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ORIGINAL ARTICLE

Chronic migraine: Genetics or environment?

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Abstract

Background: The transition from episodic migraine to chronic migraine, migraine chronification, is usually a gradual process, which involves multiple risk factors. To date, studies of the genetic risk factors for chronic migraine have focused primarily on candidate-gene approaches using healthy individuals as controls.

Aims and methods: In this study, we used a large cohort of migraine families and unrelated migraine patients (n > 2200) with supporting genotype and whole-genome sequencing data. We evaluated whether there are any genetic variants, common or rare, with a specific association to chronic migraine compared with episodic migraine.

Results: We found no aggregation of chronic migraine in families with a clustering of migraine. No specific rare variants gave rise to migraine chronification, and migraine chronification was not associated with a higher polygenic risk score. Migraine chronification was not associated with allelic associations with an odds ratio above 2.65. Assessment of effect sizes with genome-wide significance below an odds ratio of 2.65 requires a genome-wide association study of at least 7500 chronic migraine patients.

Conclusion: Our results suggest that migraine chronification is caused by environmental factors rather than genetic factors.

KEYWORDS
genetics, genotype, migraine, neurology, sequence analysis

INTRODUCTION

Migraine is a highly prevalent disorder with great socioeconomic and personal impact. It is the second-highest cause of disability worldwide [1] Migraine presents with episodic migraine (EM) attacks, but a subset of patients with chronic migraine (CM) may have daily headache. CM is defined as 15 or more headache days per month, in a period of at least 3 months, of which at least 8 days per month are migraine headaches [2] CM affects between 1.4% and 2.2% of the general population [3] and about 8% of individuals with migraine [4] Relative to EM, CM patients have worse socioeconomic status [5] and health-related quality of life [6] The transition from EM to CM is usually a gradual process, and some patients oscillate between EM and CM [7] The cause of migraine chronification is not fully clarified. Development of central sensitization or increased excitability of the trigeminal nociceptive pathways has been hypothesized to play a role in the pathophysiology [8,9] Each year 2.5% of patients with EM develop new-onset CM

*Full lists of consortia members appear in Appendix S1.

[10] The reason why only a minority of EM patients chronify while the great majority do not is still not fully understood. Some environmental factors such as medication-overuse are known, but a genetic disposition is also an obvious possibility. There have been attempts to find genetic risk factors for CM [11–17] These studies have, however, used a candidate-gene approach comparing CM with healthy controls and not with EM. No studies have to date investigated whether a genetic component promotes the development of EM into CM.

We investigated whether CM is associated with common or rare genetic variants. We hypothesized that CM is, partly, caused by a genetic disposition. We tested whether (a) patients with CM had an increased burden of rare variants and/or an increased polygenic risk score (PRS) for migraine compared with EM patients and (b) whether there were any common variants associated with CM compared with EM, using genome-wide association analysis.

Since CM is a rare subtype of migraine, recruitment of patients for genetic studies in CM is challenging, and substantial power for genetic studies is a potential issue. Patients with proposed CM (pCM), defined as patients with eight or more migraine days per month, but not 15 days with headache, in a period of at least 3 months, are two times more prevalent than CM but comparable to patients with CM in a number of clinical and sociodemographic factors [18] Therefore, we added an analysis of pCM.

METHODS

Study population

The study population consisted of 2228 patients with migraine (age ≥18 years) recruited from the Danish Headache Center, a tertiary headache treatment center. There was supporting genetic data on 1053 patients. Data collection has been described in previous studies [19–22] Patients were excluded if they (1) had headaches thought to be secondary to another disorder, (2) declined to or were cognitively unable to participate in the semi-structured interview, and (3) were of other than Danish origin. Each patient underwent a validated semi-structured interview [23,24] The interview included information about headache characteristics, aura, frequency, duration, accompanying symptoms, treatment response, precipitating and provoking factors, comorbidities, and familial occurrence. The diagnosis relied on the diagnostic criteria in the third edition of the International Classification of Headache Disorders (ICHD-3) [2] All interviews were conducted by a neurology resident or a senior medical student trained in headache diagnosis and subsequently validated by a physician, ensuring high-quality data. We analyzed patients with ICHD-3 CM, pCM, and EM. Patients with medication-overuse were not included. EM is defined, here, as eight or fewer migraine days per month, pCM is defined as eight or more migraine days per month [18] and ICHD-3 CM is defined as 15 or more headache days per month, in a period of at least 3 months, of which at least 8 days per month are migraine headaches [2]

Familial aggregation of chronic migraine

Familial aggregation analysis is a fundamental step to perform when assessing the extent of genetic background of a disease. For the analysis of aggregation of ICHD-3 CM and pCM in families we assessed 160 families with a clustering of migraine. The families had a family member count ranging from $n = 3$ to $n = 60$ (n total = 850). The frequency of ICHD-3 CM and pCM in families versus migraine patients, with no first-degree relatives with migraine, was assessed using 1378 unfamilial migraine patients. Patients without supporting genetic data were also included. Pedigrees of the families were plotted using an edited version of the kinship2 R package [25]

