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REVIEW

A review of migraine genetics: gathering genomic and transcriptomic factors

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Abstract
Migraine is a common and complex neurologic disorder that affects approximately 15–18% of the general population. Although the cause of migraine is unknown, some genetic studies have focused on unravelling rare and common variants underlying the pathophysiological mechanisms of this disorder. This review covers the advances in the last decade on migraine genetics, throughout the history of genetic methodologies used, including recent application of next-generation sequencing techniques. A thorough review of the literature interweaves the genomic and transcriptomic factors that will allow a better understanding of the mechanisms underlying migraine pathophysiology, concluding with the clinical utility landscape of genetic information and future consideration to creating a new frontier toward advancing the field of personalized medicine.

Introduction

Given its multifactorial nature, migraine is a complex disease, which comprises a number of distinct clinical symptoms and syndromes, even within the same patient. This clinical heterogeneity has led to several different pathophysiological mechanisms being proposed throughout the years, many of which complement each other. Initially, it was thought to be mainly a vascular disease, but nowadays it is believed that both neuronal and vascular mechanisms are involved, with neuronal preponderance.

The genetics of migraine has been the subject of study since the nineteenth century when Liveing (1873) and Tissot (1834) described for the first time the hereditary factor of migraine (Allan 1928). Later, important genes have been discovered, mainly related to a rare subtype of migraine with autosomal dominant inheritance and a pattern of neurological symptoms that precedes the headache (called an aura)—familial hemiplegic migraine (FHM).

This review covers the advances in the last decade of genome-wide association studies (GWAS), discussing the genomic results that emerged using next-generation sequencing (NGS) techniques and its hypothetical relationship with pathophysiology, including clinical diagnoses in which migraine is a critical symptom.

GWAS in migraine have been fruitful, allowing the identification of several loci in the genome that harbor genetic risk factors (Van Den Maagdenberg et al. 2019), which have low penetrance individually, but together might have a significant impact on disease susceptibility. Although GWAS represents a robust approach with distinct advantages regarding the identification of novel trait-associated variants, it also carries some limitations. The introduction of the NGS era made it possible to bridge some gaps left by existing techniques; with the study of exomes (whole exome sequencing—WES), genomes (whole genome sequencing—WGS) and their transcriptomes (RNA-seq) much more genetic information has emerged (Shademan et al. 2017).

These methods with clinical utility provide insight into the genetic landscape enabling future possibilities to create a new frontier toward personalized medicine.

Migraine's clinical synopsis

Migraine affects roughly 15–18% of the population, with remarkable economic burden (less work hours and less productivity) (Serrano et al. 2013; Vos et al. 2017)and social impacts (Stovner et al. 2007). Migraine is the sixth most prevalent disorder in the world, according to the latest

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Global Burden of Diseases, Injuries, and Risk Factors Study (Vos et al. 2017).

Migraine can be divided into migraine with aura (MA) and without aura (MO) and is characterized by recurring episodes of severe headache, vomiting, nausea, and hypersensitivity to sound, light and smell, often associated with neurological symptoms, such as MA (Burstein et al. 2015). It is three times more prevalent in women than men (Jensen and Stovner 2008).

Because migraine is mostly an episodic disorder, different mechanisms were associated with different phases of each episode. Episodes differ greatly from person to person, but may encompass four different stages (prodrome; aura; headache and postdrome).

The first phase of a migraine attack (prodrome) occurs up to 48 h before the beginning of the headache itself and predicts an imminent headache (Giffin et al. 2003). It is usually characterized by nonpainful symptoms, such as fatigue, mood and cognitive changes, and yawning. Other symptoms include sensory sensitivity, such as neck stiffness, photophobia, phonophobia, osmophobia or nausea (Karsan et al. 2018). The aura phase also arises before the headache and normally lasts from 5 min to 4 h, but the typical duration of aura is 30 min (Charles 2018b). It is present in one-third of migraineurs (Quintana et al. 2018) and occurs in the same ratio on both genders (Buse et al. 2013). According to the latest revision of the International Classification of Headache Disorders (ICHD-3), the aura phase consists of recurrent attacks, lasting minutes, of unilateral fully reversible visual, sensory or other central nervous system symptoms that usually develop gradually and are usually followed by headache and associated migraine symptoms ("Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd Edition," 2018).

The headache phase is frequently unilateral (one-sided), throbbing and the pain can range from mild to severe in intensity. It can also be associated with nausea and/or photophobia usually aggravated by routine physical activity (Zhang et al. 2010).

Lastly, postdrome phase, lasting up to 48 h is described as a "migraine hangover" and is usually accompanied by a resolution of the symptoms in the migraine attack (Buse et al. 2013; Quintana et al. 2018). This symptomatic phase is characterized by fatigue, concentration issues and lowered mood levels (Nicola J Giffin et al. 2016).

Pathophysiology of migraine

This disorder's pathophysiology is only partially understood, but it is thought to be due the activation of the trigeminovascular system, responsible for the sensation of pain (Hoffmann et al. 2019). Although the trigger for this phenomenon remains unidentified, some research suggests it may be caused by cortical spreading depression (CSD) (Hoffmann et al. 2019). CSD was originally defined by Aristides Leão (1944) as a slowly propagating wave of neuronal and glial depolarization that begins in the occipital cortex and slowly spreads anteriorly (Fig. 1A) (May and Goadsby 2001; Pietrobon 2018; Zhang et al. 2010).

