Genome-wide association studies in migraine: current state and route to follow

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Purpose of review
Genome-wide association studies (GWAS) have revealed over a dozen genetic factors robustly associated with the common forms of migraine. The identification of these factors, the implicated biological mechanisms, and whether they are of use in basic research and clinic practice will be discussed.

Recent findings
Several GWAS have been performed in recent years. New approaches are being tested to identify how information of genetic susceptibility factors can be used in research and the clinic. Still, we are only at the beginning of understanding how the genetic factors may be involved in migraine pathophysiology.

Summary
The identification of genetic factors that confer risk for the common forms of migraine by GWAS has given insight into the genetic underpinning of migraine pathophysiology. Still, the interpretation of the findings of GWAS is not straightforward. Various strategies are being tested to investigate which pathophysiological mechanisms are involved, how they can be studied, and what this means for clinical diagnosis, and even care.

Keywords
functional consequence, genetic risk score, genome-wide association study

INTRODUCTION

Migraine is a devastating common neurovascular brain disorder characterized by debilitating attacks of headache accompanied by nausea, vomiting, photophobia, and/or phonophobia [1]. Clinically, two main subtypes are distinguished based on the presence or absence of an aura (consisting of neurological symptoms, i.e. transient visual, sensory, motor, or speech disturbances) that can precede the headache phase: migraine with aura and migraine without aura. The disease has a strong genetic component but its pathophysiology is largely unknown, although much progress has been made in recent years [2▪].

Advances in genotyping technology allow cost-effective screening for genetic factors in large cohorts, usually of several thousand or more individuals, and have yielded thousands of susceptibility genes for hundreds of traits using what is called a genome-wide association study (GWAS) approach. In this review, we will discuss how these novel opportunities have led to the identification of an increasing number of genetic factors for the common forms of migraine. In addition, we will discuss how this knowledge can be used for research

and whether it has relevance in the clinic. Lessons learnt from similar studies in other disease areas than migraine will be discussed as well.

GENOME-WIDE ASSOCIATION STUDIES: GENETIC FACTORS IN COMMON FORMS OF MIGRAINE

Several GWAS have been performed for migraine, as part of a large collaborative effort: the International Headache Genetics Consortium (IHGC). In these studies, up to several million common variants [single-nucleotide polymorphisms (SNPs)] that cover the whole genome were tested for association with various migraine traits in very large cohorts of

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KEY POINTS

- Migraine SNP risk loci detected by GWAS all have small effect sizes, but this does not mean that they are insignificant.

- Earlier findings from candidate gene association studies in migraine could not be replicated in a much larger GWAS dataset.

- Polygenic risk score analysis of GWAS datasets indicates genetic overlap between migraine and ischemic stroke and migraine and CAD.

- Polygenic risk score analysis of GWAS datasets indicates that migraine and MDD are genetically distinct disorders and that the group of patients with migraine ánd MDD are more similar to MDD patients.

patients and controls (each exceeding several thousand cases and controls). Disease-associated SNPs ('SNP risk loci') were identified by a different distribution of allele frequencies between cases and controls that survived correction for rigorous multiple testing. Only P values below 5×10^{-8} are considered genome-wide significant – which is now convention – to ensure a 5% type I error rate (i.e. the equivalent of testing one million independent SNPs) [3]. The use of such a stringent significance threshold, coupled with the large sample sizes required to detect them, means that the SNP risk loci identified by GWAS are robust, being very unlikely to occur by chance (random sample variation). Moreover, GWAS test and (where necessary) adjust for population stratification (i.e. ancestry differences between cases and controls that may bias tests for differential allele frequency), cryptic relatedness and technical (i.e. genotyping) artefacts, which further increase the analytical robustness of GWAS findings.