Sequencing and annotation of rare variants

For analysis of rare variants we assessed whole-genome sequencing (WGS) data. Some 103 ICHD-3 CM patients were compared with 854 EM patients, and 230 pCM patients were compared with 727 EM patients. The two comparisons were performed in the migraine cohort: (1) patients who fulfilled ICHD-3 CM versus patients who did not fulfil ICHD-3 CM and (2) patients who fulfilled pCM versus patients who did not fulfil pCM. Thus, the number of EM patients was higher in the first comparison. Genomic DNA extraction from whole blood and WGS were performed in collaboration with deCODE genetics and described in detail elsewhere [26] Annovar v.2018apr16 was used to annotate variants. Rare variants were defined as variants with a minor allele frequency (MAF) <1% using gnomAD v.2 as reference. Variants were stratified based on location: downstream, exonic, intergenic, intronic, splicing, upstream, UTR3, and UTR5 genomic variants. Mutations were categorized based on the variation type: frameshift deletions, frameshift insertions, non-frameshift deletions, non-frameshift insertions, nonsynonymous single nucleotide polymorphisms (SNPs), stop-gain, and stop-loss mutations. The number of rare variants per group was used for linear regression explained in the section on statistical analysis.

Generalized linear mixed regression model, SKAT on rare variants, and statistical analyses

To analyze differences in the number of rare variants, we fitted a generalized linear mixed model using the Markov Chain Monte Carlo approach. We adjusted for the total number of variants per individual, sex, age, and kinship using the mixed modeling with genetic relationship matrices (MCMCgrm) function in the GAP v.1.1-22 R package. The total number of variants per individual was the number of detected variants when compared to the human genome assembly GRCh38 [26] The kinship matrix was calculated using the Kinship v.1.1.3 R package with pedigree data as input data including all patients with ICHD-3 CM, pCM, and EM. The difference between patient groups was calculated as the

difference in the area under the curve (AUC) between models with and without number of functional variants. The pROC v.1.14.0 R package was used for AUC analysis. *P* values were corrected using Bonferroni correction adjusting for number of tests (n = 7 or 8). To assess whether there were genes with an increased burden of rare variants (MAF <1%), that were associated with either ICHD-3 or pCM compared with EM, we used a SNP-set kernel association test (SKAT) for binary traits, rvtests v.20190205 [27,28] We adjusted for age, sex, and kinship. Bonferroni correction was used to adjust the *p* values according to the number of genes tested (n = 22,000).

Genotyping

For analysis of common variants, we assessed genotype data. Some 127 ICHD-3-defined CM patients were compared with 926 EM patients and 268 pCM patients were compared with

