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## **The migraine triad: chronification, depression, and medication overuse**

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# 7

CHAPTER

## Candidate gene association study searching for genetic factors involved in migraine chronification

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## **Abstract**

### ***Introduction***

Chronic migraine (CM) is at the severe end of the clinical migraine spectrum, but its genetic background is unknown. Our study searched for evidence that genetic factors are involved in the chronification process.

### ***Methods***

We initially selected 144 single nucleotide polymorphisms (SNPs) from 48 candidate genes, which we tested for association in two stages: the first stage encompassed 262 CM patients, the second investigated 226 patients with high-frequency migraine (HFM). Subsequently, SNPs with  $p$ -values  $<0.05$  were forwarded to the replication stage containing 531 patients with CM or HFM.

### ***Results***

Eight SNPs were significantly associated with CM and HFM in the two-stage phase. None survived replication in the third stage.

### ***Discussion***

We present the first comprehensive genetic association study for migraine chronification. There were no significant findings. Future studies may benefit from larger, genome-wide data sets or should use other genetic approaches to identify genetic factors involved in migraine chronification.

## Introduction

According to the ICHD-III beta classification criteria, a clinical diagnosis of chronic migraine (CM) is made when a patient has 15 or more days with headache per month of which at least eight days have features of migraine headache (or that are described by the patient as migraine and are relieved by migraine-specific medication). (1) CM is at the severe end of the clinical migraine spectrum with a substantially decreased quality of life and increased disability, and is strongly associated with depression, medication overuse, and/or cutaneous allodynia. (2, 3) The reported prevalence of CM is estimated to be around 0.5% to 2.0%. (4, 5) Recently, several genome-wide association studies (GWAS) have identified a dozen susceptibility gene variants and loci for episodic migraine, but until now no studies have focused on identifying genetic risk factors for CM. (6-8)

It is debatable whether it is meaningful to make a strict distinction between episodic and chronic migraine because headache frequency in patients varies from month to month and the thresholds of 15 headache days and eight migraine days, while practical, are arbitrary. (9) Genetic studies in rarer complex disease subtypes, such as CM, are particularly challenging as collecting sufficiently large numbers of well-characterized patients is difficult. Therefore, we decided to also include a group of patients with high-frequency migraine (HFM) that suffer from headache on 10 to 14 days per month, with half or more days meeting the criteria for migraine.

The aim of this study was to obtain evidence for association of variants in genes, acting in pathways possibly implicated in the chronification process of migraine as well as relevant secondary hits from GWA studies, with chronification of migraine. In total, 144 SNPs selected based on literature and previous studies were tested in a three-stage design.

## Methods

### *Participants and design of the genetic association study*

Participants included in our study were patients diagnosed with either chronic migraine (CM) or high frequency migraine (HFM), and healthy control individuals. Migraine diagnoses were based on ICHD criteria. A three-stage genetic association study was performed (Figure 1). The discovery stage included 262 CM patients and 2,879 control individuals (all patients came from the CHROMIG study (Spain) or the LUMINA study (The Netherlands)). In this stage, all 144 SNPs (in 48 genes) were tested. The selected markers filled one or more of the following criteria: i) SNPs had been positively associated to migraine and not replicated in other migraine

candidate gene association studies; ii) the corresponding genes had already been implicated in mechanisms relevant to the chronification of migraine; or iii) SNPs were identified as secondary findings in previous migraine GWA studies. In the second stage, all SNPs of the first stage that showed a p-value <0.05 were tested in a further 226 patients diagnosed with HFM vs. the same 2,879 controls (patients again came from Spain or The Netherlands). In the third stage, SNPs with p-values <0.05 in the first two stages were tested for replication in 531 patients with CM or HFM (all patients came from the CHROMIG study, Spain, the LUMINA study, The Netherlands or the HUNT study, Norway). In this stage, 2,491 different control individuals from the three countries were tested.