Two GWAS focussed on patients that were collected by specialized headache clinics [4,5]. One study focussed on patients with migraine with aura and identified a single associated SNP that pinpoints the *MTDH* gene [4]. The other study focussed on migraine without aura and identified six SNP risk loci that suggested *MEF2D*, *TGFBR2*, *PHACTR1*, *ASTN2*, *TRPM8* and *LRP1* as the likely susceptibility genes [5] – of which the latter two, along with the *PRDM16* locus, were identified the year before in a large population-based GWAS [6]. A subsequent meta-analysis of these studies, and several additional cohorts, yielded an enormous dataset of 23 285 cases and 95 425 controls that identified 12 SNP risk loci (and indirectly genes) significantly associated with migraine [7]. The 13 migraine susceptibility loci (Table 1) identified genes that are involved in neuronal (*MTDH*, *LRP1*, *PRDM16*,

MEF2D, *ASTN2*, *PHACTR1*, *FHL5*, *MMP16*), vascular (*PHACTR1*, *TGFBR2*, *c7orf10*), metalloproteinase (*MMP16*, *TSPAN2*, *AJAP1*) and pain (*TRPM8*) pathways. The most recent IHGC GWAS (currently under review) combined data of 59 674 migraine cases and 316 078 controls and detected no less than 45 independent migraine SNP risk loci that map to 38 distinct genomic regions, 28 of which have not previously been reported [8]. Although the number of loci clearly is increasing from GWAS to GWAS, there are, however, important questions to ask when interpreting the results of these GWAS, that is, how relevant are these findings to research and clinical practice and how do findings relate to those of similar studies in other diseases.

GENOME-WIDE ASSOCIATION STUDY ERA: LESSONS LEARNT AND WHAT TO DO NEXT?

Small effect sizes are the rule, which does not imply that findings are insignificant

Results from the 1000s of GWAS performed to date (GWASdb v2, http://jjwanglab.org/gwasdb [9]) have shown that, apart from a few rare exceptions, SNP risk loci have small genetic effects with an allelic odds ratio (OR) of 1.10–1.20. Migraine findings are no exception, with observed effect sizes (OR) of 1.07–1.18 for the 12 SNP risk loci reported in the 2013 IHGC migraine GWAS [7], and 1.04–1.20 for the 45 SNP risk loci in most recent IHGC GWAS [8]. It is important to realize that small effects can still reveal novel relevant insight into disease pathogenesis and do not imply that their implicated targets will have low therapeutic value. This is because the disease risk effect of each associated variant is influenced by natural selection (allele frequency changes because of favouring alleles that increase reproductive fitness), genetic drift (random variation), pleiotropy (producing more than one effect) and population history, whereas the effect of drugs that modulate the associated gene and/or pathway(s) are not. For example, the effect of statins on lowering cholesterol is 20 times 'stronger' than the genetic effect of the GWAS variants associated with cholesterol level at the *HMGCR* locus (the enzyme targeted by statins) [10].

The route from 'single-nucleotide polymorphism risk locus' to 'causal single-nucleotide polymorphism and gene' remains a major hurdle

GWAS SNPs only 'tag' the disease locus, implying that the identified associated SNP is not likely