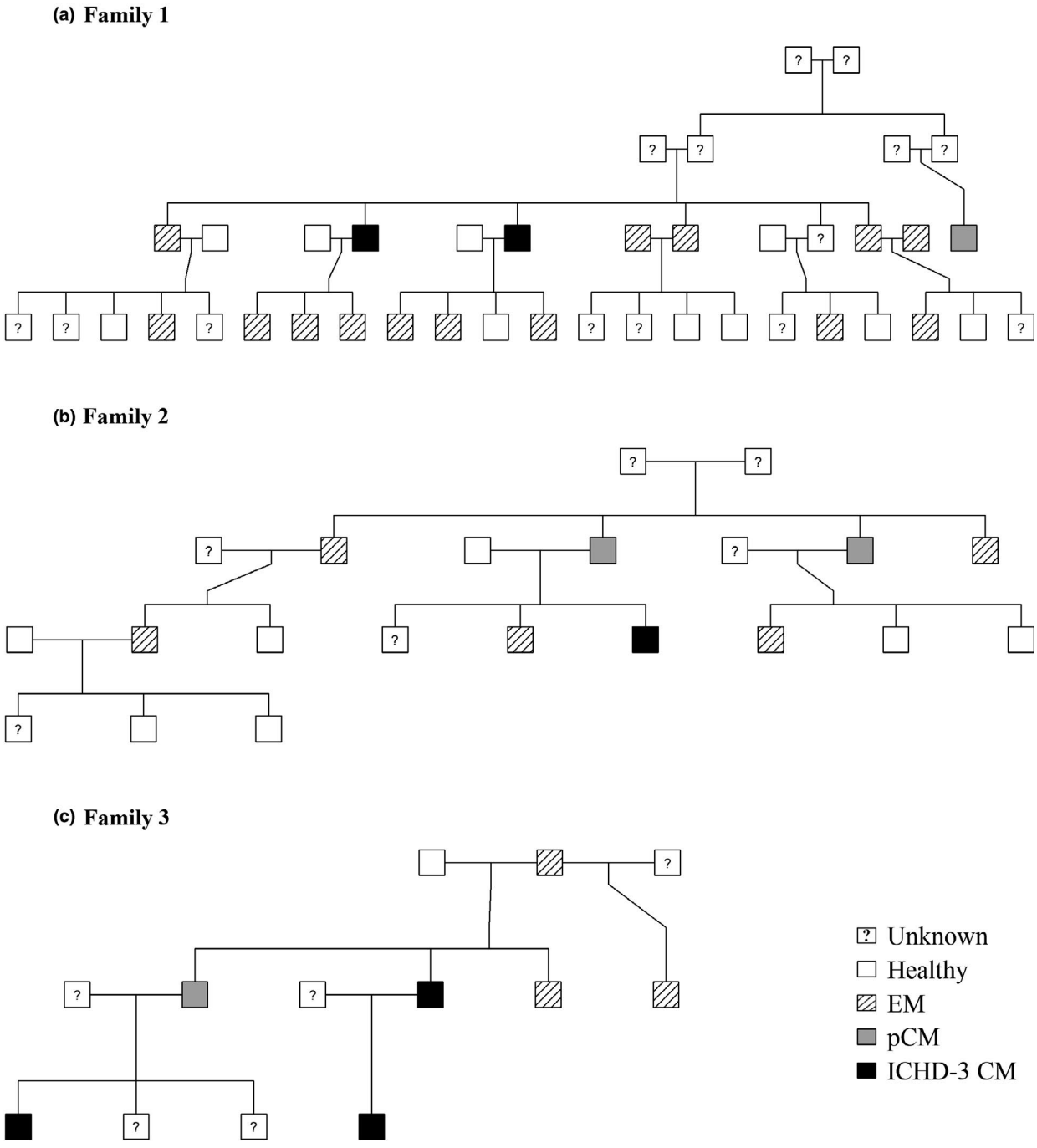

FIGURE 1 Pedigree plots of the three families with the highest clustering of ICHD-3 CM and pCM. There was no clear aggregation of ICHD-3 CM or pCM in Family 1, 2, or 3. EM, episodic migraine; ICHD-3 CM, International Classification of Headache Disorders (3rd edition) defined chronic migraine; pCM, proposed chronic migraine

785 EM patients. All patients were genotyped on the Illumina HumanOmniExpress 12v1/24v1. Imputation was based on The 1000 Genomes Project reference panel [29] The quality control of genotypes was performed using Plink2 v.1.90beta5.4. Details about genotyping and quality control is described elsewhere [30] After filtering and quality control, 6,101,288 SNPs and 1053 patients were retained for analyses.

Polygenic risk score calculation and statistical analysis

The PRS is the sum of migraine risk alleles carried by an individual, and it estimates the individual genetic risk for migraine [31–34] The PRS was calculated using the most recent genome-wide association study (GWAS) meta-analysis on migraine [35] After exclusion of the Danish cohort, there were 57,903 cases and 315,078 controls. The PRS was calculated using LDpred, which adjusts for linkage disequilibrium (LD) between markers [36] LDpred uses a Bayesian approach and the calculation of the PRS in this cohort has been described elsewhere [30] The PRS was centred and scaled to a mean of 0 and standard deviation of 1. Statistical analyses were performed using statistical software R version 3.3.2 and R Studio version 1.0.136. To examine the difference of PRS between ICHD-3 CM and EM and between pCM and EM we used a logistic regression model including age, sex, and the first 10 principal components (PCs) of the genotypes as covariates. The PCs were calculated in Plink [37] and included in the model to correct for population stratification.

Genome-wide association study for chronic migraine

We performed a GWAS for ICHD-3 CM versus EM and pCM versus EM, using Plink2 v.1.90beta5.4 and adjusting for age, sex, and PCA1-10. Quantile-quantile (QQ) plots, Manhattan plots, and data interpretation were conducted using the R package qqman v.0.1.4. Additionally, we assessed SNPs associated with CM. We performed a literature search in PubMed for available English literature of SNPs associated with CM using the following terms: “chronic migraine” AND “single nucleotide polymorphism” (9 hits) and MeshTerms: single nucleotide polymorphism OR SNP OR gene variants OR gene OR genetics AND "chronic migraine" (198 hits). After screening for relevant genes and SNPs, we verified six SNPs and three genes in our data (Table 2). SNPs will hereafter be addressed as common variants.

Standard protocol approval, registrations, and patient consents

Written informed consent was obtained from all participants. The study was approved by the Danish Ethical Standards Committee (H-2-2010-122) and the Danish Data Protection Agency (01080/GLO-2010-10).