### ***Gene and SNP selection***

We designed a candidate-gene association study focusing on genes that are likely associated with migraine or migraine comorbidities and may act as risk factors for migraine progression. To date, many association studies have been performed to identify genetic factors that confer susceptibility to common migraine (10, 11). We selected genetic variants that had been studied in Caucasian populations, especially those which were only studied once. According to these criteria, a total number of 42 SNPs in 26 genes were selected. These genes were related to: i) ion metabolism transport (*CACNB2* and *KCNB2* (12), *STX1A* (13), *EDN1*, *EDNRA* and *EDNRB*) (14-16); ii) dopamine (*DBH*) (17) and serotonin metabolism (*HTR2B*) (18); iii) hormonal metabolism (*ESR1*) (19-23); iv) vascular disease (*IL-9*, *KCNK17*, *LRP1*, *MMP12*, *MTHFD1*, *NOS3*, *SCNN1A*, *TGFB1* and *TNF*) (24-26); v) autonomous nervous system dysfunction (*GNAS1* and *KCNJ1*) (27, 28); vi) stress-response (*BDNF*) (29); vii) and psychiatric disorders related genes, specially associated with anxiety and depression syndrome (*COMT*, *CRY1*, *VIPR2*, *RGS2*, *SCN9A* and *WFS1*) (30-33). In addition, we selected candidate genes that encode molecules known to play an important role in migraine pathophysiology but had not been studied before in candidate gene association studies for migraine. In more detail, 37 TagSNPs were selected from CEU Hapmap data using a tagger pairwise tool with  $r^2 > 0.8$  (Haploview tool) that code for calcitonin-gene related peptide (*CALCA*) and its CGRP-receptor subunits (*CALCRL* and *RAMP1*). The pituitary adenylatecyclase-activating polypeptide (*PACAP*), a neuroexcitatory peptide released to periaqueductal gray matter during neurogenic inflammation, encoded by the *ADCYAP1* gene, and its receptor, encoded by *ADCYAP1R1*, were also investigated with 17 tagSNPs. *ESR1* and *ESR2*, but not *GPR30* estrogen receptors, have been previously studied in relation to migraine, so we included *GPR30*, which encodes a multi-pass membrane protein that binds estrogen. Fractalkine, a chemokine that has been associated with neuroprotection (*CX3CL1* gene), and its receptor (*CX3CR1*) were chosen as candidate genes with six tagSNPs (34). We also focused on two molecules that had previously been reported in a microarray study as probable migraine with aura biomarkers, namely alpha-phodrin (*SPTAN1*) and

hippocalcin-like protein (*HPCAL1*) (35). The former is a cytoskeletal protein of the spectrins family and the latter is a member of neuron-specific calcium binding protein family and is involved in neuronal signaling in the central nervous system. Two tagSNPs in both genes were genotyped. A tagSNP in peripherin (*PRPH*), a cytoskeletal protein localized in neurons of the peripheral nervous system, the expression of which has been associated with *GPR30*, was also studied (36). Finally, eight tagSNPs in a gene involved in circadian rhythm and metabolism regulation (*CLOCK*) were added to the panel. Overall, 77 non-previously studied gene variants in 12 genes were selected.

Finally, 25 polymorphisms extracted from the list of secondary top hits in the analysis of the first migraine GWAS (6) that was carried out by our International Headache Genetics Consortium (IHGC) were included. The list included fifteen intergenic SNPs and 10 variations that were located in gene coding regions (*ACSL5*, *C4orf22*, *DCC*, *INSIG2*, *OPCML*, *OR9Q1*, *RELN*, *SMYD3*, *STAMBPL1* and *TRPM8*). In summary, 119 SNPs were genotyped in 38 candidate genes, as well as 25 additional SNPs from GWAS data.

### Cohorts

Spanish CM and HFM patients were recruited at the Headache Unit of the Vall d'Hebron University Hospital (Barcelona). Patients with CM were diagnosed by a clinical interview and physical examination by a headache specialized neurologist, according to the ICHD-III beta classification. (1) HFM was diagnosed when patients suffered from headache on 10 to 14 days per month, from which half or more days fulfilled the criteria for migraine. Healthy controls were blood donors. Exclusion criteria for this control population were migraine, a positive family history for migraine and any type of severe or recurrent headache in first-degree relatives.

Dutch CM and HFM patients were available from the well-defined, web-based LUMINA population (Leiden University Migraine Neuro Analysis program) ([www.lumc.nl/hoofdpijn](http://www.lumc.nl/hoofdpijn)). Details of this study are described elsewhere. (37) Migraine was diagnosed according to the ICHD-III beta criteria. (1) CM was diagnosed when patients suffered from migraine and indicated that they experienced severe headache on 15 or more days per month. HFM was diagnosed when patients suffered from migraine and indicated that they experienced severe headache on 10-14 days per month. Control samples for the discovery phase were part of the population-based Rotterdam Study. (38) Control samples for the replication phase were collected via a Dutch blood bank.