Table 1. Migraine loci and genes identified by genome-wide association studies

Chromosome	Associated DNA variant (and implicated gene)	Migraine subtype[a]	Cohort[b]	P value [odds ratio [95% confidence interval]]	Reference
1	rs12134493 (near TSPAN2)	Migraine	Population and clinic-based combined	6.71×10^{-14} (1.14 [1.10–1.18])	[7]
1	rs2651899 (PRDM16)	Migraine	Population-based	3.80×10^{-9} (1.11 [1.07–1.15])	[6]
1		Migraine	Population and clinic-based combined	3.28×10^{-14} (1.09 [1.07–1.12])	[7]
1	rs10915437 (near AJAP1)	Migraine	Clinic-based	2.81×10^{-8} (0.86 [0.82–0.91])	[7]
1	rs3790455 (MEF2D)	Migraine without aura	Clinic-based	7.06×10^{-11} (1.20 [1.14–1.27])	[5]
1	rs2274316 (MEF2D)	Migraine	Population and clinic-based combined	3.14×10^{-8} (1.07 [1.05–1.10])	[7]
2	rs7577262 (TRPM8)	Migraine	Population and clinic-based combined	9.11×10^{-14} (0.87 [0.84–0.90])	[7]
2		Migraine	Clinic-based	1.09×10^{-8} (0.79 [0.73–0.86])	[7]
2		Migraine without aura	Clinic-based	8.64×10^{-11} (0.81 [0.76–0.86])	[7]
3	rs7640543 (near TGFBR2)	Migraine without aura	Clinic-based	1.17×10^{-9} (1.19 [1.13–1.26])	[5]
3	rs6790925 (near TGFBR2)	Migraine	Population and clinic-based combined	2.16×10^{-8} (1.05 [1.02–1.07])	[7]
6	rs9349379 (PHACTR1)	Migraine without aura	Clinic-based	3.20×10^{-8} (0.86 [0.81–0.91])	[5]
6		Migraine without aura	Population and clinic-based combined	2.81×10^{-10} (0.93 [0.91–0.96])	[7]
6	rs13208321 (FHL5)	Migraine	Population and clinic-based combined	1.34×10^{-11} (1.10 [1.07–1.13])	[7]
6		Migraine without aura	Clinic-based	1.58×10^{-12} (1.18 [1.13–1.24])	[7]
7	rs4379368 (c7orf10)	Migraine	Population and clinic-based combined	1.46×10^{-9} (1.11 [1.08–1.15])	[7]
7		Migraine without aura	Clinic-based	5.81×10^{-8} (1.19 [1.12–1.27])	[7]
8	rs10504861 (near MMP16)	Migraine without aura	Population and clinic-based combined	1.17×10^{-8} (0.86 [0.81–0.90])	[7]
8	rs1835740 (MTDH/AEG-1)	Migraine with aura	Clinic-based	1.69×10^{-11} (1.18 [1.13–1.24])	[4]
9	rs6478241 (ASTN2)	Migraine without aura	Clinic-based	3.86×10^{-8} (1.16 [1.11–1.23])	[5]
9		Migraine	Clinic-based	1.04×10^{-9} (1.16 [1.11–1.22])	[7]
12	rs11172113 (LRP1)	Migraine	Population-based	4.30×10^{-9} (0.90 [0.78–0.93])	[6]
12		Migraine without aura	Clinic-based	2.97×10^{-8} (0.86 [0.81–0.91])	[5]
12		Migraine	Population and clinic-based combined	2.69×10^{-19} (0.90 [0.88–0.92])	[7]
12		Migraine without aura	Clinic-based	9.96×10^{-11} (0.88 [0.84–091])	[7]

[a]Migraine: migraine subtype is not specified.
[b]Cohort type: clinic-based (i.e. cases were collected through specialized headache clinics) or population-based (i.e. cases were collected from a cohort), meta-analysis which combined both cohort types.

the disease-causing variant but in fact correlated – because of linkage disequilibrium – with the disease-causing variant. As a consequence, the majority of GWAS loci are defined by large regions of linkage disequilibrium which can comprise zero to dozens of genes. Although imputation (i.e. the statistical inference of unobserved genotypes using an external haplotype reference, such as 1000 Genomes Project data [11"]) can help refine a GWAS signal, most GWAS 'index SNPs' (defined as the SNP with the lowest P value at a GWAS locus) lie in intronic or intergenic regions. Moreover, a substantial proportion of variants within a haplotype reference cannot be accurately imputed. For example, over one million (>10%) 1000 Genomes Project variants with a minor allele frequency (MAF) at least 0.01 in Europeans are poorly imputed and fail standard quality control. Importantly, imputation quality is proportional to MAF; being generally poor for less common variants ($0.01 \leq MAF \leq 0.05$) and practically useless for variants with MAF less than 0.01. Therefore, to identify the potential 'causal' variant(s) underlying an observed GWA signal (i.e. those with the greatest effect sizes and lowest P values), the region needs to be fine-mapped using custom dense genotyping or resequencing approaches [12–15].

Recent advances in next generation sequencing technologies made it possible to sequence genomic regions exhaustively [16]. Targeted sequencing of specific regions implicated by GWA using next generation sequencing technologies can efficiently capture all variants in these regions and their potential effects can be assessed by a subsequent association study, which provides an effective approach to find the causal variant(s) affecting the concerned traits. For example, results from targeted sequencing of GWAS loci revealed an excess burden of deleterious coding mutations in late onset Alzheimer disease, and implied that late onset Alzheimer disease GWAS loci contain multiple rare, damaging mutations that can be recurrent among unrelated patients and in some instances can segregate within families [17].