As the CSD depolarization wave spreads, drastic ionic changes occur: increased extracellular K^+, reduced extracellular Na^+ and other ionic fluxes, such as protons, Cl^-, Mg^{2+} and Zn^{2+} (Somjen 2001). CSD biochemical changes may trigger the activations of meningeal trigeminal endings and

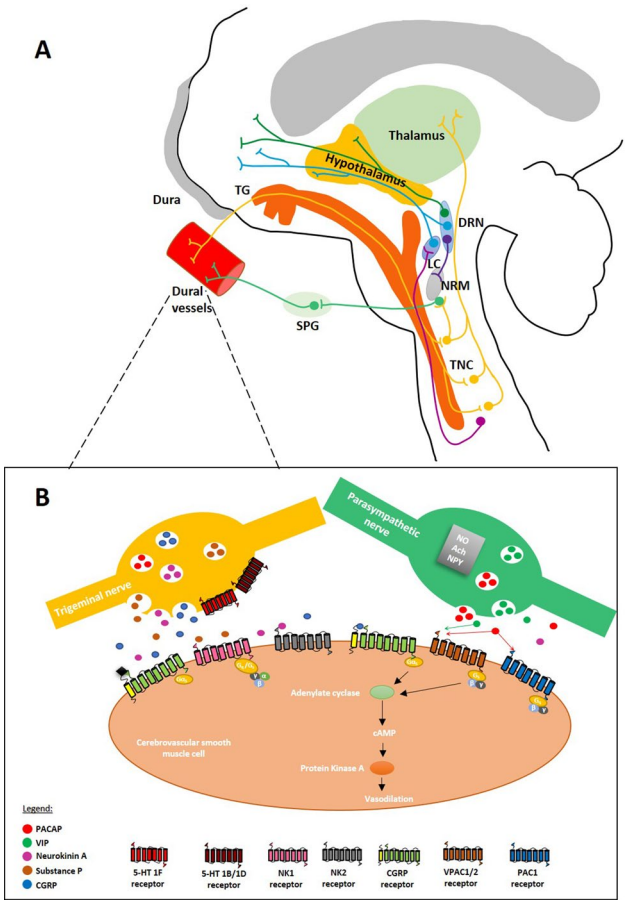

Fig. 1 **A** Cortical spreading depression (CSD) initiates with the increase of extracellular potassium ion concentration and excitatory glutamate. These changes lead to the activation of the trigeminovascular system responsible to the headache phase. Primary afferents of neurons in the trigeminal ganglion (TG) extend from the meningeal vasculature to central terminals in the TNC (orange). Second-order neurons of the TNC, in turn, project to the posterior thalamus. The sphenopalatine ganglion (SPG) (green) also provides reflex parasympathetic innervation to meningeal vessels. The peripheral and central sensitization of the trigeminal system together with the release of calcitonin gene-related peptide (CGRP) determines the sensation of pain. **B** Mechanisms inherent to the development of the neurogenic inflammation

trigeminovascular system, causing the pain during the headache phase (Costa et al. 2013). Locus coeruleus (LC) and nucleus of raphe magnum (NRM) are brainstem structures implicated in the process of trigeminal pain. This constitutes a hypothetical link between the aura [CSD which is considered the main cause (Ramachandran 2018; Somjen 2001)], and headache phases of migraine. Trigeminovascular system can be briefly described as the superficial and meningeal blood vessels that receive innervation from the nociceptive nerve fibers afferents from the trigeminal ganglion, and relay it to the trigeminal nucleus caudalis (TNC) (Pietrobon 2018). During CSD, the release of ATP, glutamate, potassium and H^+ occurs by neurons, glia or vascular cells.

The activated perivascular nerve cells also release calcitonin gene-related peptide (CGRP), substance P (SP) mediated through NK1 receptors (May and Goadsby 2001), neurokinin A (NKA) mediated through NK2 receptors, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP) and nitric oxide (NO) (Ramachandran 2018). Many of the migraine therapies are based on the release of the CGRP, and 5-HT1B/1D/1F receptors are one of the targets that inhibit CGRP release (Edvinsson et al. 2018a). Following the CGRP, the PACAP pathway may be a second neuropeptide system that might be useful to block migraine attacks (Edvinsson et al. 2018b). PAC1 receptor is specific for PACAP, while VPAC1/VPAC2 receptors bind both PACAP and VIP with similar affinity (Ivic et al. 2019). The receptor–effector neuropeptide coupling results in stimulation of adenyl cyclase and an increase in cyclic adenosine monophosphate (cAMP) resulting in vasodilatation consequential to neurogenic inflammation in the meninges (Malhotra 2016). CGRP, SP and NKA are considered to be major players in the development of neurogenic inflammation (Malhotra 2016; Ramachandran 2018) (Fig. 1B).

Genetics of migraine

Familial aggregation studies have shown that migraine is essentially caused by genetic factors, with multiple genes contributing to its liability, in addition to environmental factors (Gervil et al. 1999; Russell et al. 1995; Ulrich et al. 1999). In fact, twin studies show that heritability ranges from 40 to 50%, with a contribution of nonshared environmental factors (Honkasalo et al. 1995).

In previous studies, we performed the first familial aggregation study in Portugal, reporting a strong evidence that relatives of migraineurs have a three- to fourfold increased risk, when compared with the general population (Lemos et al. 2009). Epidemiological studies showed that an early age-at-onset of migraine in the proband was associated with higher levels of family aggregation (Stewart et al. 2006). Moreover, the presence of one or both parents affected is strongly associated with the increase in cases of MA. Pelzer et al. suggests that having even one affected parent is a clear indication of possible inherited genetic factors that may make an individual more susceptible to developing migraine attacks. However, since the frequency of migraine is higher in women and there are no gender differences, we must bear in mind that other external factors may also be playing a dominant role (e.g., sex hormones) (Pelzer et al. 2019).

