Abstract

Migraine is a common episodic neurological disorder, typically presenting with recurrent attacks of severe headache and autonomic dysfunction. Apart from rare monogenic subtypes, no genetic or molecular markers for migraine have been convincingly established. We identified the minor allele of rs1835740 on chromosome 8q22.1 to be associated with migraine (P = 5.38 x 10^{-5}, odds ratio = 1.23, 95% CI 1.150-1.324) in a genome-wide association study of 2,731 migraine cases ascertained from three European headache clinics and 10,747 population-matched controls. The association was replicated in 3,202 cases and 40,062 controls for an overall meta-analysis P value of 1.69 x 10^{-11} (odds ratio = 1.18, 95% CI 1.127-1.244). rs1835740 is located between MTDH (astrocyte elevated gene 1, also known as AEG-1) and PGCP (encoding plasma glutamate carboxypeptidase). In an expression quantitative trait study in lymphoblastoid cell lines, transcript levels of the MTDH were found to have a significant correlation to rs1835740 (P = 3.96 x 10^{-9}, permuted threshold for genome-wide significance 7.7 x 10^{-8}). To our knowledge, our data establish [...]
Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1

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Abstract

Migraine is a common episodic neurological disorder, typically presenting with recurrent attacks of severe headache and autonomic dysfunction. Apart from rare monogenic subtypes, no genetic or molecular markers for migraine have been convincingly established. We identified the minor allele of rs1835740 on chromosome 8q22.1 to be associated with migraine (p=5.12 × 10^{-9}, OR 1.23 [1.150–1.324]) in a genome-wide association study of 2,748 migraineurs from three European headache clinics and 10,747 population-matched controls. The association was replicated in 3,202 cases and 40,062 controls for an overall meta-analysis p-value of 1.60 × 10^{-11} (OR 1.18 [1.127–1.244]). rs1835740 is located between the astrocyte elevated gene 1 (MTDH/AEG-1) and plasma glutamate carboxypeptidase (PGCP). In an expression quantitative trait study in lymphoblastoid cell lines transcript levels of the MTDH/AEG-1 were found to have a significant correlation to rs1835740. Our data establish rs1835740 as the first genetic risk factor for migraine.

The recent boom of genome-wide association (GWA) studies has had a major impact on our current view of genetic susceptibility to common traits and complex disorders. However, the number of loci identified in central nervous system disorders (CNS) is underrepresented (www.genome.gov/gwastudies 1). To our knowledge no GWA studies or any common, robustly established variants have been reported for major episodic neurological disorders (ICD G40-44, migraine, epilepsy, ataxias). However, there is substantial genetic information for rare Mendelian forms of migraine, epilepsy and ataxia, which classify them as channelopathies associated with compromised neurotransmitter homeostasis2. So far there is no evidence for the contribution of ion channel variants in common forms of these diseases3,4.
Migraine is an episodic neurological disorder with complex pathophysiology, affecting 8% of males and 17% of females. Migraine ranks among the 20 most disabling diseases and has been estimated the most costly neurological disorder for society with a considerable impact on public health. Clinically, the International Classification of Headache Disorders (ICHD-II) recognizes two main common forms: i) migraine with aura (MA), (ii) migraine without aura (MO). The two forms are distinguished based on the presence of aura, a period of variable and diverse neurological symptoms that precede the headache phase. Patients may have attacks of only MO, or only MA, or a combination of both types in variable proportions. There is debate whether MA and MO attacks represent two distinct disorders, or merely are variations of a single disease entity on a common complex genetic background. Migraine headache is believed to be caused by activation of the trigeminovascular system and the aura by cortical spreading depression (CSD), a slowly propagating wave of neuronal and glial depolarization. However, these are considered to be downstream events, and it is unknown how migraine attacks are initiated.

To identify variants associated with the common forms of migraine we carried out a two-stage GWA study in six clinic-based and one population-based European migraine samples (Supplementary Figure 1). In the discovery stage, we studied 3,279 migraineurs (1,124 Finns, 1,276 Germans and 879 Dutch) recruited from headache clinics and genotyped using Illumina arrays, against population-matched controls (10,747) recruited from pre-existing population-based GWA studies (see Supplementary Note for details). In the replication stage, a further 3,202 patients and 40,062 population-matched controls from Iceland, Denmark, the Netherlands and Germany were studied.