Where targeted sequencing of migteremaaki-chraine GWAS loci indicate causal variants in intronic or intergenic regions, such causal variants are more likely influencing (in a subtle manner) gene regulation instead of directly affecting protein function (as is the case with many coding variants). Methods such as pathway-based analyses that examine the GWAS loci for a group of genes that are involved in the same biological process can be used to prioritize genes from regions identified by GWAS or to explore pathways affected by multiple GWAS loci [18–20]. Various bioinformatic tools that

integrate regulatory information [21] and mine databases, such as the genotype-tissue expression project [22], Encyclopedia of DNA Elements (ENCODE) [23], the Roadmap Epigenomics Project [24], and Post-GWAS Explorer for Functional Indels and SNPs (PExFInS) [25], a pipeline that integrates linkage disequilibrium analysis information with functional annotation from public databases, and expression quantitative trait loci mapping, have been generated and are publically accessible sources, many since this year, that can aid in the interpretation of GWAS loci and assign functional meaning to gene variants [26].

Toward understanding the biological function of causal genes in genome-wide association study loci

Our final aim of understanding the biology underlying GWAS loci requires functional validation of identified causal SNPs and associated genes for their effects on disease-relevant pathways, by modulating their expression in cellular and/or animal (e.g. zebrafish or mouse) models. Recent technological possibilities allow the development of highly sophisticated cellular disease models based on (neuronally differentiated) human induced pluripotent stem cells derived from patients, as already tried for schizophrenia and age-related macular degeneration [27,28], or of standard induced pluripotent stem cell lines, in which patient mutations are introduced with genome editing such as 'clustered regularly interspaced short palindromic repeats' RNA-guided DNA endonuclease Cas9 (CRISPR/Cas9) technology [29]. The recent report by Claussnitzer and colleagues [30""] successfully applied the CRISPR/Cas9 approach to elucidate a potential mechanistic basis for a GWAS SNP in *FTO* and obesity.

SHIFTING LANDSCAPE: FROM CANDIDATE GENE ASSOCIATION STUDIES TO GENOME-WIDE ASSOCIATION STUDY

Shifting landscape: candidate gene association study findings could not be replicated in genome-wide association study data

Prior to the availability of high-throughput low-cost genotyping required to perform GWAS, many candidate gene association studies (CGAS) have been performed for migraine that focused on genes from neurological, vascular, hormonal, and mitochondrial pathways [31]. However, almost without

exception, CGAS involve small sample sets without robust replication, thus making the risk of false-positive findings high. Indeed, a recent study that used GWAS data reported no genetic evidence for the involvement of any of the genes previously implicated by migraine CGAS in migraine patho-physiology [32].

GENOME-WIDE ASSOCIATION STUDY FINDINGS IN MIGRAINE: DO THEY HAVE CLINICAL RELEVANCE?

Genetic distinction between migraine with and migraine without aura?

There has been extensive discussion in the migraine field as to whether patients diagnosed according to ICHD criteria for migraine with aura and migraine without aura experience distinct disorders or whether their migraine subtypes are genetically related [33–36]. Results comparing the effects (OR) of the top SNPs from the 12 genome-wide significant loci [7] in the migraine with aura and only migraine without aura subgroups showed that although four SNPs had a significant heterogeneous effect size ($P < 0.05$), the allele associated with increased risk was the same across these subgroups for all 12 SNPs, and an analysis of 23 000 independent SNPs found that the significant majority of genome-wide SNP effects were in the same direction across the migraine with aura and migraine without aura subgroups [37■]. A second study using a novel gene-based (statistical) approach found the proportion of genes with a nominally significant gene-based P value (≤ 0.05) in both the migraine with aura and migraine without aura subgroups is almost double the empirically derived null expectation, producing significant evidence of gene-based overlap ($P = 1.5 \times 10^{-4}$) [38]. These results indicate that migraine with aura and migraine without aura share a similar genetic architecture – at least with regard to common (MAF > 0.01) genetic risk factors.

Prospects for understanding aetiology and comorbidities of migraines

In addition to individual migraine loci that overlap with GWAS loci for other traits, polygenic risk score (PRS) analysis – an approach that estimates genetic overlap across two traits by comparing trait values for one trait to a PRS for another trait – has enormous potential to increase our understanding of the aetiology and comorbidities of migraines.

Briefly, a PRS for a 'discovery' trait is first calculated in a 'target' sample of migraine cases and controls, as a weighted count of risk-associated SNP alleles identified in a 'discovery' GWAS for sets of SNPs with varying levels of significance (i.e. P value <0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0). By testing whether higher PRSs are estimated in cases compared with controls of the target sample via logistic regression, a genetic overlap across the two traits can be determined. Despite not differentiating between true and false-positive SNP risk effects, such PRS analyses, using 1000s of SNPs, are known to capture more genetic variance than noise compared with using a small number of highly significant SNPs [39–41].