A number of different genetic aspects contribute to migraine risk such as multiple candidate genes and epigenetic factors (Charles 2018a; Sutherland and Griffiths 2017). Family studies in some rare monogenic migraine subtypes point to a genetic predisposition to migraine. The first unequivocal evidence that migraine has a strong genetic component was found in patients with FHM, an autosomal dominant subtype of MA (de Vries et al. 2009). Three disease-causing genes were found for this specific disorder: calcium voltage-gated channel subunit alpha1 A—*CACNA1A* (FHM1); ATPase Na + /K + transporting subunit alpha 2—*ATP1A2* (FHM2) and sodium voltage-gated channel alpha subunit 1—*SCN1A* (FHM3). These three genes encode proteins that affect ion transport in the brain and regulate glutamate availability in the synapse.

However, not all FHM families are linked to one of the three known FHM *loci* which implies that there are additional FHM genes (de Vries et al. 2009), such as *PRRT2*, *SLC2A1*, *PNKD*, *SLC1A3* and *SLC4A4*. Noteworthy, all of these FHM-associated genes, when mutated, result in increased excitatory neurotransmission and cortical excitability. Nevertheless, the evidence for some of these as FHM genes is still very limited (Sutherland and Griffiths 2017).

After these FHM genes were established, researchers focused on discovering a genetic basis for the common polygenic migraine, through candidate–gene association studies in migraine case–control populations. These approaches focused on the study of DNA variants in candidate genes, which had been previously selected, based on knowledge of this disease's pathophysiology (Gasparini et al. 2013). Thus, various genes and pathways related to neurological, vascular, hormonal and mitochondrial functions have been the spotlight of researchers. This type of study often has small sample sizes, so they lack power to detect variants that have small effect size and in addition, this type of study offers a hypothesis-driven set of genes and single nucleotide polymorphisms (SNPs) for testing, which, despite appearing to be involved in the pathophysiology of the disease, may not present a true risk for its development (Bron et al. 2021; De Vries et al. 2016). Thus, many of the previous problems led to the appearance of new methodologies that were able to overcome some of the limitations presented. GWAS has begun to emerge as a fast and cost-effective genotyping technology.

Genome-wide association studies (GWAS)

GWAS have set a new paradigm in the study of common migraine genetics. These studies highlight alleles with increased frequency in the migraine population, in comparison to a control population. This kind of study design allows for the discovery of single nucleotide variants (SNV) associated with migraine, possibly targeting new migraine mechanisms. In the last decade, six large-scale migraine GWAS were published in which 38 genetic variants and 123 genetic susceptibility *loci* associated with migraine were identified (Anttila et al. 2010, 2013; Chasman et al. 2011; Freilinger et al. 2012; Gormley et al. 2016a, b; Hautakangas et al. 2021). Genes associated with neuronal, vascular, ion channel/homeostasis, pain sensing, glutamatergic transmission and nitric oxide or oxidative stress have been pinpointed (Fig. 2).

The first migraine GWAS, in 2010, reported a SNV (rs1835740) that is located between two potentially interesting candidate genes, *MTDH* (metadherin) and *PGCP* (plasma glutamate carboxypeptidase). *MTDH* downregulates EAAT2 (also known as *SLC1A2* and GLT-1), the major glutamate transporter in the brain, and indirectly can lead to an increase in *PGCP* activity. This may provide a putative mechanism for the occurrence of migraine attacks which is a tempting hypothesis, as this neurotransmitter has long been suspected to play a key role in migraine pathophysiology (Anttila et al. 2010).

Subsequently, another GWAS has reported three variants, in *TRPM8* (rs10166942), *LRP1* (rs11172113) and *PRDM16* (rs2651899) genes (Chasman et al. 2011), which have been repeatedly replicated in most of the GWAS (De Vries et al. 2016). *TRPM8* encodes a sensor for cold and cold-induced burning pain, which is primarily expressed in sensory neurons and dorsal root ganglion neurons. As migraine shares some characteristics with neuropathic pain disorders, *TRPM8* could be a pathophysiological link between both pain syndromes. *LRP1* is expressed in many tissues including brain and vasculature. It serves as a sensor of the extracellular environment and modulates synaptic transmission, which interferes with glutamate homeostasis.

PRDM16 is a transcription factor highly expressed in arterial endothelial cells (ECs) and smooth muscle cells (SMCs) (Craps et al. 2021). It plays an important role in regulating angiogenesis by communicating with adjacent ECs during cortical development (Su et al. 2020). *PRDM16* was originally identified as an oncogene near a chromosomal breakpoint associated with myelodysplastic syndrome and acute myeloid leukemia, but subsequent research has focused on its transcriptional role in brown/beige adipose tissue fate decision, craniofacial development, hematopoietic/neuronal stem cell maintenance and homeostasis (Chasman et al. 2011; Craps et al. 2021). In the context of certain cardiomyopathies, some studies demonstrated that *PRDM16* mutations can cause cardiomyopathy in individuals with the chromosome 1p36 deletion syndrome as well as in nonsyndromic forms of left ventricular non-compaction cardiomyopathy (LVNC) and dilated cardiomyopathy (DCM) (Arndt et al. 2013). Furthermore, *PRDM16* seems to play a pivotal role in sustaining arterial flow recovery in a mouse peripheral artery disease (PAD) model, preserving endothelial function (Craps et al. 2021).