Diagnoses were made by headache experts using a combination of questionnaire and individual interviews that are based on the ICHD-II. Due to the overlap between MA and MO, we analyzed the following groups: i) “all migraine”, i.e. all migraine patients irrespective of subtype, ii) “MA only”, i.e. patients who only have attacks where aura is present, iii) “both MA and MO”, i.e. patients with attacks both with and without aura and iv) “MO only”, i.e. patients with only attacks of migraine without aura.

A multi-population Cochran-Mantel-Haenszel (CMH) association analysis and a significance threshold of $p \leq 5 \times 10^{-8}$ were applied. In the initial GWA study, 2,748 cases and 10,747 controls (Table 1) passed quality control steps, and 429,912 markers were successfully genotyped (see Online Methods for details). A quantile-quantile plot of the CMH analysis (Supplementary Figure 2) and an overall inflation factor ($\lambda = 1.08$) were used as final quality control measures.

Only one marker, rs1835740 on chromosome 8q22.1, showed significant association to migraine in the multi-population CMH analysis (Figure 1, Supplementary Figure 3). Further 11 loci were found with $p$-values $\leq 5 \times 10^{-5}$ (Supplementary Table 1). The minor allele (A) of marker rs1835740 was associated with migraine with a $p$-value of $5.12 \times 10^{-9}$ and odds ratios ranging between $1.21 - 1.33$ (Table 2). Two nearby markers with the highest linkage disequilibrium (LD) to rs1835740 (rs982502: $r^2=0.59$, $p=1.54 \times 10^{-4}$ and rs2436046: $r^2=0.68$, $p=3.83 \times 10^{-5}$) also showed association to migraine (Supplementary Table 2). Haplotype analysis detected a 27 kb haplotype ($p=1.15 \times 10^{-7}$) (Supplementary Figure 4 and Supplementary Table 3). We analysed the HapMap Phase II data to demonstrate that no long-range LD to rs1835740 exists within a 5 Mb window using the ssSNPer program, strongly suggesting that the causative variant is tagged by the minor allele of rs1835740 located between two close recombination hotspots (at 98.199 Mb and 98.309 Mb, Figure 1). The 2 Mb window around rs1835740 was also imputed against the 1000 Genomes data (August 2009 release), but no other marker exceeded (Figure 1) the evidence of rs1835740 for association. Conditional analysis of the SNPs around rs1835740 showed no additional independent signals.

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(Supplementary Table 2). The proportion of genetic variance explained by the rs1835740 variant was estimated to be 1.5-2.5% depending on the heritability estimate used and the population attributable risk to be 10.7% using the methodology of Risch et al.\textsuperscript{13}

To confirm and extend our results, we performed a replication study on the only marker with genome-wide significance in the initial study, rs1835740. The replication samples were divided into the phenotypic subgroups similar to the discovery sample. Replication was successful in two “MA only” subsets (Danish: p=0.015, OR 1.29; Icelandic: p=0.038, OR 1.36), the Icelandic MO set (p=0.029, OR 1.18) as well as in the Icelandic “all migraine” group (p=0.010, OR 1.18) (Table 2). Overall, the A allele of marker rs1835740 was overrepresented (OR 1.05 – 1.36, Table 2) in each subset of all replication samples except in the Danish “MA, MO” group (OR 0.99). The effect was stronger in the MA groups than other migraine subgroups (Figure 2). It should be noted that the majority of the groups which did not reach formal replication were small with limited power. Meta-analysis was conducted using the CMH test for each diagnosis subgroup alone as well as for all migraine samples, with the latter showing a final p-value of 1.60 × 10\textsuperscript{−11} (Table 2).

Marker rs1835740 is located between two potentially interesting candidate genes, \textit{MTDH/AEG-1} and \textit{PGCP}. We analyzed the effect of the marker genotype on the expression of genes within a 2 Mb window in fibroblasts, primary T-cells and lymphoblastoid cell lines (LCL) established from umbilical cords\textsuperscript{14}. In the expression quantitative trait locus (eQTL) analysis, the rs1835740 genotype was found to have significant correlation to the transcript levels of the nearby \textit{MTDH/AEG-1} gene in LCLs (see Table 3 and Supplementary Table 4), with the risk allele A being associated with higher expression levels (Figure 3). This is in line with previous studies, which have shown expression analyses in LCL cells to be informative in neurological and neuropsychiatric traits\textsuperscript{15-17}. No significant association was detected in fibroblasts or primary T-cells. The eQTL analysis suggests rs1835740 to be a \textit{cis} regulator of \textit{MTDH/AEG-1} in LCLs.

