association studies (GWAS) have become the state-of-the-art approach to identify genetic factors involved in common disorders. Unlike candidate gene association studies that are hypothesis driven, GWAS are hypothesis free and hypothesis generating in nature. Typically they involve large cohorts of at least several thousand patients and controls and test the association with disease of hundreds of thousands of single nucleotide polymorphisms (SNPs) distributed over the genome (7). Importantly, initial association findings are always scrutinized by follow-up testing in multiple independent replication cohorts. Therefore, the GWAS approach is less susceptible to false-positive results and more powerful than candidate gene association studies. Two GWA studies that investigated large numbers of migraine cases from clinic-based cohorts and controls have been published (8,9). One study investigated migraine with aura (MA) (with 2731 cases and 10,747 controls) and revealed a single genome-wide significant migraine susceptibility locus on chromosome 8q22.1 that pinpointed the *MTDH* gene as the possible disease-causing gene in this region (8). The other study investigated migraine without aura (MO) (with 2326 cases and 4580 controls) and yielded four additional migraine susceptibility loci on 1q22, 3p24, 6p24 and 9q33 presenting evidence for involvement of the *MEF2D*, *TGFBR2*, *PHACTR1* and *ASTN2* genes, respectively (9). The latter study also confirmed genetic

associations of SNPs in the *TRPM8* and *LRP1* genes (2q37 and 12q13, respectively) that had previously been identified as migraine susceptibility loci in a population-based GWA study (with 5122 cases and 18,108 controls) (10). A recent large meta-analysis (with 23,285 cases and 95,425 controls) that studied patients from clinic-based as well as population-based cohorts confirmed these loci and provided evidence for five additional migraine susceptibility loci (11). Notably, none of these genome-wide significant gene loci overlapped with genes that had been selected for candidate gene association studies in migraine.

The availability of GWAS data provides a unique opportunity to re-evaluate key findings from previous genetic studies in a much larger data set. We investigated 27 genes. Twenty-one genes were previously reported to be associated with migraine in candidate genes-based association studies. Three genes had been identified by positional cloning studies in families with familial hemiplegic migraine (FHM), a monogenic subtype of MA (12–14). Three genes came from direct sequencing of candidate genes in families and patients with monogenic migraine or common migraine (15–17). As the majority of the original studies investigated migraine patients who had been collected via specialized headache centers (i.e. patients who are clinic-based), we restricted our investigations to GWAS data of clinic-based migraine patients only (8,9,11).

Materials and methods

Selection of candidate genes for re-evaluation in the International Headache Genetics Consortium (IHGC) GWA data set

Genes were selected for re-evaluation in the IHGC GWAS data set based on the results of a literature search of candidate gene association studies in migraine. We included studies that had investigated at least 300 migraine patients and 300 controls of Caucasian origin. From these studies, we selected only those genes for which at least nominally significant, uncorrected *p* values ($p < 0.05$) were reported for one or more SNPs (see list of genes in Table 1). In addition, we selected genes from non-GWA genetic studies of migraine, namely the three FHM genes (*CACNA1A* (12), *ATP1A2* (13) and *SCN1A* (14)) and three genes in which possibly causal mutations had been identified by a candidate gene sequencing approach, i.e. *SLC1A3* (which encodes the EAAT1 glutamate transporter) (15), *SLC4A4* (which encodes the NBCe1 protein) (16), and *KCNK18* (which encodes the TRESK protein) (17) (Table 2).

Table 1. Summary of candidate gene association studies performed for migraine that reported at least nominal evidence for association ($p < 0.05$ for a single SNP) and that contained at least 300 cases and controls.