A third GWAS, primarily in MO, confirmed the previous variants found in *TRPM8*, *LRP1* and *PRDM16*, and additionally identified four migraine susceptibility *loci* (*MEF2D*, *ASTN2*, *TGFBR2*, *PHACTR1*). The MEF2D is

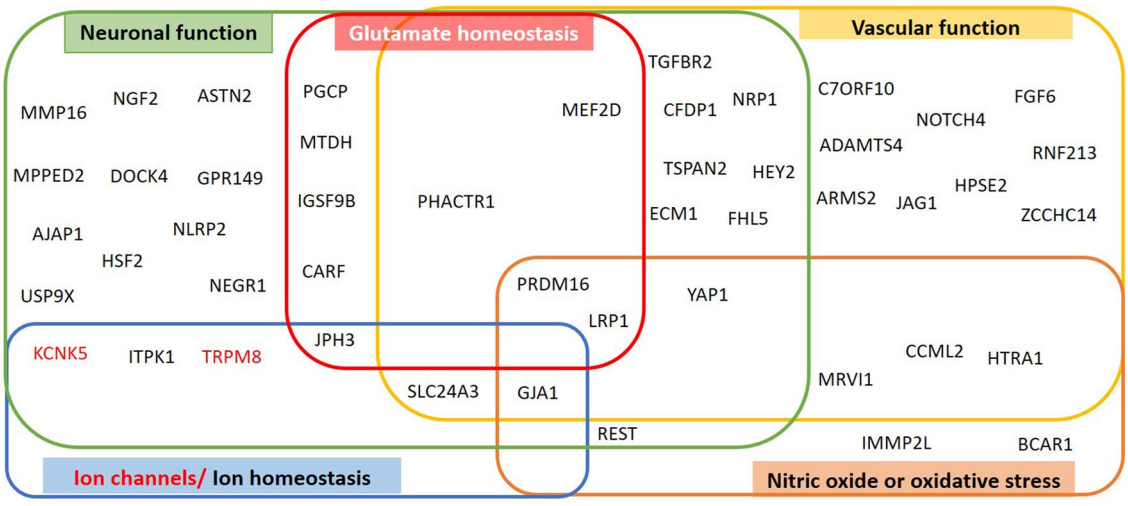

Fig. 2 Summary of all genes described by GWAS and their respective functions in migraine. Genes associated with neuronal, vascular, ion channel/homeostasis, glutamatergic transmission and nitric oxide or oxidative stress

highly expressed in brain and regulates neuronal differentiation; therefore, its deregulation might affect neuronal excitatory neurotransmission. *ASTN2* has a role in the glial-guided migration that seems important for development of the laminar architecture of cortical regions in the brain; however, their role in migraine pathophysiology remains unclear. *TGFBR2* may be involved in systemic vascular disease through a TGF-β signaling pathway, as well as *PHACTR1*. Additionally, *PHACTR1* may also play a dual role in migraine, vascular or neuronal role through aberrant synaptic transmission (Freilinger et al. 2012).

In 2013, another GWAS identified five new *loci* associated with migraine susceptibility (near *AJAP1*, near *MMP16*, near *TSPAN2*, in *C7ORF10*, and in *FHL5*) and seven confirmed previously reported *loci* associated with migraine (*PRDM16, MEF2D*, *TRPM8*, *TGFBR2, PHACTR1, ASTN2* and *LRP1).*

AJAP1 is expressed in the brain and has been associated with tumor invasion and the regulation of metalloproteinase activity, as well as *MMP16*. *TSPAN2* mediates signal transduction events involved in the regulation of cell development, activation, growth and motility. *C7ORF10* has been associated with excretion of glutaric acid; *FHL5* encodes a transcription factor that regulates the cyclic AMP (cAMP) and plays a role in synaptic plasticity and memory formation. Several of the SNVs associated with migraine are located in known transcription factor binding motifs, supporting the idea that alterations in genetic regulation might be causative in migraine pathology (Anttila et al. 2013).

A meta-analysis studied 22 GWAS and mapped 38 distinct genomic regions significantly associated with migraine. Of these 38 significant GWAS loci for migraine, 13 are related to vascular functions, reinforcing the involvement of vascular and smooth muscle dysfunction in the pathogenesis of migraine. Contrary to the hypothesis that migraine is a potential channelopathy, only two loci contained ion channel genes (*KCNK5* and *TRPM8*) and three other harbor genes involved more generally in ion homeostasis (*SLC24A3*, near *ITPK1*, and near *GJA1*) (Gormley et al. 2016a). Regardless of channelopathy's hypothesis, the pathophysiological link among migraine, these genes and pain and vascular pathways cannot be ruled out.

A genetic overlap between GWAS of coronary artery disease (CAD) and migraine (Anttila et al. 2013; Peden et al. 2011; Schunkert et al. 2011) unraveled a causal non-coding variant (rs9349379) in *PHACTR1 l*ocus (6p24), which interferes with the development of five associated vascular diseases (CAD, migraine, cervical artery dissection, fibromuscular dysplasia and hypertension). The authors used a CRISPR-edited stem cell-derived endothelial cells technology and demonstrated that the SNP regulates expression of endothelin 1 (*EDN1*), a gene upstream of *PHACTR1*. The G allele of this variant leads to an increased expression of *EDN1*, and consequently to vasoconstriction, which increases the risk of CAD and decreases the risk for the other vascular conditions mentioned above, including migraine (Gupta et al. 2017).

Finally, the largest GWAS meta-analysis on migraine to date reported 123 risk loci migraine susceptibility, from which it was possible to highlight three variants associated with MA, rs12598836 in *HMOX2*, rs10405121 in *CACNA1A* and rs11031122 in *MPPED2*, while two variants are associated with MO, rs7684253 in the locus near *SPINK2* and rs8087942 in the locus near *FECH*. Furthermore, they conducted a phenome-wide association scans (PheWAS) and, on the top of the categories, most of the variants identified were associated with cardiovascular diseases and blood pressure. This evidence corroborates those mentioned above, reinforcing the importance of the link between migraine and several vascular conditions (Hautakangas et al. 2021).