The location of the associating sequence variant, rs1835740, between two genes involved in glutamate homeostasis, \textit{PGCP} and \textit{MTDH/AEG-1}, suggests that this region contains elements that could regulate either or both of these flanking genes, the eQTL analysis pointing to the latter gene. Although \textit{MTDH/AEG-1} has mainly been studied in carcinogenesis\textsuperscript{18}, previous studies in cultured astrocytes have shown that \textit{MTDH/AEG-1} down-regulates EAAT2/GLT1, the major glutamate transporter in the brain. Furthermore, mice lacking the EAAT2 gene have been shown to suffer from lethal spontaneous epileptic seizures\textsuperscript{23}. Despite the limitations to extrapolate eQTL findings from LCL cells directly to brain tissue the data suggests a plausible link between the identified variant and glutamate regulation. This is a tempting hypothesis as this neurotransmitter has long been suspected to play a key role in migraine pathophysiology\textsuperscript{24}.

Although the evidence provided here is indirect, accumulation of excess glutamate in the synaptic cleft through down-regulation of EAAT2/GLT1 or through increased \textit{PGCP} activity (or both), would provide an intriguing putative mechanism for the occurrence of migraine attacks. It is reasonable to speculate that this accumulation can increase susceptibility to migraine through increased sensitivity to CSD, the likely mechanism for the migraine aura\textsuperscript{9, 10}, as well as through glutamate involvement in central sensitization, which has been postulated to be the underlying mechanism of allodynia during a migraine attack\textsuperscript{25}.

This and our previous study\textsuperscript{3} did not yield evidence for association of ion channel genes to common forms of migraine. Thus, even if the contribution of ion channel genes is well established in Mendelian forms of paroxysmal neurological disorders, such as familial hemiplegic migraine (FHM)\textsuperscript{26-29}, their direct role in more common forms remains open.
Interestingly, previous studies suggested that the imbalance of glutamate release and clearance is a key component of the pathogenesis of FHM, where the underlying mutation is in \textit{CACNA1A}, \textit{ATP1A2} or \textit{SCN1A}\textsuperscript{30,31}. The results of the present study support the hypothesis that complementary pathways such as the glutamate system may tie the Mendelian channelopathies with pathogenetic mechanisms of more common forms of episodic neurological disorders, such as migraine. Mutations in the functionally related EAAT1 transporter have been identified in other episodic phenotypes (such as episodic ataxia \textsuperscript{6,32}, and a non FHM1/2 hemiplegic migraine/episodic ataxia/seizure phenotype \textsuperscript{33}), providing an example of the link between EAAT transporters to episodic disorders. Future studies should be conducted to specifically test this hypothesis.

In summary, we have identified the first robust genetic association to migraine. As our cases were mainly selected from specialized headache clinics, subsequent studies are needed to establish the contribution of \textit{rs1835740} in population-based migraine cohorts. These population based cohorts may represent a different severity spectrum and thus, possibly, also a somewhat different underlying combination of genetic susceptibility variants. The effect of \textit{rs1835740} is stronger in MA than MO, but further studies are needed to confirm the role of the variant in different migraine subgroups. The variant explains only a small fraction of the overall genetic variance in migraine and future GWA studies, perhaps with different ascertainment schemes, will likely identify additional loci explaining more of the genetic variance.

**Online Methods**

**Study design**

We jointly analyzed patient samples from three migraine with aura collections from Finland, Germany and the Netherlands with population-matched controls obtained from pre-existing studies. This initial phase was followed by a replication study of the top SNP, \textit{rs1835740}, in migraine samples from Denmark, Iceland, the Netherlands and Germany. Characteristics of each study sample are described in Table 1, and the recruitment and ascertainment of cases and controls are described in the Supplementary Note.

**Initial genome-wide association (GWA) study genotyping**

DNA was extracted from patient blood samples using standard methods. Genotyping of the GWA study samples was done at the Wellcome Trust Sanger Institute on the Illumina 610K (Finns, Germans) and 550K (Dutch) single nucleotide polymorphisms (SNP) microarrays following the Infinium II protocol from the manufacturer (Illumina Inc., San Diego, USA). Genotype calling was performed using the Illuminus software\textsuperscript{34}.