The Norwegian patients were recruited from the population-based HUNT-2 (1995-97) and HUNT-3 (2006-08) studies, in which all inhabitants (age  $\geq 20$  years) of the Nord-Trøndelag county of Norway were invited to participate. (39, 40) Migraine

was diagnosed based on a modified version of the most recent ICHD criteria at the time of each study, and this questionnaire-based headache classification has been validated by interview diagnoses. (39, 40) Migraineurs reporting headache on 7 or more days per month were classified as HFM, and those reporting headache on 15 or more days per month were classified as CM. Controls were recruited from the same two studies, and participants fulfilling criteria for migraine were excluded from the control population.

## Genotyping

### *Spanish cohorts*

Venous blood samples of subjects that fulfilled inclusion criteria were collected in EDTA tubes and conserved at -80°C until DNA extraction. DNA was extracted from blood lymphocytes at the *Centre de Regulació Genòmica* (CRG, Barcelona, Spain) with the Chemagen® extraction kit (Perkin Elmer, Germany) and at the *Departament de Genètica* (Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain) by a standard salting out procedure. (41) Quantity and quality of DNA samples were controlled spectrophotometrically with NanoDrop ND1000 (Nanodrop, Wilmington, DE, USA). Genotyping of SNPs in the discovery sample set was performed with VeracodeGoldenGate technology (Illumina, CRG, Barcelona, Spain). For the replication phase, an additional 70 CM and HFM patients and 394 controls were recruited under the same criteria and procedures that were used for the discovery sample. Blood sampling and DNA extraction were performed in the same way. Genotyping was performed with a Taqman® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) using the 7900HT Sequence Detection System (SDS, Applied Biosystems) in 384-well plates and following the manufacturer's protocol.

### *Dutch cohort*

Peripheral blood samples were collected in EDTA tubes. Subsequently, DNA was isolated using a standard salting out method. Genotyping of the samples had been previously performed as part of two genome-wide association studies for common migraine. (6, 7) Genotyping of the replication cohort was performed with a Taqman® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). A standard PCR reaction was carried out using the TaqMan Universal PCR Master Mix. Genotyping clusters were analysed using the Lightcycler LC-480 machine and LightCycler®480 1.5.0 software, version 1.5.0.39 (Roche Applied Science, Penzberg, Upper Bavaria, Germany) in 384-well plates following the manufacturer's protocol.

### ***Norwegian cohorts***

DNA from all Norwegian samples was extracted from blood using two kits: Autopure Kit (Qiagen, Duesseldorf, Germany) and Masterpure Kit (Medinor, Oslo, Norway), both based on a salting out procedure. Quantity and quality of DNA samples were controlled regularly by monitoring every eighth sample spectrophotometrically with NanoDrop ND1000 and ND8000 (Nanodrop). Genotyping of the replication cohort was performed with a Taqman® SNP Genotyping Assay (Applied Biosystems). A standard PCR reaction was carried out using the TaqMan Universal PCR Master Mix. Genotyping clusters were analyzed using the Lightcycler LC-480 machine and LightCycler®480 1.5.0 software, version 1.5.0.39 (Roche Applied Science, Penzberg, Germany) in 384-well plates following the manufacturer's protocol. A part of the Norwegian sample for replication had previously been genotyped with the Illumina 670k platform, as part of a genome-wide association study of migraine (8), and was used for *in silico* replication for the current study. We used the Illuminus calling algorithm, with the following filters for genotyped SNPs: minimum call rate per SNP and per individual (0.97), Hardy-Weinberg equilibrium p-value higher than 1.00E-06 and MAF>0.01. For those SNPs that were not directly genotyped, imputation was performed with Impute v.2.1.2 in a standardized pipeline, using HapMap2 data from CEU population as the reference panel.

### ***Statistical analyses***

We performed power calculations for all three steps of our design, assuming an additive model, an effect allele frequency of 0.20 and effect sizes ranging from 1.2-1.4. We added the outcome of these power calculations to the supplementary information. Statistical analyses were performed using PLINK v1.07 (42) and SNPTEST v2.2.0. (43) GTOOL v0.7.5 was used to combine different cohorts. First, the entire panel of SNPs was tested for the Hardy-Weinberg equilibrium (HWE) for each cohort considering  $p < 0.05$  as the threshold. Then, both allele and genotype frequencies were compared between cases and controls, considering additive, genotypic (co-dominance), dominant and recessive models. Subsequently, a meta-analysis was performed using GWAMA v2.1. For all analyses, the threshold for statistical significance was defined as a p-value below 0.05. Approval was obtained from local medical ethics committees and written informed consent was obtained from all participants.