All these GWAS studies have reported some migraine susceptibility genes, which were replicated in the following studies, revealing that some genes may be associated with different pathophysiological pathways. The recurrence of these genes significantly associated with migraine among the various studies reinforces the robustness of these results involved in migraine pathophysiology, as well as the overlap of pathways involved in it. The interaction of genes and different pathways can be found in Fig. 2.

GWAS have become the standard approach to unravel genetic variations underlying complex diseases and subsequently to generate polygenic risk scores (PRSs). PRSs calculate the additive effect of several SNPs of disease (Kogelman et al. 2019a). The PRS approach relies on the theory that phenotypic variation explained by genetic components is caused by an additive effect of multiple common gene variants with small individual effect sizes (polygenic effect) that is traditionally identified by GWAS. Additionally, pleiotropy is characterized by the possibility of associating more than one phenotype with a genetic variant, which may indicate that different diseases are genetically correlated (Chalmer et al. 2018).

A PRS study was performed in 1806 migraine patients composed of seven SNPs for severe migraine traits. This study demonstrated that PRS results (based on variants previously identified in GWAS studies) predict a risk on the susceptibility for MO but not for MA (Esserlind et al. 2016). Furthermore, a study conducted by Gormley and their collaborators in 8319 individuals across 1589 migraine families concluded that the PRS explained 1.6% of the phenotypic variance in the population cases and 3.5% in the familial cases (including 2.9% for MO, 5.5% for MA, and 8.2% for FHM). The results demonstrate a significant contribution of common polygenic variation to the familial aggregation of migraine. In this sense, some studies have shown that rare forms of migraine with aura (FHM and sporadic hemiplegic

migraine—SHM) can be explained by common polygenic variants, rather than highly penetrant, rare variants (Gormley et al. 2018).

However, GWAS lack a straightforward analysis mainly because of these reasons: most SNVs are in intronic and intergenic regions affecting gene regulation instead of protein function directly; the inability to assess rare genetic variants; this type of study requires a substantial sample size for detecting significant SNPs; and some SNVs are in linkage disequilibrium (LD) with the true susceptibility variant (Tolner et al. 2015).

GWAS present a high power to identify common variants of high or moderate effect size (Di Lorenzo et al. 2012), but a significant proportion of the genetic variance and heritability observed for the common forms of migraine remains unexplained. Therefore, rarer variants with a higher impact can be missed by a GWAS approach (de Boer et al. 2020).

To answer these questions raised and to understand how GWAS loci influence traits and disorders including migraine, many different fine-mapping approaches have been developed. Fine-mapping aims to determine genetic variants associated to complex traits, allowing to define causal variants (Cano-Gamez and Trynka 2020; Schaid et al. 2018). Since most loci have small effect size (allelic odds ratio of 1.03–1.28), genotyping of large numbers of individuals is required for potential clinical use in migraine risk prediction (Van Den Maagdenberg et al. 2019).

Due to the complexity of local genomic effects, the association of variants to genes and respective pathways is difficult, because of two main reasons: first, most studies refer to index SNP or lead SNP (the SNP with the lowest *p* value in a genomic region), but there may be many nearby SNPs that affect regulatory regions; the second is about assuming that index SNP is linked to the closest gene, under evidence that regulatory effects tend to largely act on short distances (this evidence depends on a number of factors, such as the size and gene density of the identified locus (Van Den Maagdenberg et al. 2019).

Different fine-mapping methods have been developed, combining statistical and functional evidence. Firstly, association test statistics can be combined with LD information to prioritize a credible set of SNPs which may play a causal role in disease susceptibility. Second, it is important to identify affected genes (since many susceptibility SNPs are found in both intronic and intergenic regions), by connecting the variants with the respective genes through complementing functional annotation with information from projects such as ENCyclopedia of DNA Elements (ENCODE), NIH Roadmap Epigenomics, and FANTOM5, which have characterized regulatory regions and expression quantitative trait loci (eQTL) (Sutherland et al. 2019).

With the identification of risk genes, it will be necessary to proceed with in silico studies to predict the role of these genes in migraine pathophysiology. This methodology was already applied in the studies carried out by Gupta and their collaborators, mentioned above (Gupta et al. 2017).

Exome/genome sequencing

As GWAS technology only detects common variants with a low effect size, NGS has been developed to detect rare variants which are expected to have a larger effect size and may shed further light on migraine pathophysiology. In Table 1, it is possible to find a vast list of genes associated with migraine by "genome-wide" approaches (GWAS, WES/WGS and RNA-seq). Although the aforementioned studies link NGS to migraine in some way, to the best of our knowledge, no study has been done to date using the WGS to specifically identify migraine-associated variants.

The decision to choose WES or WGS needs to take into account some factors such as research budget, research goals, gene–disease models, analysis and storage burden and overall data quality. In Table 2, it is possible to find the advantages and disadvantages of GWAS versus WES/WGS (Bertier et al. 2016; Zhang and Lupski 2015).

Nowadays, the application of NGS represents a great asset to investigate genetic variation in the genome since it allows the finding of both common and rare variants either in monogenic or polygenic disorders.

WES is a powerful genetic technique to reveal all the nucleotide sequences in the protein coding regions—exons, and WGS performs the screening of total genome (including all non-coding regions) in a single experiment. WES is mightily advanced in the areas of disease diagnosis, prognosis and personalized treatment.

Currently, several studies have been developed using genomics approach. A study that conducted exome and genome sequencing in members of families with ROSAH syndrome symptoms (characterized by retinal dystrophy, optic nerve edema, splenomegaly, anhidrosis, and migraine) detected an *ALPK1* missense pathogenic variant (c.710C > T, [p.Thr237Met]), responsible for the onset of the disease. Functional studies suggested that the *ALPK1* variant has a critical role in centrosome and cilia biology (Williams et al. 2019).