Two recent examples examined the genetic overlap between migraine and comorbid traits. The first study utilized several approaches to compare GWAS results between migraine (23 285 cases and 95 425 controls) [7] and ischemic stroke (12 389 cases and 62 004 controls) [42], including identification of overlapping genome-wide significant loci and PRS analysis, and found that shared genetic factors contribute to the increased risk of stroke in patients with migraine, with a particularly strong overlap between migraine without aura and both large artery stroke and cardioembolic stroke [43■]. A second, similar, study comparing GWAS results between migraine and coronary artery disease (CAD) suggests that shared biological processes contribute to risk of migraine and CAD, but surprisingly this commonality is restricted to migraine without aura and the impact is in opposite directions [44]. Although further work is required to understand the specific biological pathways underlying these associations, these results point to mechanisms with potential roles in migraine pathogenesis, including platelet dysfunction (*MRVI1*), endothelial dysfunction (*PHACTR1*) and insulin homeostasis (*GIP*).

Another application of PRS analysis utilising three independent GWAS datasets revealed a significant correlation in genetic risk across migraine and major depressive disorder (MDD) [45]. The observed patterns of prediction suggest that the 'pure' forms of migraine and MDD are genetically distinct disorders and that the subgroup of individuals with comorbid MDD and migraine were genetically most similar to MDD patients. These results suggest that, at least in a subset of individuals, migraine may be a 'result' of MDD; and that migraine with and migraine without comorbid MDD may have different genetic aetiologies.

Use of genome-wide association study findings for disease risk prediction

Further to GWAS SNP risk loci having small genetic effects with negligible clinical utility, Dudbridge

[40] examined the power and predictive accuracy of PRS analysis for discrete and continuous traits, and showed that although current GWAS are well-powered to detect significant association between a trait and PRS, useful levels of prediction may only be approached when a PRS is estimated from very large samples, that is, up to an order of magnitude greater than currently available. For moderately heritable traits like migraine, 'several hundred thousand' (in the worst case millions of) cases and controls will likely be required to obtain clinically useful prediction in the population at large. Nonetheless, PRS studies using currently available GWAS data hold great potential for identifying risk subgroups [46]. Indeed, PRS analysis has recently been used to help identify: individuals at high-risk for childhood-onset schizophrenia [47]; MDD patients likely to suffer from bipolar spectrum features [48]; patients with extreme treatment-resistant schizophrenia [49]; children likely to benefit from intervention (designed to reduce the early risk behaviours of poor achievement and aggressive behaviour) in terms of delayed age of first tobacco use [50]; and individuals who are more likely to suffer from more severe schizophrenia [51].

BEYOND CURRENT GENOME-WIDE ASSOCIATION STUDY IN MIGRAINE

The continued discovery of new risk loci for common forms of migraine and improved risk prediction by PRS is only limited by availability of additional large GWAS datasets. The tireless efforts by the IHGC to genotype all known large migraine sample collections are nearing completion, so further increases in GWAS sample size are most likely to come from large 'private' (e.g. 23andMe and Kaiser Permanente) and 'public' (e.g. UK Biobank and Generation Scotland) datasets. Improved genotyping technology and reduced costs will continue to make such large-scale GWAS possible, which will eventually lead to a deep understanding of the biology underlying migraine and to ultimately make accurate risk prediction a reality.

CONCLUSION

GWAS already yielded over a dozen SNP risk loci and suggested novel genes and pathways to be involved in migraine pathophysiology that point toward neuronal and vascular mechanisms of disease. Considerable increases in the number of cases and controls will further increase the number of loci drastically. Major challenges remain before GWAS findings can live up to their high 'a priori' expectation. Foremost, causal variants that really confer migraine risk still need to be identified for most, if not all, SNP risk loci and realistic approaches to unravel their functional consequences still need to be developed. Also with respect to predicting disease, the current GWAS dataset does not suffice and needs to be increased by at least one perhaps two orders of magnitude. Regardless, migraine genetics has come a long way and future prospects look bright that they at one moment in time will bear fruit.

Conflicts of interest

There are no conflicts of interest.

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