RESULTS

Sample characteristics

The male:female ratio in ICHD-3 CM was 1:5.3 and similar in pCM patients (1:5.3) and EM patients (1:4.1) (p = 0.449). The average age of patients with ICHD-3 CM was 42.6 years, for pCM 44.8 years, and for EM 44.1 years. There was no significant difference in mean age between ICHD-3 CM, pCM, and EM patients (p = 0.996).

Power calculations

We conducted separate power calculations for the burden test, PRS analysis, and the CM GWAS. Below we list the power calculations of the three analyses. Given our sample size, we could reject the null hypothesis of no genetic difference between ICHD-3 CM and

TABLE 1 Rare variant burden analysis

	ICHD-3 CM vs EM		pCM vs EM	
Genomic annotation	***P* value**	***P* value adj**	***P* value**	***P* value adj**
Rare variant category				
Downstream	0.73	1	0.73	1
Exonic	0.47	1	0.33	1
Intergenic	0.54	1	0.29	1
Intronic	0.22	1	0.24	1
Splicing	0.41	1	0.81	1
Upstream	0.15	1	0.079	0.63
UTR3	0.29	1	0.17	1
UTR5	0.61	1	0.24	1
Rare mutation category				
Frameshift deletions	0.96	1	0.62	1
Frameshift insertions	0.33	1	0.17	1
Non-frameshift deletions	0.58	1	0.38	1
Non-frameshift insertions	0.49	1	0.32	1
Nonsynonymous SNPs	0.94	1	0.66	1
Stop-gain	0.94	1	0.71	1
Stop-loss	0.28	1	0.74	1

Note: In the first column the rare variants and mutation groups are listed. In the second to fifth columns the p values and adjusted p values from the mixed modeling with genetic relationship matrices (MCMCgrm) area under the curve (AUC) analysis are listed for ICHD-3 CM vs EM and pCM vs EM.

Abbreviations: adj, adjusted; EM, episodic migraine; ICHD-3 CM, International Classification of Headache Disorders (3rd edition) defined chronic migraine; pCM, proposed chronic migraine.

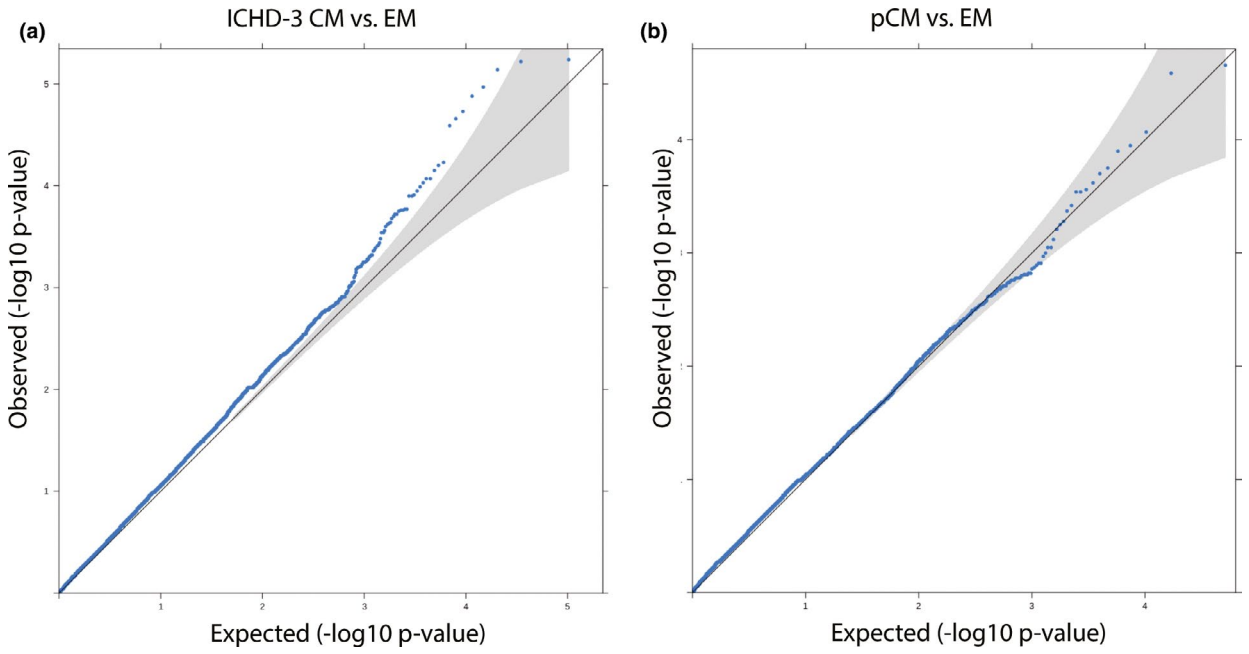

FIGURE 2 Quantile-quantile (QQ) plots from the single nucleotide polymorphism (SNP)-set kernel association test (SKAT). QQplots of expected versus observed *p* values from SKAT analysis comparing (a) pCM versus EM and (b) ICHD-3 CM versus EM. EM, episodic migraine; ICHD-3 CM, International Classification of Headache Disorders (3rd edition) defined chronic migraine; pCM, proposed chronic migraine [Colour figure can be viewed at wileyonlinelibrary.com]