Gene	Cases (n) ^a Migraine (MA/MO)	Controls (n)	Associated allele with phenotype (p value) ^b	Reference
<i>MTHFR</i>	652 (465/187) 413 (187/226) 2961 (2170/791) 477 (124/353)	320 1212 3844 1402	677T: NS ($p = 0.017/-$) 677T: — ($p < 0.006/NS$) 677T: NS ($p = 0.0005/NS$) 677T: NS ($p = 0.02/-$)	Lea et al. 2004 (18) Scher et al. 2006 (19) Rubino et al. 2009 (20) Samaan et al. 2011 (21)
<i>DBH</i>	830 (588/242) 650 (650/-)	500 650	—1021T: $p = 0.004$ ($p = 0.011/NS$) rs2097629: — ($p = 0.01/-$)	Fernandez et al. 2009 (22) Todt et al. 2009 (23)
<i>DRD1</i>	543 (318/225)	561	rs251937: $p = 0.0261$ ($-/-$)	Corominas et al. 2009 (24)
<i>DRD2</i>	650 (650/-) 543 (318/225)	650 561	rs7131056: — ($p = 0.006/-$) rs2283265: $p = 0.0030$ ($p = 0.037/p = 0.0081$)	Todt et al. 2009 (23) Corominas et al. 2009 (24)
<i>DRD3</i>	543 (318/225)	561	rs3732790: $p = 0.0033$ ($-/-$)	Corominas et al. 2009 (24)
<i>SLC6A3</i>	543 (318/225)	561	rs40184: — ($p = 0.03/-$)	Todt et al. 2009 (23)
<i>TH</i>	543 (318/225)	561	rs2070762: $p = 0.0035$ ($NS/P = 0.036$)	Corominas et al. 2009 (24)
<i>EDNRA</i>	850 (850/-)	890	rs2048894: — ($p = 0.015/-$) rs5334: — ($p = 0.046/-$)	Tikka-Kleemola et al. 2009 (25)
<i>EDNRB</i>	850 (850/-)	890	rs2329047: — ($0.035/-$)	Tikka-Kleemola et al. 2009 (25)
<i>STX1A</i>	569 (407/129)	720	rs941298: $p = 0.004$ ($NS/p = 0.008$)	Tropeano et al. 2012 (26)
<i>TRPV1</i>	1040 (490/650)	1037	rs222741: $p = 0.03$ (NS/NS)	Carreno et al. 2012 (27)
<i>TRPV3</i>	1040 (490/650)	1037	rs7217270: NS ($p = 0.02/NS$)	Carreno et al. 2012 (27)
<i>FSHR</i>	356 (198/158)	374	Rs6166: NS ($p = 0.03/NS$)	Oterino et al. 2008 (28)
<i>ESR1</i>	484 (360/124) 898 (898/-)	484 900	594A: $p = 0.003$ ($p = 0.01/p = 0.02$) rs6557170, rs2347867, rs6557171, rs4870062 and rs1801132 (p values 0.007–0.034)	Colson et al. 2004 (29) Kaunisto et al. 2006 (30)
	356 (198/158)	374	rs1801132: $p = 0.03$ ($p = 0.045/NS$)	Oterino et al. 2008 (28)
<i>ESR2</i>	356 (198/158)	374	2039G: NS ($p = 0.01/NS$)	Oterino et al. 2008 (28)
<i>PGR</i>	509 (371/138)	454	PROGINS ins: $p = 0.02$ ($NS/p = 0.008$)	Colson et al. 2005 (31)
<i>TNFA</i>	299 (38/261)	306	308G: $p < 0.001$ ($NS/p < 0.001$)	Rainero et al. 2004 (32)
<i>SLC6A4</i>	546 (257/289)	770	STin2: $p = 0.002$ (NS/NS)	Schürks et al. 2010 (33)
<i>TPH2</i>	503 (214/289)	515	Haplotype block with five SNPs: $p = 0.04$ ($p = 0.4/p = 0.006$)	Jung et al. 2010 (34)
<i>LTA</i>	439 (65/327)	382	—294C: $p = 0.0002$ ($p = 0.0006/p = 0.0008$)	Lee et al. 2007 (35)
<i>INSR</i>	827 (377/450)	765	c.2946-713A: NS ($p = 0.002/NS$) c.2842 + 1451A: NS ($p = 0.007/NS$) c.3255T: NS ($p = 0.008/NS$)	McCarthy et al. 2001 (36)
	1278 (1278/-)	1337	c.2842 + 1451T: — ($p = 0.005/-$)	Netzer et al. 2008 (37)

MA: migraine with aura; MO: migraine without migraine; NS: not significant; —: not tested/not available; SNP: single nucleotide polymorphism; Ins: insertion; Del: deletion; VNTR: variable number of tandem repeats. ^aNumber of cases and ^b p values are given for all migraine cases combined or, when specified between brackets, for migraine with aura cases only and/or migraine without aura cases only.

GWAS data sets

GWAS data of 2849 MA patients and 2326 MO patients from five clinic-based cohorts were collected via specialized headache centers in Finland, the Netherlands, and Germany (8,9,11). Migraine diagnoses were based on a combination of questionnaires and/or individual interviews according to the International Classification of Headache Disorders, second edition (ICHD-II) guidelines (38) (Table 3).

Since the patients of nearly all candidate gene association studies came from clinic-based cohorts, we chose to investigate GWAS data only from clinic-based cohorts and not from population-based cohorts. An additional reason for including only clinic-based cohorts is that phenotypic information is less detailed and/or accurate in population-based cohorts, which would probably increase clinical and genetic heterogeneity. In all GWAS samples, standard quality control measures were applied; SNPs with call rates $< 97\%$,

Table 2. Migraine candidate genes from family studies.