## Results

For this study, 144 SNPs in genes already implicated in migraine or that had surfaced as interesting secondary hits in GWA studies (see Supplementary Data) were used in a three-stage association design (Figure 2). In the first stage, SNPs were tested in 262 patients with CM vs. 2,879 control individuals. Nominal significant associations ( $p$ -value  $<0.05$ ) were obtained for 30 SNPs (see also Supplemental Table 2). These 30 SNPs were taken forward to the second stage with 226 patients with HFM and the same control data set, where eight SNPs showed a nominally significant association; rs5742912 (in *SCNN1A*), rs3792603 (in *CLOCK*), rs2956 (in *CALCA*), rs858745 (in *CALCRL*), rs302680 (in *RAMP1*), rs2267730 and rs2299908 (in *ADCYAP1R1*), and rs217693 which is an intergenic SNP. These eight SNPs were taken forward to the replication stage and were genotyped in three replication cohorts from Spain (70 patients with CM or HFM and 394 controls), The Netherlands (210 patients with CM or HFM and 896 controls), and Norway (162 patients with CM or HFM and 495 controls). The availability of GWA data allowed testing of seven of the eight SNPs in 89 additional Norwegian patients with CM or HFM and 706 controls. Subsequently, a combined meta-analysis of the association results from these replication cohorts with 531 patients with CM or HFM and 2,491 controls was performed but showed no statistically significant associations.

## Discussion

Here we present the first comprehensive genetic association study in chronic and high-frequency migraineurs testing 144 SNPs from 48 genes in 1,019 patients with chronic or high frequency migraine, without significant associations. Patient numbers in each cohort were relatively small, largely because of the rarity of chronic migraine, which makes it difficult to collect large enough patient samples. As (chronic) migraine is a complex genetic disorder, it is likely that multiple genetic variants, each with relatively small effect, contribute to disease susceptibility, suggesting that large numbers of patients and controls are needed to reach sufficient power to detect a genetic association. We attempted to address this challenge in two ways. First, to increase overall numbers, we decided not only to include chronic migraine patients, but also high frequency migraineurs, as we consider the cut off values for a diagnosis of chronic migraine rather arbitrary and instead favour the idea that migraine chronification has a broader spectrum with respect to the number of headache days.<sup>(9)</sup> Second, by selecting only candidate genes (and SNPs therein) we reduced the massive correction for multiple testing that is needed for unbiased genome wide association approaches. Considering the negative results, our approach may still have had insufficient statistical power or we may have selected SNPs irrelevant to migraine chronification. As even large international collaborations, such as the International Headache Genetics

Consortium (IHGC), have difficulties to collect large enough cohorts of well-characterized patients with chronic and high frequency migraine, we feel that studies like ours will probably remain underpowered in the immediate future. We are working in the International Headache Genetic Consortium on unifying the criteria to select patients so that future studies will be able to count on larger and better phenotyped cohorts.