Furthermore, two variants in *TRESK* (a two-pore-domain K + channel encoded by *KCNK18* gene) have been associated with migraine: *TRESK*-MT and *TRESK*-C110R. Although both variants result in a non-functioning TRESK potassium channel, only TRESK-MT frameshift variant has been shown to segregate perfectly with the MA phenotype in a large pedigree and leads to hyperexcitability of trigeminal ganglion neurons. This can be explained by the fact that this variant produces a second *TRESK* protein (*TRESK*-MT2), which co-assembles and inhibits two other potassium channel subfamily K members (TREK1 and TREK2) leading to

Table 1 Summary table of genes associated with migraine by "*genome-wide*" approaches

Authors	Year	Method	Sample size	Phenotype	Genes	Pathways
Anttila et al.	2010	GWAS	Discovery stage: 2748 migraineurs; 10,747 controls Replication stage: 3202 migraineurs; 40,062 controls	MA MA	*MTDH* *PGCP*	Glutamate homeostasis
Chasman et al.	2011	GWAS	5122 migraineurs 18,108 controls	Migraine related	*TRPM8* *LRP1* *PRDM16*	Pain related Neurotransmission Unknown
Freilinger et al.	2012	GWAS	2326 MO patients 4580 controls Replication test: 2508 MO patients; 2652 controls	MO MO MO MO	*MEF2D* *ASTN2* *TGFBR2* *PHACTR1*	Neurotransmission TGF-β signaling pathway
Anttila et al.	2013	GWAS	23,285 migraineurs 95,425 controls	MA/MO MO MA/MO MO MA/MO	*AJAP1* *MMP6* *TSPAN2* *C7ORF10* *FHL5*	Tumor invasion; metalloproteinase activity Neurotransmission Metalloproteinase activity Excretion of glutaric acid cAMP regulation
Gormley et al.	2016	GWAS	59,674 migraineurs 316,078 controls	MA/MO	*SLC24A3* *ITPK1* *GJA1*	Ion homeostasis
Gupta et al.	2017	Phenome-wide association analysis (PheWAS)	112,338 individuals	Migraine related	*PHACTR1/EDN1*	Vascular endothelial function
Gerring et al.	2018	GWAS blood gene expression	83 migraineurs 83 controls	Migraine related	*NFKBIZ* *TNFSF10* *TNFAIP3* *CXCR4* *ABCB1* *NFIL3*	Immune and inflammatory processes
Guo et al.	2020	GWAS Transcriptome-wide association study (TWAS)	59,674 cases 316,078 controls	Migraine related	*ITGB5* *SMG6* *ADRA2B* *ANKDD1B* *KIAA0040*	Endothelial function; Neurogenic inflammation; calcium homeostasis
Hautakangas et al.	2021	GWAS	102,084 cases 771,257 controls	MA MO	*HMOX2, CACNA1A* and *MPPED2* *SPINK2* and *FECH*	Vascular and neuronal involvement
Rasmussen et al.	2020	WGS RNA-seq	262 MA, 213 MO, 145 (MO/MA), 254 controls Replication study: 1930 sporadic migraine patients (312 MA, 1087 MO and 531 MO/MA)	MA/MO	*ATXN1* *FAM153B* *CACNA1B*	Glutamate signaling Voltage-gated calcium channel
Perry et al.	2016	Transcriptome	Calvarial periosteum of 1 patient with chronic migraine; 2 patients headache free	Chronic migraine	*IL6* *SOCS3* *IFNB* *CXCR4* *CCL2* *NFKBIA*	Inflammatory processes

Table 1 (continued)

Authors	Year	Method	Sample size	Phenotype	Genes	Pathways
Gazerani et al.	2019	miRNA microarray	Review study	MA/MO MA/MO MO MO MO MO	miR-34a-5p miR-382-5p miRNA-27b miRNA-181a miRNA-let-7b miRNA-22	Inflammation and vascular endothelial function Stress response
Renthal et al.	2018	Single cell- RNA-seq	2039 individual human brain cells	Migraine related	*CACNA1A* *SCN1A* *NOTCH3*	Ion channels
Starobova et al.	2018	RNA-seq	Review study	Pain related	*Neuropeptide Y(NPY)* *SCN9A* *SNC10A* *SCN11A*	Ion channels
Jeong et al.	2018	RNA-seq	20 RNA samples	Chronic migraine	*LRRC8* *WSCD1*	Immune response, glutamate signaling pathway and reactive oxygen species process regulation
Williams et al.	2019	WES, WGS	WES—2 families with affected and non-affected members WGS—1 family with both affected and non-affected members	Migraine related	*ALPK1*	Centrosomal cilia functions Innate immune response Inflammation: NF-kB signaling
Royal et al.	2019	RT-PCR	Animal and cell models	Migraine related	*TRESK*	Neuronal excitability
Ibrahim et al.	2020	WES	16 individuals (without mutations in FHM genes)	Migraine related	*ATP10A* *ATP7B* *CACNA1C* *CACNA1I*	ATPase Voltage-Gated calcium channel
Kogelman et al.	2019	RNA-seq	17 MO and 9 MA female patients; 20 female controls	MA MA	*NMNAT2* *RETN*	–
Kogelman et al.	2020	RNA-seq	17 MO and 10 MA female patients	MA, MO	*CPT1A* *SLC25A20* *ETFDH* *MAML2* *ADAM15* *ADAM17* *CARD9* *SH2D2A* *CD300C*	Fatty acid oxidation Notch signaling pathways Immune-related pathways
Vgontzas et al.	2020	Single cell RNA-seq	Two single-cell RNA sequencing datasets	MA, MO	*HCK* *ARHGEF26* *WSCD1* *TSPAN2* *NEGR1* *SLC24A3* *GPR182* *NOTCH4* *MYO1A* *HELLS*	Central nervous System Neurovascular cell types Peripheral Nervous System

downregulation of trigeminal ganglion neuron excitability. Thereby, TREK1 and TREK2 can be important potential molecular targets in migraine pathophysiology (Royal et al. 2019).