Gene	Relation to migraine	Reference
<i>CACNA1A</i> (FHM1)	A linkage study identified <i>CACNA1A</i> as the first FHM gene. <i>CACNA1A</i> encodes the $\alpha 1$ pore-forming subunit of $\text{Ca}_v2.1$ calcium channels.	Ophoff et al. 1996 (12)
<i>ATP1A2</i> (FHM2)	A linkage study identified <i>ATP1A2</i> as the second FHM gene. <i>ATP1A2</i> encodes the $\alpha 2$ subunit of sodium-potassium pumps.	De Fusco et al. 2003 (13)
<i>SCN1A</i> (FHM3)	A linkage study identified <i>SCN1A</i> as the third FHM gene. <i>SCN1A</i> encodes the $\alpha 1$ subunit of neuronal $\text{Na}_v1.1$ sodium channels.	Dichgans et al. 2005 (14)
<i>SLC1A3/EAAT1</i>	A mutation in a single case with SHM that was identified through sequencing of the coding exons of <i>SLC1A3</i> and presented first evidence for <i>SLC1A3</i> as an SHM gene. <i>SLC1A3</i> encodes the EAAT1 glutamate transporter.	Freilinger et al. 2010 (15)
<i>SLC4A4/NBCE1</i>	Homozygous mutations in <i>SLC4A4</i> were reported in two sisters with reported hemiplegic migraine, in addition to proximal renal tubular acidosis and ocular abnormalities, and presented first evidence for <i>SLC4A4</i> as a migraine gene. <i>SLC4A4</i> encodes the $\text{Na}^+-\text{NCO}_3^-$ cotransporter NBCE1.	Suzuki et al. 2010 (16)
<i>KCNK18/TRESK</i>	A mutation in <i>KCNK18</i> in a single family with familial migraine was identified in a candidate gene sequencing approach and presented first evidence for <i>KCNK18</i> as a migraine gene. <i>KCNK18</i> encodes for the ion channel TRESK.	Lafrenière et al. 2010 (17)

FHM: familial hemiplegic migraine; SHM: sporadic hemiplegic migraine; MA: migraine with aura.

MAF <1% and/or excessive deviation from Hardy-Weinberg equilibrium (with $p < 10^{-6}$) in either cases or controls were excluded. Individuals with a genotyping rate <97% were excluded from the analyses (for more details, see Anttila et al. (11)).

Genome-wide marker data from 13,972 individuals from several pre-existing non-overlapping control cohorts that were population-matched to the cases were used as controls. The majority of the control cohorts were unselected for migraine status, implicating that they are expected to contain cases at the same frequency as the general population (Table 3). In the meta-analysis, SNPs missing from one of the studies, those with a MAF <1%, and/or those showing excess heterogeneity ($I^2 > 0.75$) were excluded.

Power calculation and significance threshold

Data for the selected genes were extracted from the existing GWAS data from an interval containing the candidate gene and the flanking region 500 kb in each direction, to have a reasonable chance of covering possible regulatory effects for the targeted genes. The threshold for evaluating the significance of SNPs located in the tested gene regions was 2.19×10^{-6} , based on a Bonferroni correction for the number of unique SNPs that were tested (0.05/22,774). Our GWAS sample (5175 cases and 13,972 controls) has 99% power to detect association with an SNP under the assumption of an allele frequency (AF) of at least

0.05 and a relative risk of 1.5 or higher (allelic test, Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc>) (19)). These thresholds are in line with published candidate gene association studies. On a more stringent level, we have 84% power to detect a variant with a relative risk of 1.4. See Supplemental Table 1 for power calculations at a range of different allele frequencies (0.05–0.4) and relative risks (1.15–1.5).

Effect size estimation

We used the Genetic Power Calculator to estimate the genotype frequencies for a marker with similar MAF and odds ratio (OR) as the *MTHFR* C677T risk allele, while assuming a disease prevalence of 12%, and using the sample size of the current study (5175 cases and 13,972 controls). A chi-square test for the resulting genotype frequencies was converted to a p value using a two-degree of freedom (df) chi-square test.

Results

We used GWAS data of clinic-based migraine patients (8,9,11) to re-evaluate 21 genes from migraine candidate gene association studies that had analyzed at least 300 migraine cases and controls and yielded associations of at least nominal p values (Table 1). Six additional genes were included that came from other non-GWA studies, i.e. either candidate gene sequencing

Table 3. Description of the cohorts included in the previously published GWA studies for clinic-based migraine.