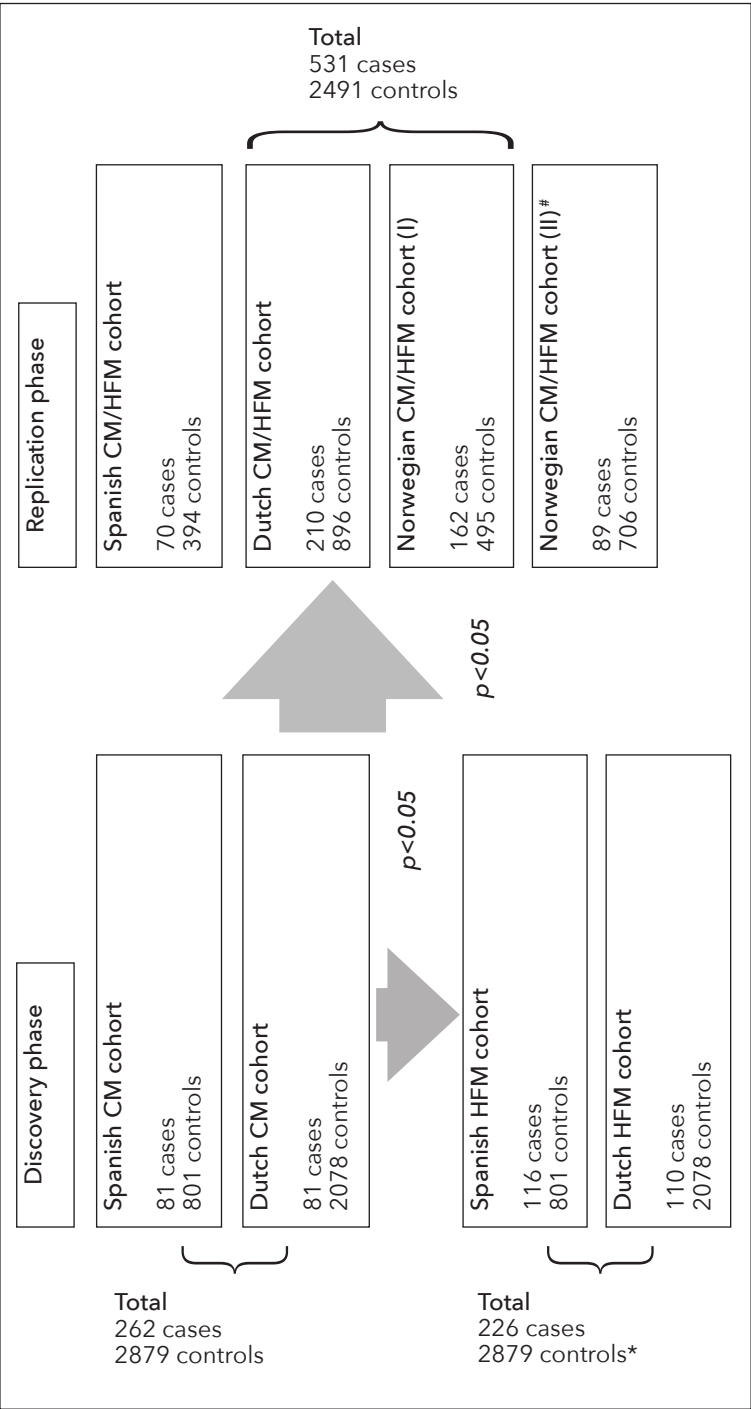
Chronic migraine is severely disabling and difficult to manage, as affected patients experience substantially more-frequent headaches, comorbid pain and affective disorders, and fewer pain-free intervals, than do those with episodic migraine. (4) Furthermore, the relationship of chronic migraine with cutaneous allodynia has been investigated, indicating that cutaneous allodynia is a clear risk factor for migraine chronification. (3) Different models have been proposed to explain this relationship. Further investigations in the basic mechanisms of cutaneous allodynia, and its relationship with migraine chronification, could lead to new potential genes which should be studied in future designs.

Clinical and genetic studies have shown that migraine is a multifactorial disorder with complex interaction between multiple predisposing genetic and modulating non-genetic factors. GWAS have identified 13 gene variants pointing, among others, at pathways involved in glutamatergic neurotransmission and synaptic function. (8) Translating results from GWAS to pathophysiological mechanisms, however, remains one of the biggest challenges in molecular biology as gene effect sizes are small and their interactions are complex.

We suggest that for future designs it is relevant to consider the outcome of withdrawal from medication, as the vast majority of CM patients is (over)using acute headache medication. In this study, we did not have sufficient data to include this aspect in the analysis. However, future studies would benefit from subdividing chronic migraineurs into patients responsive to withdrawal therapy and returning to episodic migraine after withdrawal of their medication, and patients in which such withdrawal has no or less effect on attack frequency. Lastly, although the problem of statistical power will remain problematic in association studies for chronic and high frequent migraine, we would like to put forward that perhaps other genetic approaches are more fruitful in detecting genes and pathways involved in chronic migraine, such as gene expression studies, epigenetic studies or the analysis of rare variants.