EM and pCM and EM with a power of 0.8 for the three different analyses:

1. For burden test of rare variants we could reject the null hypothesis for a difference in mutation burden of rare, functional variants above 1.11 for ICHD-3 CM and 1.07 for pCM (α-level = 2.5×10^{-6}). The median mutation rate of all genes in our in-house dataset (n > 2200 individuals) was 1.11 (SD = 2.7) and n = 22,000 genes.
2. For PRS analysis of common variants we could reject the null hypothesis for mean differences of PRS scores greater than 0.46 for ICHD-3 CM and 0.41 for pCM (α-level = 0.05). We assumed that the PRS scores were normally distributed with a SD = 2.
3. For CM GWAS of common variants associated with CM/pCM we could reject the null hypothesis of no genetic difference for allelic association with an odds ratio (OR) above 2.65 and below 0.162 (α-level = 5×10^{-8}).

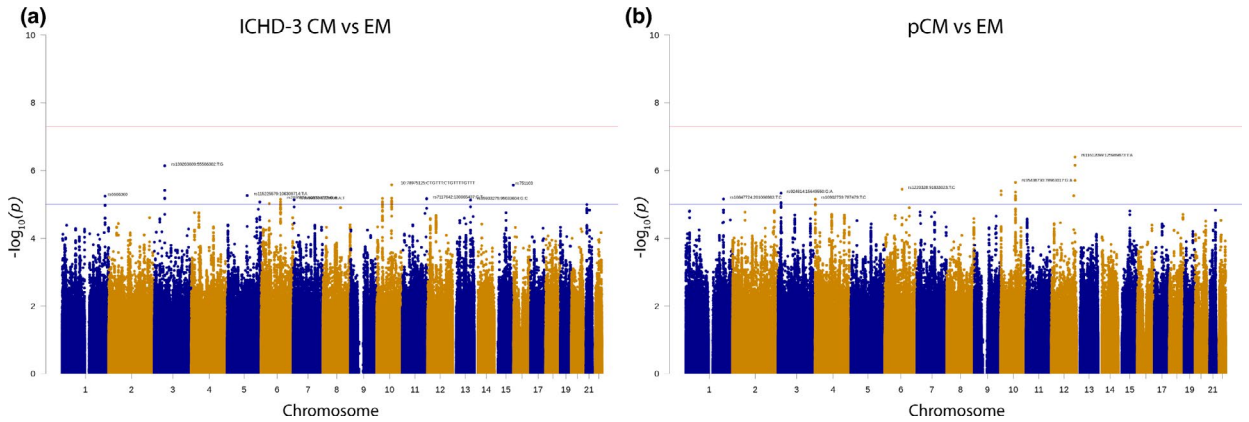

FIGURE 3 Manhattan plots from a genome-wide association study (GWAS) between ICHD-3 CM versus EM and pCM versus EM. Manhattan plots of *p* values per single nucleotide polymorphism (SNP) for autosomal chromosomes from GWAS between (a) ICHD-3 CM versus EM and (b) pCM versus EM. EM, episodic migraine; ICHD-3 CM, International Classification of Headache Disorders (3rd edition) defined chronic migraine; pCM, proposed chronic migraine [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Verification of previously reported common variants and genes associated with chronic migraine