Cohort	Ethnicity	Gender distribution	Reference
GWA study for MA (Anttila et al. 2010, 2013 (8,11))			
Clinic-based MA patients (n = 820)	European (Dutch)	82.2% female 17.8% male	van Oosterhout et al. 2011 (39)
Unselected controls (n = 4774)	European (Dutch)	58.2% female 41.8% male	Hofman et al. 2011 (40)
Clinic-based MA patients (n = 997)	European (German)	81.1% female 18.9% male	Anttila et al. 2010, 2013 (8,11)
Unselected controls (n = 1105)	European (German)	60.8% female 39.2% male	Krawczak et al. 2006 (41); Anttila et al. 2010, 2013 (8,11)
Clinic-based MA patients (n = 1032)	European (Finnish)	80.2% female 19.8% male	Kallela et al. 2001 (42)
Migraine-free and unselected controls (n = 3513)	European (Finnish)	52.6% female 47.4% male	Naukkarinen et al. 2010 (43); Barker et al. 2005 (44)
GWA study for MO (Freilinger et al. 2012 (9); Anttila et al. 2013 (11))			
Clinic-based MO patients (n = 1208)	European (German)	87% female 13% male	Freilinger et al. 2012 (9) Anttila et al. 2013 (11)
Unselected controls (n = 2564)	European (German)	55.1% female 44.9% male	Muglia et al. 2010 (45); Wichmann et al. 2005 (46); Schmermund et al. 2002 (47)
Clinic-based MO patients (n = 1118)	European (Dutch)	85.8% female 14.2% male	van Oosterhout et al. 2011 (39)
Unselected controls (n = 2016)	European (Dutch)	54.2% female 45.8% male	Hofman et al. 2011 (46)

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

studies (*KCNK18*, *SLC1A3*, *SLC4A4*) in common migraine and/or hemiplegic migraine or linkage studies in FHM (*CACNA1A*, *ATPIA2*, *SCN1A*) (Table 2). Within the 27 gene regions we investigated 22,774 SNPs for association with migraine, applying a significance threshold for individual SNPs of 2.19×10^{-6} .

None of the SNPs, including the specific SNPs reported in the original publications (Supplemental Table 2), surpassed the significance threshold (Table 4, Supplemental Information). When analyzing MA and MO together, the best p value was seen for SNP rs805287 ($p = 1.08 \times 10^{-4}$) that is located within the surrounding region of the *TNFA* and *LTA* genes. However, this SNP is located in a gene-dense region over 130 kb downstream of both genes (Figure 1(a)) and lies within the major histocompatibility complex locus, where overall levels of noise are higher because of the complex linkage disequilibrium structure (49). When analyzing MA and MO separately, for MA, again the best p value was observed with an SNP (rs630379; $p = 9.68 \times 10^{-6}$) at the border of the region surrounding the *TNFA* and *LTA* genes (Supplemental Information). For MO, the best p value was seen for an SNP (rs13024246, $p = 2.76 \times 10^{-5}$) located in the *FSHR* gene region (Figure 1(b)) but this SNP was located far away from the originally selected gene. Only one gene region, namely that of the *DRD3* gene, showed a potentially interesting peak (with best associated SNP rs1486008, $p = 2.88 \times 10^{-4}$; OR = 1.19) within the previously implicated migraine gene (Figure 1(c)).

Although the chance of observing associations that are gender-specific is limited, as the vast majority of the migraine patients are women, we performed a gender-specific analysis for the total migraine group. Analyzing males and females separately did not reveal SNPs with gender-specific signals surpassing the significance threshold (Supplemental Table 3).

Discussion

For this study, we used the data of clinic-based GWA cohorts from the IHGC (8,9,11) to re-evaluate key findings from previously published candidate gene association studies (and other genetic non-GWAS studies) in migraine. Our study included GWAS data from 5175 migraine patients and 13,972 controls and shows no significant association with migraine for any of the 27 genes (Table 4), despite the fact that our study had sufficient power (>95%) to significantly detect genetic association signals for variants with an MAF >0.05 and a relative risk >1.4, as commonly presumed in the much smaller migraine candidate gene association studies. Only a few single SNPs for some of the 27 selected gene regions showed moderate evidence of association. Notably, none of the p values of the