**Replication study genotyping**

For the replication study, Danish cases and 459 migraine-free controls were genotyped using the Centaurus platform (Nanogen Inc., San Diego, CA, USA), and 904 additional controls were genotyped at deCODE genomics using Illumina HumanHap650 BeadArray\textsuperscript{TM}. The Icelandic cases and controls were genotyped using the Illumina HumanHap 317K, 370K, 610K or 1M bead arrays at deCODE genomics. The Dutch replication cohort was genotyped using the TaqMan technology (Applied Biosystems, Life Technologies, Foster City, CA, USA) at Leiden University Medical Center. The German replication cases were genotyped using Illumina HumanHap 610K at Munich with external replication.
Expression study

The GenCord resource, a collection of cell lines derived from umbilical cords of 75 newborns of Western European origin born at the maternity ward of the University of Geneva Hospital, was used. Sample collection was performed on full term or near full term pregnancies to ensure homogeneity for sample age. Three cell types were derived: 1) primary fibroblasts, 2) LCLs and 3) primary T-cells. Total RNA was extracted from these cells and two one-quarter scale Message Amp II reactions (Ambion) were performed for each extraction with 200 ng of total RNA. 1.5 μg of cRNA was hybridized to Illumina’s WG-6 v3 Expression BeadChip array to quantify transcript abundance. Intensity values were log2 transformed and normalized independently for each cell type using quantile normalization for sample replicates, and median normalization across all individuals. Each cell type was renormalized using the mean of the medians of each cell type expression values. DNA samples were extracted from umbilical cord tissue LCLs with the Puregene cell kit (Gentra-Qiagen, Venlo, the Netherlands) and genotyping was performed using the Illumina 550K SNP array (Illumina Inc., San Diego, USA) to obtain the SNP genotypes for the samples.

Statistical analysis of initial genome-wide scan data

Stringent per-SNP and per-sample limits were implemented in order to obtain high-quality data. Quality control measures were: exclusion of samples with call rates <97%, non-comparable ancestry as measured using multidimensional scaling plots from PLINK, possible contamination as identified by being an extreme heterozygosity outlier, and cryptic relatedness (low-level relatedness to a large number of samples), and non-cryptic relatedness of pi-hat >12.5%. From the initial 3,279 cases and 12,369 controls, altogether 2,748 cases and 10,747 controls passed all quality control criteria, while 531 cases and 1,622 controls were excluded. The majority of case exclusions were due to quality issues on the 550K chips, and the majority of control exclusions were due to low-level relatedness in the Dutch control set. SNPs were excluded for having a minor allele frequency of <1% or for departing from Hardy-Weinberg equilibrium with p < 10^{-6} in cases or controls. Only completely overlapping SNPs from the three populations were used, leaving a total of 429,912 SNPs for analysis. To ascertain whether the control samples were properly matched to the cases, a population-specific and overall genomic inflation factors (λ) was estimated using the median χ² value from a 1-degree of freedom allelic χ² test. For the Finns, λ = 1.05, for Germans λ = 1.07, for the Dutch λ = 1.09, and overall λ = 1.08, suggesting reasonably well-matched controls in each case. Differences between cases and controls were assessed between each SNP and disease using a two-tailed Cochran-Mantel-Haenszel (CMH) test for 2x2xK stratified data (K = 3), as implemented in PLINK v1.06. To exclude long-range LD for the identified variant, we used the program ssSNPer to demonstrate that no SNP within a 5 Mb window had high LD to rs1835740 in HapMap Phase II data.

Conditional analysis for secondary effects

In addition to rs1835740, two other SNPs on 8q22.1, rs2436046 and rs982502, showed a CMH p-value < 10^{-3} (main paper Table 2 and Figure 2). Based on our data, rs2436046 (r² = 0.68) and rs982502 (r² = 0.59) are in moderate LD with rs1835740. To evaluate whether these signals were independent from the top SNP association signal, the association between migraine and SNP alleles was tested using logistic regression and conditioning on rs1835740 as implemented in PLINK v1.06. Conditioning on rs1835740, no evidence of additional independent signals was found either for rs2436046 or rs982502 (p = 0.89 and p = 0.47) (Supplementary Table 3), suggesting that the moderate association of rs2436046 and rs982502 observed in the CMH test is the result of these SNPs being in LD with rs1835740.
Meta-analysis of initial and replication samples

The CMH test was used for meta-analysis, with a nominal covariate used to distinguish each sample collection from the others. For the replication in Icelandic and Danish samples, association analysis was carried out using a likelihood procedure\textsuperscript{37}, and results were adjusted for relatedness by dividing the chi-square statistics by an inflation factor estimated through simulation\textsuperscript{38}.