Genomic position (HG38)	Gene/SNP	Replication cohort/ cases	Discovery cohort/ controls	Country	Race-ethnicity	Study approach	Comment	Reference	ICHD 3-CM vs EM (GWAS)	pCM vs EM (GWAS)	Meta-analysis[34]	ICHD-3 CM vs EM (SKAT)	pCM vs EM (SKAT)
	HLA-A*33:03	218 CM patients	6055 healthy individuals	Taiwan	Han Chinese	CGA	Associated with CM with MOH, but not CM without MOH	Huang et al, 2020	–	–	–	0.96	0.72
	HLA-B*58:01	218 CM patients	6055 healthy individuals	Taiwan	Han Chinese	CGA	Associated with CM with MOH, but not CM without MOH	Huang et al, 2020	–	–	–	0.17	0.14
	HLA-C*03:02	218 CM patients	6055 healthy individuals	Taiwan	Han Chinese	CGA	Associated with CM with MOH, but not CM without MOH	Huang et al, 2020	–	–	–	0.29	0.23
chr2:233916448	TRPM8/rs10166942	332 CM patients	252 CM patients	Taiwan	Han Chinese	CGA	Associated with CM and allodynia	Ling et al, 2019	0.083	0.20	2.22×10^{-27}	–	–
chr9:13363161	DBH 19-bp I/D polymorphism/ rs141116007	130 CM patients	204 healthy individuals	Italy	Caucasian	CGA	Correlation between DBH 19-bpI/ Dpolymorphism and MO in CM patients	Barbanti et al, 2019	NA	NA	NA	–	–
chr11:14972978	CALCA/rs3781719	120 CM patients who were OnabotA responders	36 CM patients who were OnabotA non-responders	Spain	Caucasian	CGA	Related to response to OnaboA in CM patients	Moreno-Mayordomo et al, 2019	0.15	0.21	0.0059	–	–
chr17:3592080	TRPV1/rs222749	120 CM patients who were OnabotA responders	36 CM patients who were OnabotA non-responders	Spain	Caucasian	CGA	Related to response to OnaboA in CM patients	Moreno-Mayordomo et al, 2019	0.39	0.61	0.69	–	–
chr6:154039219	OPRM1/A118G	119 CM patients with aura who are A/A carriers	34 CM patients with aura who are A/G or G/G carriers	Australia	Caucasian	CGA	64.7% of the G carriers had high head pain severity scores compared to 37% of the A/A carriers	Menon et al, 2012	NA	NA	NA	–	–
chr15:74749576	CYP1A2*1F/ rs762551	75 patients with CM and overuse of triptans	51 patients with CM and no overuse of triptans	Italy	Caucasian	CGA	Association with triptan overuse in CM patients	Gentile et al, 2010	0.02239	0.80	0.059	–	–

Abbreviations: CGA, candidate-gene approach; CM, chronic migraine; EM, episodic migraine; HLA, human leukocyte antigen; ICHD-3 CM, International Classification of Headache Disorders (3rd edition) defined chronic migraine; MO, medication-overuse; MOH, medication-overuse headache; OnabotA, OnabotulinumtoxinA; pCM, proposed chronic migraine; SKAT, SNP-set Kernel Association Test; SNP, single nucleotide polymorphism.

Familial aggregation of chronic migraine

Among the migraine family members (n = 850), 5.4% had ICHD-3 CM (n = 46) and 8.9% had pCM (n = 76). Among the unfamilial migraine patients (n = 1378), 10.6% had ICHD-3 CM (n = 146) and 22.6% had pCM (n = 312). The higher values were probably because all were patients seeking treatment in our tertiary headache treatment center. There was no clear aggregation of ICHD-3 CM nor pCM in the families. Among 160 families with migraine, three families had more than one family member with ICHD-3 CM or pCM (Figure 1).

Rare variant burden analysis

We assessed the burden of rare functional variants in patients with ICHD-3 CM and pCM compared with patients with EM. We fitted a generalized linear mixed model using the Markov Chain Monte Carlo approach. The genomic variants were stratified based on position: downstream, exonic, intergenic, intronic, splicing, upstream, UTR3, and UTR5. The genomic mutations were stratified based on type of variance: frameshift deletions, frameshift insertions, non-frameshift deletions, non-frameshift insertions, nonsynonymous SNPs, stop-gain, and stop-loss mutations. The results from the rare variant burden analysis are listed in Table 1. We found no differences between ICHD-3 CM and EM nor between pCM and EM for any of the categories of rare variants and mutations (boxplots of the percentile distribution of the different types of rare variants and rare mutations are presented in Figures S1 and S2).

SNP-set kernel association test (SKAT)

Next, we assessed whether there were genes with increased burden of rare variants in patients with either ICHD-3 CM or pCM compared with EM. We found that no genes were significantly associated with neither ICHD-3 CM nor pCM compared with EM after correcting for genome-wide significance (p values adjusted >0.05) (Figure 2).

Polygenic risk score

We assessed the migraine (PRS distribution in patients with ICHD-3 CM (PRS mean = 0.20, SD = 1.05) and pCM (PRS mean = 0.20, SD = 1.01) in comparison to patients with EM (PRS mean = 0.16, SD = 0.93). There was no difference between the PRS of ICHD-3 CM patients and EM patients (p = 0.803, OR = 1.02, 95% CI 0.842–1.25) nor between the PRS of pCM patients and EM patients (p = 0.684, OR = 1.03, 95% CI 0.889–1.20).

Genome-wide association study on chronic migraine

We compared ICHD-3 CM with EM and pCM with EM. We found no genome-wide significant common variants associated with neither pCM nor ICHD-3 CM when compared to EM (Figure 3 and Figure S3).

We then assessed the six genetic variants and three genes that have previously been associated with CM (Table 2). All the studies used a candidate-gene approach and compared CM patients with healthy controls. Of all the common variants we found a nominal association for rs762551 (p = 0.022). We compared the six variants with the most recent migraine GWAS meta-analysis [35] and rs10166942 was already associated with migraine ($p = 2.22 \times 10^{-27}$).