SNPs reported in the original publications surpassed the significance threshold (Supplemental Table 2), nor translated to the originally reported effect sizes. For example, the T-allele of the *C677T* polymorphism in the *MTHFR* gene, which showed significant association with migraine in various candidate gene association studies, did not show up in our study. Assuming an effect size of 1.5, which is in line with previously reported effect sizes for this variant in migraine (18–21, 50), and an MAF of 31% in the European population (51), our study would have produced a p value below 1.46×10^{-63} . However, the T-allele showed no association with migraine in our study ($p = 0.56$ for migraine; $p = 0.51$ for MA; $p = 0.11$ for MO); the lowest observed p value in the *MTHFR* gene region in our study was 7.18×10^{-4} for SNP rs11121783. Also for the other SNPs of the originally reported associations, our study should have produced low p values; well below the set threshold of 2.19×10^{-6} , if the reported effect sizes would have replicated. These poor replication results indicate the limited value of small-scale genetic association studies at the single-gene or single-marker level, and emphasize the importance of using large, well-powered studies that are properly designed. This finding is in line with a recent review that supports the statistical observation that low power due to small sample sizes not only decreases the chance to detect a true effect, but also increases the chance that a significant finding does not reflect a true effect (52).

Based on current knowledge of effect sizes of common variants for many common diseases, the vast majority of the candidate gene association studies in the literature lacked sufficient power to detect an effect that can be realistically expected for a common allele in a common disorder like migraine. Therefore, the most probable reason for the lack of replication is that the results of the candidate gene association studies most likely represent false-positive findings. Although we did not show significant evidence for any of the genes previously implicated in common migraine as genetic migraine risk factors, we cannot, however, exclude the possibility that some of the previous findings are true-positive findings reflecting effects specific to a particular patient pool (such as individual families, in whom alleles that are rare in the general population can predominate). Possible additional reasons that could explain why we did not detect associations are that: (1) rare variants that may play a role may not be captured, either in candidate gene association studies or GWAS platforms, because of specific LD patterns that are not sufficiently reflected in the surrounding common markers; or (2) variants located in these candidate gene regions may play a role that have effect sizes too low to be detected, even with the current sample size, and will surface only with sample sizes

Figure 1. Regional association plot (generated using LocusZoom) for SNPs within the (a) *TNFA* and *LTA* gene region and their association with migraine; (b) the *FSHR* gene region and their association with migraine without aura (MO); and (c) the *DRD3* gene region and their association with migraine. The plots show the chromosomal position (based on NCBI build 36) for the SNP in the respective region against $-\log_{10} p$ values. The SNP with the highest association signal is represented as a purple diamond. Other SNPs are color coded according to the extent of LD with that specific SNP. SNP: single nucleotide polymorphism; NCBI: National Center for Biotechnology Information; LD: linkage disequilibrium.

Table 4. The most significant association results for the 27 gene regions that contain the previously proposed migraine gene and its 1 Mb window.