Imputation

For each cohort, imputation of the untyped markers in the 2 Mb region around rs1835740 was carried out using IMPUTE v2 with recommended options\textsuperscript{39}. Haplotypes from the 1,000 Genomes Project (August 2009 release) and haplotypes from HapMap Phase 3 (www.hapmap.org) were used as reference panels.

eQTL analysis

Association between genotypes and expression was analyzed with Spearman rank correlation for all SNPs with a 2 Mb window centered on the transcription start site of the gene. Significance was assessed by comparing the observed p-values at a 0.001 threshold with minimum p-values from each of 10,000 permutations of the expression values relative to genotypes\textsuperscript{35}.

URLs

Control populations: Finland – Health2000 study, www.nationalbiobanks.fi; Finland – Helsinki Birth Cohort study, www.nationalbiobanks.fi; Germany – KORA S4/F4 study, www.helmholtz-muenchen.de/kora; Germany – PopGen study, www.popgen.de; Germany – HNR study, www.recall-studie.uni-essen.de; Illumina iControlDB – www.illumina.com; the Netherlands – Rotterdam I and III studies, www.epib.nl/research/ergo.htm; the Netherlands – Lumina study, www.lume.nl/hoofdpijn. Other URLs: International Headache Genetics Consortium – www.headache genet i c s. org; ssSNPer – http://gump.qimr.edu.au/general/daleN/ssSNPer/; GWAS plotter – broadinstitute.org/node/555; HapMap Phase 2 and 3 data – www.hapmap.org

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cochran-Mantel-Haenszel association results for combined analysis of the three study populations between 97.5 and 98.5 Mb on chromosome 8q22.1

Diamonds show position and p-value for each marker in the region, with colors representing extent of linkage disequilibrium (measured in $r^2$) with marker rs1835740, and blue circles indicate locations and p-values of imputed markers. For rs1835740, p-values are shown for both the original genome-wide association study and the meta-analysis of all migraine samples in the study (denoted by asterisk). The blue graph shows the local recombination rate based on HapMap Phase II data\textsuperscript{11}. Red line denotes the threshold for genome-wide significance ($p \leq 5 \times 10^{-8}$). Figure was generated using a modified version of the script available at broadinstitute.org/node/555.
Figure 2. Forest plot of migraine risk for individuals carrying the A allele of marker rs1835740 in each study population
For each dataset, the horizontal line indicates 95% confidence interval, and the number above the line indicates the point estimate of the odds ratio. MA only – patients whose attacks are always accompanied with aura, Both MA, MO – patients with attacks with and without aura, MO only – patients whose attacks never include aura.
Figure 3. A box-plot of the quantified expression values for *MTDH/AEG-1* ordered based on sample genotype of rs1835740

Normalised expression levels in lymphoblastoid cell lines using Illumina's WG-6 v3 Expression BeadChip array are shown. In each group, the small pyramid indicates median value, the shaded area represents the lower and upper quartiles, and the crosses show the minimum and maximum values in the expression data.
Table 1

Study populations used in the two stages of the study.