We were able to assess common genetic variants in the CM GWAS with relatively low effect sizes, below an OR of 2.65. Depending on the expected risk allele frequency, 7500 cases with ICHD-3 CM or pCM are needed to assess genetic variants with an OR >1.2 for common alleles (MAF 50%) and 20,000 cases for less frequent alleles (MAF 10%) (Figure 4).

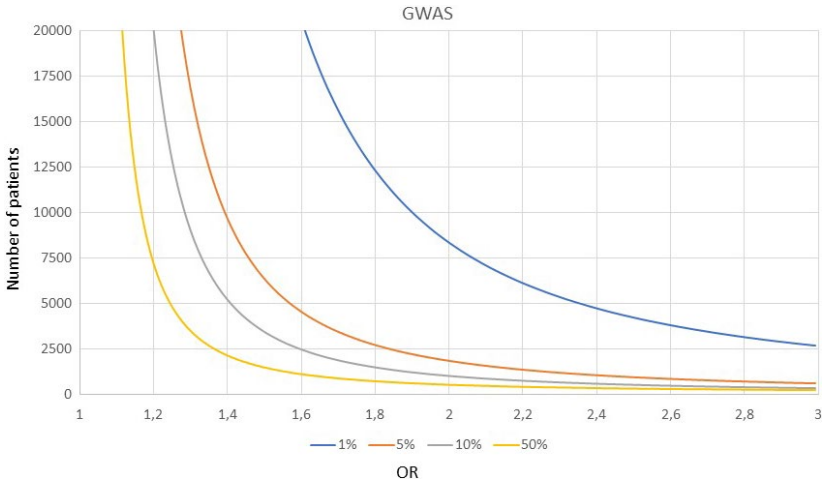

FIGURE 4 Power calculation for chronic migraine (CM) genome-wide association study (GWAS). Power for the CM GWAS is dependent on the expected risk allele frequency of the common alleles (minor allele frequency [MAF] 1%, 5%, 10%, 50%). The figure shows an estimated power for all frequencies, and the number of CM patients needed (y-axis) given the effect size or odds ratio (OR) (x-axis) of the common alleles, assuming a 1:2 CM patient:control ratio [Colour figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

Rare and common gene variants do not give rise to migraine chronification

In the present study we found that rare and common genetic variants could not explain the transition from EM to CM. We found that there was no aggregation of ICHD-3 CM or pCM [18] in families with a known clustering of migraine. The burden test of rare functional variants showed that no specific categories of rare functional variants give rise to the transformation from EM to ICHD-3 CM nor pCM. The SKAT analyzes the enrichment of rare variants in genes; however, no genes were found to harbor rare variants that predispose to ICHD-3 CM or pCM. Figure 2 showed a slight inflation of the SKAT *p* values for the ICHD-3 versus EM analysis. The inflation is not substantial and is not present in the analysis of the pCM versus EM. We corrected for age, sex, and PCs. The inflation is most likely related to lack of power as the inflation disappears when the sample size is increased, as is seen for pCM. Therefore, we could exclude that genes that harbor on average 1.1 mutations in ICHD-3 CM patients and 1.07 mutations in pCM patients compared with EM patients can cause migraine chronification. The PRS analysis showed that a cumulative effect of many common migraine variants does not cause the transformation from EM to ICHD-3 CM or pCM. The PRS analysis suggests that patients with ICHD-3 CM or pCM on average have a higher genomic burden of common migraine variants compared with patients with EM. Thus, we could exclude an effect of common variants for mean differences above 0.46 for ICHD-3 CM and 0.41 for pCM. Finally, we conducted a GWAS for common variants associated with ICHD-3 CM and pCM. We could reject the premise that the transformation from EM to ICHD-3 CM or pCM is caused by common alleles with an OR above 2.65 and below 0.162. This relatively large effect size, which was detectable in the GWAS of CM, may be considered too large for a disorder with a population frequency of only 1.4%–2.2%.