Figures and tables

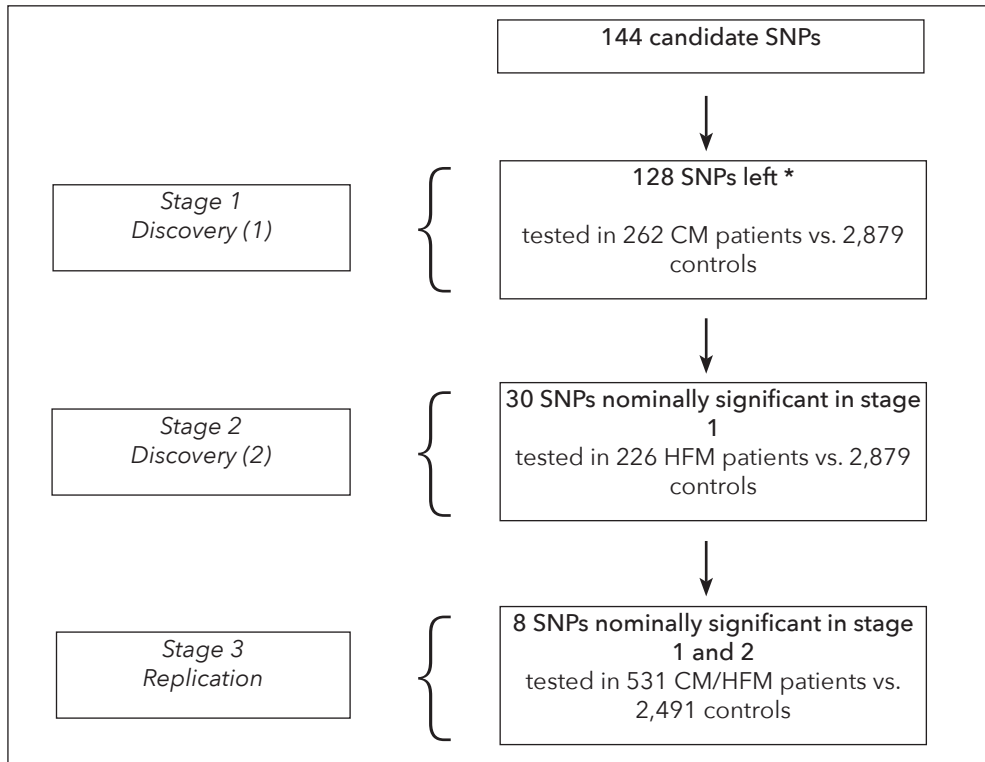
Figure 1: Three-stage gene association cohort study design



\*In stages 1 and 2, the same group of 2879 controls was used.

# The second Norwegian cohort was previously genotyped as part of a GWAS (5). This cohort was used for *in silico* replication in the present study. The other cohorts in stage 3 were genotyped using a *Taqman* genotyping assay.

Figure 2: SNP selection study flow



\* 16 SNPs excluded due to genotyping failure, low HWE, or low rate of successful genotypes.  
Nominally significant:  $p < 0.05$

Table 1: Results of the replication phase (stage 3)

| General SNP information |                |            |             |             | Stage 1              | Stage 2               | Stage 3                    |                     |             |             |         |
|-------------------------|----------------|------------|-------------|-------------|----------------------|-----------------------|----------------------------|---------------------|-------------|-------------|---------|
| SNP                     | Chr.(position) | Gene       | Ref. allele | Alt. allele | Discovery phase (CM) | Discovery phase (HFM) | Replication phase (CM/HFM) |                     |             |             |         |
|                         |                |            |             |             | p-value              | p-value               | p-value                    | OR (95%CI)          | Cohorts (N) | Samples (N) | Effects |
| rs5742912               | 12 (6458350)   | SCNN1A     | G           | A           | 0.035                | 0.003                 | 0.31                       | 0.872 (0.672-1.132) | 3           | 2,108       | -/?/-/- |
| rs3792603               | 4 (56302058)   | CLOCK      | G           | A           | 0.006                | 0.004                 | 0.69                       | 1.024 (0.911-1.151) | 4           | 2,943       | -/+/-/+ |
| rs217693                | 14 (62402801)  | intergenic | G           | A           | 0.045                | 0.019                 | 0.10                       | 0.911 (0.816-1.018) | 4           | 2,930       | -/-/-   |
| rs2956                  | 11 (14989121)  | CALCA      | T           | A           | <0.001               | 0.029                 | 0.90                       | 0.993 (0.895-1.102) | 4           | 2,967       | -/+/-/+ |
| rs858745                | 2 (188216807)  | CALCRL     | T           | C           | <0.001               | 0.004                 | 0.12                       | 1.129 (0.971-1.313) | 4           | 2,925       | +/-/+/- |
| rs302680                | 2 (238791396)  | RAMP1      | G           | A           | 0.040                | 0.010                 | 0.89                       | 1.009 (0.887-1.147) | 4           | 2,937       | +/-/+/- |
| rs2267730               | 7 (31122630)   | ADCYAP1R1  | T           | C           | 0.043                | 0.036                 | 0.36                       | 0.956 (0.868-1.052) | 4           | 2,845       | -/+/-/- |
| rs2299908               | 7 (31138096)   | ADCYAP1R1  | G           | A           | 0.026                | 0.046                 | 0.34                       | 0.946 (0.843-1.061) | 4           | 2,871       | -/-/+/- |

Genomic position in basepairs according to Build 37.