|                          | Total | Men   | Women | Patients with both MA and MO attacks | Patients with MA attacks only | Patients with MO attacks only |
|--------------------------|-------|-------|-------|--------------------------------------|------------------------------|-------------------------------|
| **Initial GWA study**    |       |       |       |                                      |                              |                               |
| Finland                  |       |       |       |                                      |                              |                               |
| Cases                    | 1064  | 19.8% | 80.2% | 94.4%                                | 5.6%                         | 0.0%                          |
| Controls                 | 3513  | 47.4% | 52.6% | -                                    | -                            | -                            |
| Germany                  |       |       |       |                                      |                              |                               |
| Cases                    | 1029  | 18.9% | 81.1% | 70.2%                                | 29.8%                        | 0.0%                          |
| Controls                 | 2317  | 45.1% | 54.9% | -                                    | -                            | -                            |
| the Netherlands          |       |       |       |                                      |                              |                               |
| Cases                    | 655   | 17.2% | 82.8% | 65.9%                                | 34.1%                        | 0.0%                          |
| Controls                 | 4917  | 41.7% | 58.3% | -                                    | -                            | -                            |
| Total GWAS               |       |       |       |                                      |                              |                               |
| Cases                    | 2748  | 18.8% | 81.2% | 78.5%                                | 21.5%                        | 0.0%                          |
| Controls                 | 10747 | 44.3% | 55.7% | -                                    | -                            | -                            |
| **Replication stage**    |       |       |       |                                      |                              |                               |
| Iceland                  |       |       |       |                                      |                              |                               |
| Cases                    | 900   | 22.5% | 77.5% | 63.0%                                | 21.8%                        | 15.2%                         |
| Controls                 | 35221 | 57.4% | 42.6% | -                                    | -                            | -                            |
| Denmark                  |       |       |       |                                      |                              |                               |
| Cases                    | 1116  | 22.4% | 77.6% | 26.3%                                | 43.3%                        | 30.5%                         |
| Controls                 | 1353  | 44.5% | 55.5% | -                                    | -                            | -                            |
| the Netherlands          |       |       |       |                                      |                              |                               |
| Cases                    | 349   | 18.3% | 81.7% | 59.8%                                | 40.2%                        | 0.0%                          |
| Controls                 | 2082  | 43.9% | 56.1% | -                                    | -                            | -                            |
| Germany                  |       |       |       |                                      |                              |                               |
| Cases                    | 837   | 11.6% | 88.4% | 0.0%                                 | 0.0%                         | 100.0%                        |
| Controls                 | 1406  | 37.3% | 62.7% | -                                    | -                            | -                            |
| Total replication        |       |       |       |                                      |                              |                               |
| Cases                    | 3202  | 19.1% | 80.9% | 33.8%                                | 25.6%                        | 41.0%                         |
| Controls                 | 40062 | 55.6% | 44.4% | -                                    | -                            | -                            |
| **Overall meta-analysis**|       |       |       |                                      |                              |                               |
| Cases                    | 5950  | 19.0% | 81.0% | 54.4%                                | 23.7%                        | 22.1%                         |
| Controls                 | 50809 | 53.2% | 46.8% | -                                    | -                            | -                            |
Table 2

Association results for marker rs1835740 using the CMH test.