Gene	Location of the study region Chromosome (position ^a)	All migraine		MA		MO	
		SNP with best p value	p value (OR)	SNP with best p value	p value (OR)	SNP with best p value	p value (OR)
MTFR	1 (11268374–12288702)	rs2745282	9.72×10^{-3} (1.08)	rs374450	0.02 (1.11)	rs11121783	7.18×10^{-4} (0.61)
DBH	9 (134991306–136014287)	rs611983	9.85×10^{-4} (1.23)	rs12683493	1.85×10^{-3} (1.15)	rs10124218	0.01 (0.88)
DRD1	5 (174300281–175303769)	rs265999	1.12×10^{-3} (0.89)	rs2471014	8.61×10^{-4} (0.87)	rs703746	7.82×10^{-4} (0.87)
DRD2	11 (112285527–113351211)	rs723079	9.42×10^{-4} (1.13)	rs17602285	1.16×10^{-3} (0.76)	rs12285469	1.37×10^{-3} (0.87)
DRD3	3 (114830247–115880589)	rs1486008	2.88×10^{-4} (1.19)	rs1354348	1.21×10^{-4} (1.35)	rs2399496	4.81×10^{-3} (1.11)
EDNRA	4 (148121357–149185556)	rs7668569	6.58×10^{-3} (0.90)	rs6535562	4.03×10^{-3} (1.76)	rs7668569	0.02 (0.87)
EDNRB	13 (76867617–77947665)	rs11838546	1.03×10^{-3} (0.71)	rs11617089	0.02 (1.37)	rs4366606	3.32×10^{-3} (1.58)
ESR1	6 (151670147–152966101)	rs7738912	9.16×10^{-4} (1.20)	rs2459110	8.23×10^{-3} (0.92)	rs7738912	4.39×10^{-4} (1.35)
ESR2	14 (63269500–64330881)	rs17101394	8.75×10^{-3} (1.10)	rs9944101	0.01 (1.09)	rs10145970	3.04×10^{-3} (1.47)
TRPV1	17 (3415490–3447085)	rs1488689 ^b	3.06×10^{-4} (1.11)	rs8067395 ^b	1.57×10^{-3} (0.90)	rs2455858 ^b	1.30×10^{-3} (0.86)
TRPV3	17 (3363236–3408039)	rs1488689 ^b	3.06×10^{-4} (1.11)	rs8067395 ^b	1.57×10^{-3} (0.90)	rs2455858 ^b	1.30×10^{-3} (0.86)
TPH2	12 (70618893–70712488)	rs7485207	2.83×10^{-3} (1.09)	rs7485207	3.32×10^{-3} (1.12)	rs941195	2.74×10^{-3} (1.17)
STX1A	7 (72751476–72771924)	rs6951030	7.03×10^{-4} (0.90)	rs2293757	9.13×10^{-4} (1.21)	rs2237279	0.01 (0.90)
FSHR	2 (48543156–49735134)	rs13024246	9.63×10^{-4} (0.91)	rs7591064	2.45×10^{-3} (0.88)	rs13024246	2.76×10^{-5} (0.84)
PGR	11 (99905565–101005754)	rs7123823	2.62×10^{-3} (1.39)	rs581136	8.29×10^{-3} (1.09)	rs2187361	1.94×10^{-3} (1.12)
INSR	19 (6563266–7745011)	rs10500204	1.22×10^{-3} (1.09)	rs2352958	1.39×10^{-4} (0.88)	rs7245562	2.36×10^{-3} (1.25)
TH	11 (1641735–2649611)	rs7108541	9.04×10^{-3} (0.92)	rs7109219	5.44×10^{-3} (1.10)	rs231361	5.67×10^{-3} (1.13)
SLC6A3	5 (945910–1998538)	rs6554667	3.03×10^{-3} (1.19)	rs4398676	2.55×10^{-3} (1.11)	rs27072	5.84×10^{-4} (1.18)
SLC6A4	17 (25049032–26086841)	rs6505176	8.75×10^{-3} (0.93)	rs216459	0.04 (1.07)	rs9904033	1.40×10^{-3} (1.16)
TNFA	6 (31152271–32154091)	rs805287 ^b	1.08×10^{-4} (0.90)	rs630379 ^b	9.68×10^{-6} (0.86)	rs2442736 ^b	3.99×10^{-3} (1.14)
LTA	6 (31148072–32150077)	rs805287 ^b	1.08×10^{-4} (0.90)	rs630379 ^b	9.68×10^{-6} (0.86)	rs2442736 ^b	3.99×10^{-3} (1.14)
CACNA1A	19 (12678257–13978274)	rs10418748	3.48×10^{-3} (1.08)	rs4461194	1.08×10^{-3} (1.11)	rs2112464	0.01 (0.90)
ATPIA2	1 (157852172–158879998)	rs11585055	1.82×10^{-3} (1.23)	rs2854248	5.02×10^{-3} (1.10)	rs11585055	4.05×10^{-3} (1.32)
SCN1A	2 (166053916–167138395)	rs4335960	3.06×10^{-3} (1.15)	rs6432879	4.30×10^{-3} (0.89)	rs4335960	4.08×10^{-3} (1.24)
SLC1A3	5 (36142214–37224193)	rs12654646	5.37×10^{-4} (1.14)	rs12654646	2.76×10^{-3} (1.16)	rs6892066	2.48×10^{-3} (1.12)
KCNK18	10 (118446990–119459800)	rs17551306	9.81×10^{-4} (1.11)	rs7910681	2.68×10^{-5} (1.15)	rs1681750	4.19×10^{-3} (0.75)
SLC4A4	4 (7171867–73156663)	rs11737727	2.01×10^{-3} (1.16)	rs7673438	1.54×10^{-3} (1.15)	rs3733488	0.01 (1.19)

SNP: single nucleotide polymorphism; MA: migraine with aura; MO: migraine without aura. ^aChromosomal position is based on build 36. ^bSame SNP.

on the order of several hundreds of thousands cases and controls.

In conclusion, our analysis shows no evidence for the involvement of any of the selected 27 genes in migraine pathophysiology of common migraine. For future

studies, other approaches should be considered to identify migraine susceptibility genes. This finding is in line with experiences of candidate gene association studies in other common diseases (53).

Article highlights

- Re-evaluation of previously reported migraine candidate gene hits shows no evidence for involvement in migraine pathology in a genome-wide association (GWA) data set.
- Small-scale genetic association studies lacking proper replication appear of limited value.