In a study performed by Ibrahim et al., it was possible to analyze 16 individuals with neurological symptoms including migraine following head injuries, which were screened by WES. In seven of the migraineurs, two had ATPase

Table 2 Advantages and disadvantages of GWAS versus WES/WGS

	GWAS	WES versus WGS	
Study design	Case/control	Case/control and family studies	
Genetic markers/PROS	Described SNPs Common variants (> 5%) Across all genomes Less costly than WGS and WES	Coding regions Reduced costs compared to WGS	Detects both coding and non-coding variants Detect copy number changes and structural variants
		Common and rare variants	
CONS	Detects only common SNPs Large case/control cohorts Fine-mapping studies to identify causal variants Multiple testing correction	Difficult to capture sections of DNA with a high GC nucleotide percentage, leading to false positives and negatives	High cost
		Genetic variants need validation using Sanger sequencing It requires large computational resources for the analysis and for the data storage Identification and interpretation of variants of unknown significance (VUS) Discovery of incidental findings	

related variants: *ATP10A* (p.Ala881Val) and *ATP7B (*p.Leu795Phe) (Ibrahim et al. 2020). Interestingly, another study had already suggested that *ATP10A* imprinting is linked to MA (Russo et al. 2005). Additionally, a novel *CACNA1C* variant (p.Ile662Leu) in an individual carrying the *ATP7B* variant was associated with prolonged migraine attacks symptoms, and in another case a predicted deleterious rare variant (p.Arg111Gly) was reported in the *CACNA1I* gene associated with severe migraine symptoms (Ibrahim et al. 2020).

Rasmussen et al. carried out a WGS study, to distinguish FHM individuals without known variants in the genes *CACNA1A, ATP1A2 and SCN1A*, from common forms of migraine. Thus, it was possible to conclude that individuals with FHM without any known variant are more likely to accumulate rare frameshift indels in multiple genes (Andreas Hoiberg Rasmussen et al. 2020a,b).

RNA-sequencing (RNA-seq)

Despite great efforts, difficulties inherent to WGS studies are rooted in the fact that every individual carries millions of DNA variants in their genome (Lek et al. 2016).These variants only explained a fraction of the total heritability of migraine, since many susceptibility *loci* are in non-coding genomic regions, and these regions can regulate the expression of downstream genes and/or splicing patterns (Vaz-Drago et al. 2017), as mentioned in the above studies.

The RNA-seq technique enables functionally testing the effects of these non-coding variants as well as coding variants: quantify the level of gene expression, identify novel transcripts, alternative splicing and gene fusion events that can also be associated with headache mechanisms (Wang et al. 2009). In recent years, some transcriptomic studies have been carried out, to understand if there is any change in the gene expression associated with the migraine's pathophysiology. Renthal (2018), Vgontzas and Renthal (2020) performed studies using single-human brain cell transcriptomics to determine where migraine susceptibility genes are expressed. They reported that 70% of the neuronal migraine-associated genes were significantly enriched in inhibitory neurons, while 30% were enriched in excitatory neurons. Nevertheless, many genes (such as *CACNA1A* and *SCN1A*) are expressed in both excitatory and inhibitory neurons. Additionally, they concluded that both genes linked to MA and MO were expressed across multiple cell types, but approximately 17.7% of genes display tissue-selective enrichment within the central nervous system (*HCK, ARHGEF26, WSCD1, TSPAN2, NEGR1, SLC24A3*), neurovascular cell types *(GPR182, NOTCH4*) and the peripheral nervous system (*MYO1A, HELLS*).

Perry and collaborators focused on the expression of inflammation and immune response genes in the calvarial periosteum of chronic migraine patients and found 37 differently expressed genes, of which 26 genes are upregulated and 11 genes downregulated. The study identified some genes as key drivers in the inflammatory pathophysiology of the periosteum: *IL6, SOCS3, IFNB, CXCR4, CCL2* and *NFKBIA* (Perry et al. 2016).

Gerring et al. compared migraineurs with controls and demonstrated that 36 genes were differentially expressed (with particular evidence to *NFKBIZ*, *TNFSF10*, *TNFAIP3*, *CXCR4*, *ABCB1* and *NFIL3* genes) and these genes are related to immune and inflammatory processes (Gerring et al. 2016, 2018).

Additionally, some authors performed a RNA-seq in the blood of migraineurs (with and without aura) and controls and reported that *CD163* gene was differentially expressed in all comparisons, whereas *NMNAT2* and *RETN* genes were differentially expressed between MA and controls (Kogelman et al. 2019b). Then, the same group of researchers performed transcriptomic studies using RNA-seq of migraineurs during migraine attack. They identified 33 differentially expressed genes between two phases of migraine:

during the attack and after the treatment. Most of them are genes related to fatty acid oxidation *(CPT1A, SLC25A20* and *ETFDH)*, Notch signaling pathways (*MAML2, ADAM15* and *ADAM17*) and immune-related pathways (*CARD9*, *SH2D2A*, *CD300C*) (Kogelman et al. 2020).