Modifiable and non-modifiable risk factors of chronic migraine

The transformation from EM to CM seems complex and may involve multiple risk factors. Clinical evidence suggests that migraine chronification does not occur abruptly, and that the rate of chronification varies per individual [38] A transition staging model of the clinical course in migraine has been suggested by Bigal et al. [39] Here, migraine patients transition between three states: low-frequency EM (0–9 headache days/month), high-frequency EM (10–14 headache days per month), and CM, and it was suggested that the rates of transmission may be determined by a variety of risk factors. Epidemiological studies have identified such risk factors and have divided these into non-modifiable and modifiable risk factors [40,41] Non-modifiable risk factors include female sex, age, race, and lower socioeconomic status. Modifiable risk factors include frequency of migraine attacks, overuse of acute migraine medication, depression, comorbid pain disorders, obstructive sleep apnea, caffeine overuse, stressful life events, and obesity. It has been suggested that an assessment of the modifiable risk factors in clinical practice could make an impact in preventing migraine chronification. The most important risk factor for CM is probably overuse of acute migraine medication, which is defined as intake of analgesics on >15 days per month or triptans on >10 days per month [2]. It is a general belief that medication-overuse may lead to migraine chronification [42–44] and clinicians have since the 1930s reported that migraine chronification occurs during a period of frequent use of analgesics in patients with a pre-existing headache syndrome [45–48] Up to 63% of patients with CM remit to EM because of medication reduction [49,50] However, some patients do not improve after withdrawal of the offending drug. This suggests that environmental factors alone may not explain the transformation from EM to CM.

Assessment of the genetic component is complex

A genetic component to CM has not been properly studied to date. Single-candidate-gene variants were studied, and CM was analyzed in association with cofactors such as medication-overuse-headache, allodynia, response to OnabotulinumtoxinA, and triptan overuse. A study performed by Louter et al. in 2015 [17] is the first to investigate multiple SNPs in CM patients. The authors tested CM and high-frequency EM versus healthy controls. In total, 144 SNPs were selected based on the literature and previous studies. No genetic variants were associated with CM. No studies have, to date, investigated both common and rare variants in patients with CM and pCM using whole-genome sequencing data and compared the results to EM. The identification of genetic risk factors for migraine in general is challenging and, most likely, both rare and common variants contribute to the genetic makeup of migraine. Moreover, a high number of patients is necessary for GWAS studies. The same challenges apply to CM.

A multicenter effort is necessary

We conducted this study using four genetic methods to investigate whether rare and common variants can dispose to the transition from EM to ICHD-3 CM or pCM, but we found no major genetic component to ICHD-3 CM or pCM. However, the sample size of our study did not allow identification of common variants with relatively low effect sizes. To assess low effect sizes with genome-wide significance below an OR of 2.65 it is necessary to carry out a large GWAS on at least 7500 CM patients using EM patients as controls (Figure 4). Given these large numbers, single-center studies will be underpowered. There is a need for a large multicenter study to remove any remaining possibilities of there being a genetic factor in migraine chronification.

It is commonly accepted that migraine does not affect fecundity in the population, which prompts no negative or positive selection of the genetic risk alleles for migraine. This observation supports the possible existence of common variants with low effect sizes.

Thus, given our results, if there are indeed any genetic risk factors for CM, these are most likely low-risk variants with a putative additive effect. Notably, epigenetic factors such as DNA methylation have been suggested as a mechanism behind CM [51] however, a multicenter research effort is needed to provide sufficient statistical power for such studies also.

Strengths and limitations

The strengths of our analysis include the use of a validated semi-structured interview conducted by trained healthcare professionals, and the assessment of both common and rare variants. Although this study utilizes one of the world's largest clinical migraine cohorts with supporting genetic data, the sample size does not allow us to exclude whether there are SNPs with an OR below 2.65 for CM. Further, we did not have the possibility of replicating our negative findings in other cohorts.

Our results rule out a major genetic disposition to ICHD-3 CM and pCM. We call for a multicenter approach in the International Headache Genetics Consortium to conduct a GWAS and an epigenome-wide association study to assess whether there are indeed any genetic risk factors or a gene × environment effect for CM.

CONCLUSION

The development of episodic migraine into chronic migraine is unlikely to be caused by genetic factors. If such factors have escaped identification, they are likely to be weak.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Members of the 23andMe Research Team are employed by and hold stock or stock options in 23andMe, Inc.

AUTHOR CONTRIBUTION

Mona Ameri Ameri Chalmer: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (supporting); Investigation (lead); Methodology (equal); Project administration (lead); Resources (equal); Software (equal); Supervision (equal); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Andreas Høiberg Rasmussen:** Conceptualization (equal); Data curation (supporting); Formal analysis (equal); Funding acquisition (supporting); Investigation (supporting); Methodology (equal); Project administration (supporting); Resources (supporting); Software (equal); Supervision (supporting); Validation (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting). **Lisette J. A. Kogelman:** Conceptualization (supporting); Data curation (supporting); Formal analysis (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Software (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Jes Olesen:** Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (equal); Software (supporting); Supervision (equal); Validation (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review and editing (equal). **Thomas Folkmann Hansen:** Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (equal); Project administration (supporting); Resources (equal); Software (equal); Supervision (equal); Validation (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

Summary statistics of the genome-wide association study (GWAS) are available upon request. Individual data are available from the corresponding author upon reasonable request and require both a material transfer agreement and memorandum of understanding in order to obtain ethical and data protection agency approval.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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