| Diagnosis | n (cases) | n (controls) | Case alleles (MAF) | Control alleles (MAF) | P-value | OR  | 95% CI     |
|-----------|-----------|--------------|--------------------|-----------------------|---------|-----|------------|
| **GWA study** |           |              |                    |                       |         |     |            |
| Finland   | All migraine | 1 064        | 3 513              | 548/1 576 (0.258)     | 1 553/5 461 (0.221) | 0.000447| 1.22| 1.093-1.368 |
| Germany   | All migraine | 1 029        | 2 317              | 515/1 537 (0.251)     | 998/3 652 (0.216)    | 0.00142 | 1.22| 1.079-1.378 |
| the Netherlands | All migraine | 655       | 4 917              | 329/963 (0.255)       | 2 086/7 742 (0.212)  | 0.000876| 1.26| 1.098-1.437 |
| Combined GWA |           |              |                    |                       |         |     |            |
| MA only   |            | 589          | 10 747             | 313/859 (0.267)       | 4 637/16 385 (0.216) | 3.07 × 10⁻⁵| 1.33| 1.164-1.528 |
| Both MA,MO|            | 2 142        | 10 747             | 1 071/3 193 (0.251)   | 4 637/16 385 (0.216) | 2.69 × 10⁻⁶| 1.21| 1.115-1.304 |
| All migraine |            | 2 748        | 10 747             | 1 392/4 076 (0.255)   | 4 637/16 385 (0.216) | 5.12 × 10⁻⁹| 1.23| 1.150-1.324 |
| **Replication study** |           |              |                    |                       |         |     |            |
| Denmark   | MA only    | 483          | 1 353              | 244/722 (0.253)       | 562/2 144 (0.208)    | **0.015**| 1.29| 1.050-1.583 |
|           | Both MA,MO | 293          | 1 353              | 121/465 (0.206)       | 562/2 144 (0.208)    | 0.951  | 0.99| 0.785-1.255 |
|           | MO only    | 340          | 1 353              | 153/527 (0.225)       | 562/2 144 (0.208)    | 0.333  | 1.11| 0.900-1.362 |
|           | All migraine | 1116        | 1 353              | 518/1 714 (0.232)     | 562/2 144 (0.208)    | 0.069  | 1.15| 0.989-1.344 |
| Iceland   | MA only    | 137          | 35 221             | 70/204 (0.255)        | 14 212/56 230 (0.202) | **0.0380**| 1.36| 1.017-1.812 |
|           | Both MA,MO | 196          | 35 221             | 82/310 (0.209)        | 14 212/56 230 (0.202) | 0.7256 | 1.05| 0.812-1.350 |
|           | MO only    | 567          | 35 221             | 261/873 (0.230)       | 14 212/56 230 (0.202) | **0.0292**| 1.18| 1.017-1.376 |
|           | All migraine | 900        | 35 221             | 413/1 387 (0.229)     | 14 212/56 230 (0.202) | **0.010**| 1.18| 1.041-1.334 |
| the Netherlands | MA only   | 212          | 2 082              | 100/324 (0.236)       | 909/3 255 (0.218)    | 0.406  | 1.11| 0.873-1.399 |
|           | Both MA,MO | 137          | 2 082              | 66/208 (0.241)        | 909/3 255 (0.218)    | 0.382  | 1.14| 0.853-1.513 |
|           | All migraine | 349       | 2 082              | 166/532 (0.238)       | 909/3 255 (0.218)    | 0.250  | 1.12| 0.925-1.350 |
| Germany   | MO only    | 837          | 1 046              | 396/1 278 (0.240)     | 629/2 183 (0.224)    | 0.3206 | 1.08| 0.932-1.241 |
|           | MO only *  | 837          | 541                | 396/1 278 (0.240)     | 218/864 (0.201)      | **0.0307**| 1.23| 1.019-1.480 |
| **Meta-analysis** |           |              |                    |                       |         |     |            |
| All MA only |           | 1 421        | 49 403             | 727/2 109 (0.256)     | 20 320/78 464 (0.206) | 6.98 × 10⁻⁸| 1.29| 1.173-1.408 |
| All Both MA,MO |           | 2 768        | 49 403             | 1 340/4 176 (0.243)   | 20 320/78 464 (0.206) | 1.09 × 10⁻⁵| 1.17| 1.089-1.248 |
| All MO only |           | 1 744        | 37 980             | 810/2 678 (0.232)     | 15 403/60 557 (0.203) | 0.0105 | 1.12| 1.028-1.230 |
| All migraine |           | 5 950        | 50 809             | 2 885/8 987 (0.243)   | 20 948/8 064 (0.206) | **1.60 × 10⁻¹¹**| 1.18| 1.127-1.244 |
The German replication control set consists of several small samples. The largest of these had a considerably deviating MAF (0.238, n=865) compared to other German (average MAF 0.216, n=3,260) and Central European control sets (average MAF 0.212, n=9,560). Thus values with both including and excluding the outlier control sample are presented. The meta-analysis value includes all control samples (without outlier control group, “All MO samples” p-value 0.00107, OR 1.18 [1.068-1.298] and “All migraine” $8.43 \times 10^{-13}$, OR 1.20 [1.143 - 1.264]. Values on line denoted by * are calculated after excluding the outlier control sample.
Association of rs1835740 genotype with gene expression levels.

| SNP     | Gene     | Strand | SNP coordinate | Gene start | Distance | SRC p-value |
|---------|----------|--------|----------------|------------|----------|-------------|
| rs1835740 | UQCRB    | −      | 98 236 089     | 97 311 911 | 924 178  | 0.0013226   |
| rs1835740 | MTDH/AEG-1 | +      | 98 236 089     | 98 725 583 | 489 494  | **0.0000396** |
| rs1835740 | HRSP12   | −      | 98 236 089     | 99 183 743 | 947 654  | 0.0028748   |

* Genes with nominal or higher p-values of expression association to rs1835740 genotype in the Spearman rank correlation test are shown.

* indicates surpassing the significance threshold $7.7 \times 10^{-5}$ (corresponding to a 0.001 permutation threshold after 10,000 permutations). SRC = Spearman rank correlation. Gene start refers to the location of 5' end of the gene, if on positive strand, 3' end if on negative. Locations and distances in basepairs, according to NCBI build 36. P-values in bold surpass the level of genome-wide significance.