Jeong et al. conducted a RNA-seq in a mouse model of chronic migraine triggered by NTG (pharmacological agent nitroglycerin which induces hyperalgesia and allodynia in mice) and found differentially expressed genes involved in immune response, in glutamate signaling pathway and in reactive oxygen species process regulation. They concluded that some genes with region-specific NTG, namely *LRRC8* and *WSCD1*, had already been associated with migraine in humans (Jeong et al. 2018).

Some studies have demonstrated that miRNAs plays an important role in epigenetic-related mechanisms (Wang et al. 2009) through the study of possible changes in miRNA in the blood of patients versus healthy controls. During migraine attacks, some authors revealed an acute upregulation in miR-34a-5p and miR-382-5p expression (Gazerani and Vinterhøj 2016). Additionally, miRNA-34a-5p is correlated with inflammation and vascular endothelial stress response, whereas miRNA-382-5p is found principally in neurons and cerebrospinal fluid (CSF), appearing only in small amounts in blood. Thus, the presence of miRNA-382-5p in the blood suggests that it can cross the blood–brain barrier (Gazerani 2019).

Furthermore, a pilot study performed in MO patients and healthy controls concluded that miRNA-27b was upregulated and miRNA-181a, miRNA-let-7b and miRNA-22 were downregulated when compared with controls (Tafuri et al. 2015).

Since blood pressure (BP) and migraine are strongly associated, Guo and collaborators performed a transcriptome-wide association study (TWAS), to demystify the genetic components and pathophysiological pathways shared between these two conditions. They identified five loci (*ITGB5, SMG6, ADRA2B, ANKDD1B and KIAA0040)* related to vascular development and endothelial function, neurogenic inflammation and calcium homeostasis (Guo et al. 2020).

In another study, demonstrating the results of combining WGS from migraine families with RNA sequencing data obtained from the brain and vascular tissue, it was possible to identify three genes (*ATXN1, FAM135B* and *CACNA1B*) involved in the pathophysiology of migraine (Rasmussen et al. 2020a,b).

Whole-transcriptome sequencing, besides allowing gene expression quantification and differential gene expression analysis, allows to identify novel transcripts, alternative splicing and gene fusion events that can also be associated with migraine mechanisms and which cannot be discerned by any other method.

Furthermore, transcriptomic information provides an avenue for biomarker discovery and several advancements in blood-based biomarker development in other neurologic disorders have occurred in recent years (Olsson et al. 2016; Santiago et al. 2018; Sun et al. 2015).

Migraine biomarkers

Throughout the years, many studies have been looking for a specific biomarker, such as neurotransmitters, neuropeptides, gliotransmitters and hormones, including cytokines, homocysteine, serotonin, hypocretin-1, CGRP, and glutamate (Atkinson et al. 2001).

Despite that CSF is believed to be the most biofluid that reflects biochemical changes in the brain, a meta-analysis of migraine biomarkers in CSF revealed that the most important compounds found in the CSF were also altered in the blood, such as glutamate, CGRP and β-endorphin (β-EP) (Van Dongen et al. 2017).

Quantification of miRNAs in blood has been studied as potential biomarkers of migraine, with potential applications for patient diagnosis and monitoring of treatment (Gazerani 2019).

Conclusions

In the last decades, genetic studies of migraine have focused on unravelling common variants underlying the pathophysiological mechanisms of this disorder, using the GWAS approach. However, to address some gaps by GWAS studies, a new era in the genetics of migraine has begun with the improvement of NGS methodologies, which allows discriminating common and rare variants in both exonic, intronic and intergenic regions, affecting gene regulation instead of protein function directly.

FHM (a monogenic form of migraine) is associated with several genetic variants within genes coding ion channels/ion transport as well as neurotransmission modulation at synaptic regions. These variants have a large effect size and a strong family component involved, but also present genetic heterogeneity. The introduction of WES/WGS methodology has also made it possible to further advance in the genetics of FHM families in whom diagnosis has not yet been possible to establish.

On the other hand, polygenic migraine forms are most likely due to the contribution of multiple variants with small effect at many genetic loci. Regarding these complex forms of migraine, GWAS brought an important initial breakthrough in the discovery of associated genes with neurological and vascular pathways, with the premise of subsequent transcriptomics and functional studies.

NGS has started revealing a more complete depiction of migraine with many different genes associated with its liability, although no single gene causes the disorder independently. We consider that migraine is likely to include a number of different disease etiologies matching a polygenic model.

It is important to keep in mind that migraine displays a heterogeneous behavior in treatment effectiveness and only 50% of migraineurs adequately respond to drugs (Pomes et al. 2019). This variability could be due in part to the genetic differences in the capability of individuals to metabolize certain drugs. Variants detected in drug-metabolizing enzymes (mostly cytocrome P450—CYPs) and drug transporters proteins have been identified as important contributors to this complexity in drug response (Capi et al. 2018). Since the patient's response (efficacy and toxicity) to a drug is affected by DNA and RNA variations, it is extremely important to study the pharmacogenomics of migraine (Viana et al. 2014).

It is mandatory to proceed with a large-scale screening study intertwining genetic variants and transcripts that will allow a better understanding of the mechanisms underlying migraine pathophysiology, by identifying novel transcripts, differential gene expression, alternative splicing, and gene fusion events.

Combining WGS/WES and RNA-seq is not only a powerful method to address functional genetic variation, but also provides the possibility to translate into effective therapies through the interaction of environmental factors and epigenetic regulation of gene expression (Renthal 2018). Validated genomic profile diagnostics in a clinically well-characterized cohort using novel NGS approaches will certainly help clinicians to deal with migraine patients and may provide rich insight into the mechanistic underpinnings of this prevalent and disabling neurological condition. Although for the complex common forms, the path that will lead to the development of biomarkers is still long, this could aid in determining patients who would benefit more from the different therapies and monitoring new treatments.

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Declarations

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