Funding

This work was supported by grants from the Netherlands Organization for the Health Research and Development (ZonMw) no. 90700217 and VIDI (ZonMw) no. 91711319 (to G.M.T.); the Netherlands Organisation for Scientific Research (NWO) VICI (918.56.602) and Spinoza (2009) grants (to M.D.F.); the EuroHead project (LSM-CT-2004-504837); the EUROHEADPAIN project (grant number 602633); and the Center for Medical Systems Biology (CMSB) established in the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (NGI/NWO), project nr. 050-060-409 (to R.R.F., M.D.F. and A.M.J.M.v.d.M.). The Wellcome Trust (grant number 098051 to AP); the Academy of Finland (grant number 251704 to AP, and 139795 to MW); the Academy of Finland, Center of Excellence in Complex Disease Genetics, (grant numbers 213506 and 129680 to AP); ENGAGE Consortium, (grant agreement HEALTH-F4-2007-201413); EU/SYNSYS- Synaptic Systems (grant number 242167 to AP); the Sigrid Juselius Foundation (to AP); the Folkhälsan Research Foundation (to MW); the Medicinska Understödsföreningen Liv & Hälsa (to MW); the Helsinki University Central Hospital (to MK, VAr).

Conflict of interest

None declared.

References

1. de Vries B, Frants RR, Ferrari MD, et al. Molecular genetics of migraine. *Hum Genet* 2009; 126: 115–132.
2. Maher BH and Griffiths LR. Identification of molecular genetic factors that influence migraine. *Mol Genet Genomics* 2011; 285: 433–446.
3. Montagna P. Molecular genetics of migraine headaches: A review. *Cephalalgia* 2000; 20: 3–14.
4. Bevan S and Markus HS. Genetic profiles in ischaemic stroke. *Curr Atheroscler Rep* 2013; 15: 342.
5. McCarroll SA and Hyman SE. Progress in the genetics of polygenic brain disorders: Significant new challenges for neurobiology. *Neuron* 2013; 80: 578–587.
6. Petrovski S and Kwan P. Unraveling the genetics of common epilepsies: Approaches, platforms, and caveats. *Epilepsy Behav* 2013; 26: 229–233.
7. Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 2010; 363: 166–176.
8. Anttila V, Stefansson H, Kallela M, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. *Nature Genet* 2010; 42: 869–873.
9. Freilinger T, Anttila V, de Vries B, et al. Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nat Genet* 2012; 10: 777–782.
10. Chasman DI, Schürks M, Anttila V, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nat Genet* 2011; 43: 695–698.
11. Anttila V, Winsvold BS, Gormley P, et al. Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nat Genet* 2013; 45: 912–917.
12. 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.
13. 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.
14. 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.
15. Freilinger T, Koch J, Dichgans M, et al. A novel mutation in *SLC1A3* associated with pure hemiplegic migraine. *J Headache Pain* 2010; 11(Suppl 1): 90.
16. Suzuki M, Van Paesschen W, Stalmans I, et al. Defective membrane expression of the Na⁺/HCO₃[−] cotransporter NBCe1 is associated with familial migraine. *Proc Natl Acad Sci USA* 2010; 7: 15963–15968.
17. Lafrenière RG, Cader MZ, Poulin JF, et al. A dominant-negative mutation in the *TRESK* potassium channel is linked to familial migraine with aura. *Nat Med* 2010; 16: 1157–1160.
18. Lea RA, Ovcarić M, Sundholm J, et al. The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. *BMC Med* 2004; 2: 3.