Ref. allele, Reference allele; Alt. allele, Alternative allele; OR, odds-ratio; CI, confidence interval.

Stage 1 included 262 CM cases and 2879 controls, stage 2 included 226 HFM cases and 2879 controls (same controls as stage 1), stage 3 included 531 CM/HFM cases and 2491 controls

Effects: Direction of individual effects in the four replication cohorts, depicted in the following order: *Spanish CHROMIG (Taqman) / Norwegian HUNT (in silico) / Norwegian HUNT (Taqman) / Dutch LUMINA (Taqman)*. (+) risk addition; (-) risk reduction; (?) not calculated (due to missing data).

## Supplemental Information

Supplemental table 1: power calculations

Stage 1:

| MAF | Prevalence of CM | RR   | Cases | Controls | Power (alpha 0.05) |
|-----|------------------|------|-------|----------|--------------------|
| 0.2 | 0.02             | 1.2  | 262   | 2,879    | 39%                |
| 0.2 | 0.02             | 1.25 | 262   | 2,879    | 54%                |
| 0.2 | 0.02             | 1.3  | 262   | 2,879    | 69%                |
| 0.2 | 0.02             | 1.4  | 262   | 2,879    | 90%                |

Stage 2:

| MAF | Prevalence of HFM | RR   | Cases | Controls | Power (alpha 0.05) |
|-----|-------------------|------|-------|----------|--------------------|
| 0.2 | 0.07              | 1.2  | 226   | 2,879    | 35%                |
| 0.2 | 0.07              | 1.25 | 226   | 2,879    | 49%                |
| 0.2 | 0.07              | 1.3  | 226   | 2,879    | 63%                |
| 0.2 | 0.07              | 1.4  | 226   | 2,879    | 85%                |

Stage 3:

| MAF | Prevalence of CM/HFM | RR   | Cases | Controls | Power (alpha 0.05) |
|-----|----------------------|------|-------|----------|--------------------|
| 0.2 | 0.09                 | 1.2  | 531   | 2,491    | 73%                |
| 0.2 | 0.09                 | 1.25 | 531   | 2,491    | 79%                |
| 0.2 | 0.09                 | 1.3  | 531   | 2,491    | 91%                |
| 0.2 | 0.09                 | 1.4  | 531   | 2,491    | 99%                |

MAF= minor allele frequency

CM/HFM= chronic/high frequency migraine patients

RR= Relative Risk

Supplementary table 2: SNPs that showed nominal association (p-value <0.05) in the CM cohort and were subsequently tested in the HFM cohort

| Gene                                                                                                                                          | SNP        | Alternative allele | Reference allele | MAF CM (N=262) | MAF HFM (N=226) | MAF controls (N=2,879) | Association test (CM/HFM) | CM uncorrected p-value | HFM uncorrected p-value |
|-----------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------|------------------|----------------|-----------------|------------------------|---------------------------|------------------------|-------------------------|
| GPR30<br>SCNN1A<br>COMT<br>TNF<br>CLOCK<br>STX1A<br>EDNRA<br>TRPM8<br>intergenic<br>intergenic<br>intergenic<br>intergenic<br>CALCA<br>CALCRL | rs3808353  | A                  | G                | 0.130          | 0.140           | 0.150                  | Gen/Gen                   | 0.035                  | 0.62                    |
|                                                                                                                                               | rs5742912  | G                  | A                | 0.036          | 0.048           | 0.035                  | Add/Add                   | 0.023*                 | 0.003*                  |
|                                                                                                                                               | rs4680     | G                  | A                | 0.517          | 0.467           | 0.464                  | Rec/Rec                   | 0.005                  | 0.67                    |
|                                                                                                                                               | rs1800750  | A                  | G                | 0.048          | 0.031           | 0.024                  | Add/Add                   | <0.001                 | 0.33                    |
|                                                                                                                                               | rs3792603  | G                  | A                | 0.279          | 0.200           | 0.246                  | Rec/Dom                   | 0.006*                 | 0.004*                  |
|                                                                                                                                               | rs941298   | A                  | G                | 0.262          | 0.274           | 0.315                  | Add/Add                   | 0.014                  | 0.063                   |
|                                                                                                                                               | rs1801708  | A                  | G                | 0.331          | 0.299           | 0.306                  | Rec/Rec                   | 0.012                  | 0.94                    |
|                                                                                                                                               | rs17862920 | T                  | C                | 0.074          | 0.088           | 0.106                  | Add/Add                   | 0.022                  | 0.236                   |
|                                                                                                                                               | rs10888075 | T                  | G                | 0.204          | 0.153           | 0.146                  | Add/Add                   | <0.001*                | 0.66                    |
|                                                                                                                                               | rs7753655  | G                  | A                | 0.250          | 0.287           | 0.298                  | Add/Add                   | 0.015                  | 0.67                    |
|                                                                                                                                               | rs217693   | A                  | G                | 0.242          | 0.256           | 0.209                  | Dom/Add                   | 0.045*                 | 0.019*                  |
|                                                                                                                                               | rs8060725  | A                  | C                | 0.311          | 0.288           | 0.281                  | Rec/Rec                   | 0.028                  | 0.75                    |
|                                                                                                                                               | rs1374111  | G                  | A                | 0.382          | 0.367           | 0.334                  | Dom/Rec                   | 0.014                  | 0.051                   |
|                                                                                                                                               | rs2956     | A                  | T                | 0.570          | 0.484           | 0.435                  | Add/Add                   | <0.001*                | 0.029*                  |
|                                                                                                                                               | rs858745   | T                  | C                | 0.213          | 0.190           | 0.156                  | Rec/Rec                   | <0.001*                | 0.004*                  |
|                                                                                                                                               | rs17464221 | T                  | C                | 0.333          | 0.317           | 0.304                  | Rec/Rec                   | 0.045                  | 0.97                    |
| RAMP1                                                                                                                                         | rs302679   | G                  | T                | 0.225          | 0.215           | 0.199                  | Rec/Rec                   | 0.029                  | 0.062                   |
|                                                                                                                                               | rs302680   | G                  | A                | 0.179          | 0.170           | 0.151                  | Rec/Rec                   | 0.040*                 | 0.010*                  |
|                                                                                                                                               | rs302673   | A                  | G                | 0.391          | 0.347           | 0.344                  | Dom/Dom                   | 0.008                  | 0.76                    |
|                                                                                                                                               | rs6741923  | C                  | G                | 0.328          | 0.280           | 0.280                  | Rec/Rec                   | 0.003                  | 0.39                    |
|                                                                                                                                               | rs757855   | C                  | G                | 0.406          | 0.387           | 0.377                  | Rec/Rec                   | 0.014                  | 0.27                    |
|                                                                                                                                               | rs6746771  | G                  | T                | 0.122          | 0.080           | 0.085                  | Add/Add                   | 0.004                  | 0.71                    |
|                                                                                                                                               | rs6717794  | G                  | A                | 0.351          | 0.291           | 0.312                  | Dom/Dom                   | 0.009                  | 0.26                    |
|                                                                                                                                               | rs4663804  | T                  | C                | 0.363          | 0.443           | 0.412                  | Dom/Dom                   | 0.007                  | 0.41                    |
|                                                                                                                                               | rs895572   | C                  | T                | 0.502          | 0.425           | 0.447                  | Rec/Rec                   | 0.001                  | 0.70                    |
|                                                                                                                                               | rs1080519  | T                  | C                | 0.256          | 0.192           | 0.222                  | Rec/Rec                   | 0.001                  | 0.65                    |
| ADCYAP1R1                                                                                                                                     | rs17723231 | T                  | C                | 0.287          | 0.261           | 0.251                  | Add/Add                   | 0.027                  | 0.55                    |
|                                                                                                                                               | rs2249714  | T                  | C                | 0.343          | 0.314           | 0.334                  | Rec/Rec                   | 0.031                  | 0.42                    |
|                                                                                                                                               | rs2267730  | T                  | C                | 0.432          | 0.430           | 0.471                  | Rec/Add                   | 0.043*                 | 0.036*                  |
|                                                                                                                                               | rs2299908  | A                  | G                | 0.232          | 0.179           | 0.223                  | Rec/Dom                   | 0.026*                 | 0.046*                  |
|                                                                                                                                               |            |                    |                  |                |                 |                        |                           |                        |                         |

\*Statistically significant in both discovery sets. \*Corrected p-value <0.05.

Genomic position in basepairs according to Build 37.

CM: chronic migraineurs; HFM: high-frequency migraineurs; MAF: Minor Allele Frequency.

Association model analysis: Gen (genotypic); Dom (dominant); Rec (recessive); Add (additive).

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