19. Scher AI, Terwindt GM, Verschuren WM, et al. Migraine and *MTHFR* C677T genotype in a population-based sample. *Ann Neurol* 2006; 59: 372–375.
20. Rubino E, Ferrero M, Rainero I, et al. Association of the C677T polymorphism in the *MTHFR* gene with migraine: A meta-analysis. *Cephalalgia* 2009; 29: 818–825.
21. Samaan Z, Gaysina D, Cohen-Woods S, et al. Methylenetetrahydrofolate reductase gene variant (*MTHFR* C677T) and migraine: A case control study and meta-analysis. *BMC Neurol* 2011; 11: 66.
22. Fernandez F, Colson N, Quinlan S, et al. Association between migraine and a functional polymorphism at the dopamine beta-hydroxylase locus. *Neurogenetics* 2009; 10: 199–208.
23. Todt U, Netzer C, Toliat M, et al. New genetic evidence for involvement of the dopamine system in migraine with aura. *Hum Genet* 2009; 125: 265–279.
24. Corominas R, Ribases M, Camiña M, et al. Two-stage case-control association study of dopamine-related genes and migraine. *BMC Med Genet* 2009; 21: 10:95.
25. Tikka-Kleemola P, Kaunisto MA, Hämäläinen E, et al. Genetic association study of endothelin-1 and its receptors EDNRA and EDNRB in migraine with aura. *Cephalalgia* 2009; 29: 1224–1231.
26. Tropeano M, Wöber-Bingöl C, Karwautz A, et al. Association analysis of *STX1A* gene variants in common forms of migraine. *Cephalalgia* 2012; 32: 203–212.
27. Carreño O, Corominas R, Fernández-Morales J, et al. SNP variants within the vanilloid *TRPV1* and *TRPV3* receptor genes are associated with migraine in the Spanish population. *Am J Med Genet B Neuropsychiatr Genet* 2012; 159B: 94–103.
28. Oterino A, Toriello M, Cayón A, et al. Multilocus analyses reveal involvement of the *ESR1*, *ESR2*, and *FSHR* genes in migraine. *Headache* 2008; 48: 1438–1450.
29. Colson NJ, Lea RA, Quinlan S, et al. The estrogen receptor 1 G594A polymorphism is associated with migraine susceptibility in two independent case/control groups. *Neurogenetics* 2004; 5: 129–133.
30. Kaunisto MA, Kallela M, Hämäläinen 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.
31. Colson NJ, Lea RA, Quinlan S, et al. Investigation of hormone receptor genes in migraine. *Neurogenetics* 2005; 6: 17–23.
32. Rainero I, Grimaldi LM, Salani G, et al. Association between the tumor necrosis factor- α -308 G/A gene polymorphism and migraine. *Neurology* 2004; 62: 141–143.
33. Schürks M, Rist PM and Kurth T. Sex hormone receptor gene polymorphisms and migraine: A systematic review and meta-analysis. *Cephalalgia* 2010; 30: 1306–1328.
34. Jung A, Hüge A, Kühlenbäumer G, et al. Genetic *TPH2* variants and the susceptibility for migraine: association of a *TPH2* haplotype with migraine without aura. *J Neural Transm* 2010; 117: 1253–1260.
35. Lee KA, Jang SY, Sohn KM, et al. Association between a polymorphism in the lymphotoxin- α promoter region and migraine. *Headache* 2007; 47: 1056–1062.
36. 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.
37. Netzer C, Freudenberger J, Heinze A, et al. Replication study of the insulin receptor gene in migraine with aura. *Genomics* 2008; 91: 503–507.
38. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 2nd edition. *Cephalalgia* 2004; 24: 1–160.
39. van Oosterhout WP, Weller CM, Stam AH, et al. Validation of the web-based LUMINA questionnaire for recruiting large cohorts of migraineurs. *Cephalalgia* 2011; 31: 1359–1367.
40. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol* 2011; 26: 657–686.
41. Krawczak M, Nikolaus S, von Eberstein H, et al. PopGen: Population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. *Community Genet* 2006; 9: 55–61.
42. Kallela M, Wessman M and Färkkilä M. Validation of a migraine-specific questionnaire for use in family studies. *Eur J Neurol* 2001; 8: 61–66.
43. Naukkarinen J, Surakka I, Pietiläinen KH, et al. Use of genome-wide expression data to mine the “Gray Zone” of GWA studies leads to novel candidate obesity genes. *PLoS Genet* 2010; 6: e1000976.
44. Barker DJ, Osmond C, Forsén TJ, et al. Trajectories of growth among children who have coronary events as adults. *N Engl J Med* 2005; 353: 1802–1809.
45. Muglia P, Tozzi F, Galwey NW, et al. Genome-wide association study of recurrent major depressive disorder in two European case-control cohorts. *Mol Psychiatry* 2010; 15: 589–601.
46. Wichmann HE, Gieger C, Illig T, et al. KORA-gen—resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* 2005; 67(Suppl 1): S26–S30.
47. Schmermund A, Möhlenkamp S, Stang A, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: Rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. *Am Heart J* 2002; 144: 212–218.
48. Purcell S, Cherny SS and Sham PC. Genetic Power Calculator: Design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; 19: 149–150.

49. Gourraud PA, Khankhanian P, Cereb N, et al. HLA diversity in the 1000 genomes dataset. *PLoS One* 2014; 9: e97282.
50. Schürks M, Rist PM and Kurth T. *MTHFR* 677C > T and *ACE* D/I polymorphisms in migraine: A systematic review and meta-analysis. *Headache* 2010; 50: 588–599.
51. The International HapMap Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; 467: 52–58.
52. Button KS, Ioannidis JP, Mokrysz C, et al. Power failure: Why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; 14: 365–376.
53. Hirschhorn JN, Lohmueller K, Byrne E, et al. A comprehensive review of genetic association studies. *Genet Med* 2002; 4: 45–61.