

Migraine genetics: current findings and future lines of research

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Abstract In the last two decades, migraine research has greatly advanced our current knowledge of the genetic contributions and the pathophysiology of this common and debilitating disorder. Nonetheless, this knowledge still needs to grow further and to translate into more effective treatments. To date, several genes involved in syndromic and monogenic forms of migraine have been identified, allowing the generation of animal models which have significantly contributed to current knowledge of the mechanisms underlying these rare forms of migraine. Common forms of migraine are instead posing a greater challenge, as they may most often stem from complex interactions between multiple common genetic variants, with environmental triggers. This paper reviews our current understanding of migraine genetics, moving from syndromic and monogenic forms to oligogenic/polygenic migraines most recently addressed with some success through genome-wide association studies. Methodological issues in study design and future perspectives opened by biomarker research will also be briefly addressed.

Keywords Aura · CADASIL · Cortical spreading depression · Headache · Migraine · Trigeminovascular

Introduction

Migraine is a complex neurological disorder, characterized by recurrent and severe attacks of headache, lasting from 4 to 72 h, with associated sensory and dysautonomic symptoms, such as

nausea and vomiting, photophobia and phonophobia. Transient, focal neurological symptoms mostly involving the visual system occur prior to or during migraine attacks, a phenomenon known as “aura” affecting approximately 15–30 % of cases. Migraine is thus subdivided into two major categories, namely “migraine with aura” (MA) and “migraine without aura” (MO), in accordance with the classification criteria of the International Headache Society listed in Box 1 [1]. However, often a single patient suffers from both types of headache or switches from one category to the other during the course of the illness [1]. The prevalence of migraine is estimated at a striking 18 % in females and 6 % in males, making it one of the most common and frequently invalidating human disorders [2, 3]. Its peak incidence occurs between 25 and 55 years of age [2, 3].

Box 1: diagnostic criteria for headache disorders [1].

Migraine with aura (MA)

- A. At least two attacks fulfilling criteria B and C
- B. One or more of the following fully reversible aura symptoms:
 1. Visual
 2. Sensory
 3. Speech and/or language
 4. Motor
 5. Brainstem
 6. Retinal
- C. At least two of the following four characteristics:
 1. At least one aura symptom spreads gradually over ≥ 5 min, and/or two or more symptoms occur in succession
 2. Each individual aura symptom lasts 5–60 min
 3. At least one aura symptom is unilateral
 4. The aura is accompanied, or followed within 60 min, by headache
- D. Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack has been excluded

Migraine without aura (MO)

- A. At least five attacks fulfilling criteria B–D
- B. Headache attacks lasting 4–72 h (untreated or unsuccessfully treated)
- C. Headache has at least two of the following four characteristics:
 1. Unilateral location
 2. Pulsating quality
 3. Moderate or severe pain intensity
 4. Aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
- D. During headache at least one of the following:
 1. Nausea and/or vomiting
 2. Photophobia and phonophobia
- E. Not better accounted for by another ICHD-3 diagnosis

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Migraine is viewed as a neurovascular disease caused by a primary brain dysfunction, leading to activation and sensitization of the trigeminovascular system and to the release of vasoactive neuropeptides. Cortical spreading depression (CSD), originating in the occipital region, is thought to represent the neurobiological underpinning of visual aura [4]. In animal models, CSD indeed activates the trigeminovascular system, triggering a series of cortical, meningeal, and brainstem events consistent with current knowledge about the pathophysiology of migraine attacks [4, 5].

Family and twin studies of migraine

The common observation that migraine tends to recur in families has long suggested that genetic factors may play a significant role in the disease. The existence and “relative weight” of genetic contributions to a complex disorder can be assessed using classical approaches, including family, twin, and adoption studies. To our knowledge, no adoption study of migraine has been published to this date. On the other hand, family and twin studies indicate that at least 50 % of patients have a first-degree relative affected by a similar condition [6–11]. Comparing familial recurrence rates with the above-mentioned incidence in the general population yields a relative risk (RR) estimated at 4.0 and 1.4 for MA and MO, respectively [10, 11]. Finally, several population-based twin studies have contrasted concordance rates for migraine between monozygotic (MZ) and dizygotic (DZ) twins to produce heritability estimates [6–8]. Mulder et al. [6] studied the prevalence and heritability of migraine in 29,717 twin pairs from six countries enrolled in Genome EUtwin Project [12]. This study did not distinguish between MA and MO, which were analyzed collectively [6]. The prevalence of migraine was highest (32–34 %) in Danish and Dutch women, and lowest (10–13 %) in Finland, a result compatible with shared environmental factors explaining a large proportion of variance. MZ concordance rates were 1.5–2.0 times higher than those recorded in DZ twins, with heritability estimated at 34–57 % (on average 50 %). Hence, both family and twin studies collectively support sizable genetic contributions to migraine pathogenesis.

The quest for single genes in migraine genetics research

Genetic contributions to any human disease can range from monogenic or oligogenic forms, due to rare or even “private” genetic variants endowed with high penetrance (i.e., phenotypic expression), to polygenic and multifactorial conditions stemming from complex epistatic interactions between multiple common genetic variants with low penetrance and small effect size, as well as from gene-environment interactions. In

the latter framework, environmental factors can trigger the onset and progression of the disease by influencing epigenetic profiles directly regulating the expression of relevant genes, further increasing heterogeneity [13, 14]. In the case of migraine, higher RR rates and heritability estimates, as well as the results of molecular studies (see below), generally support greater contributions by rare variants to MA, whereas MO more frequently stems from multifactorial and polygenic mechanisms. Within the framework of gene-environment interactions, a significant role has been reliably demonstrated for several environmental factors, including female sex hormones, early and recent stress (such as low maternal care and short-lasting, intense stressful periods, respectively), decreased sensory thresholds for visual, auditory, and olfactory stimuli [13, 14]. Instead, a more controversial role has been hypothesized for weather and climate conditions, electromagnetic fields, smoking, pollution, and molds [13, 14]. An additional layer of complexity is conferred by the frequent association of migraine with other disorders, including anxiety, depression, ADHD, sleep disorders, epilepsy, as well as atopic and cardiovascular diseases [15, 16]. These comorbidities can conceivably stem from shared genetic and/or environmental underpinnings to a different extent in different patients [17, 18].

To this date, several approaches have been used to identify genetic variants either causing migraine or conferring vulnerability to the disease. The recent advent of array-based technologies and second-generation DNA sequencing has provided novel powerful tools to genetic analysis. In general, rare variants are sought by DNA sequencing in multigenerational families with many affected individuals. These studies were previously performed using a linkage approach, followed by refinement of the linkage region and targeted Sanger sequencing of candidate genes. While these approaches remain viable, whole-exome sequencing (WES) and whole-genome sequencing (WGS) can now significantly hasten the discovery of rare pathogenic variants with high penetrance. On the other hand, common variants have been typically sought using case-control or family-based association. SNP arrays now allow an unbiased search of common variants conferring disease vulnerability in large samples of cases and controls through genome-wide association studies (GWAS). Applying “classical” approaches, several forms of MA, such as familial hemiplegic migraine (FHM), have now been elucidated, with well-defined underlying causal variants identified and functionally tested. On the other hand, common forms of migraine are still less well understood at the genetic level.

We hereby review current molecular genetic evidence for MA and MO. The present work does not represent a systematic review but rather aims to (a) provide thorough coverage of this area of investigation, spanning syndromic migraines, rare monogenic forms, up to more recent GWAS data on common predisposing variants; (b) discuss methodological issues on

experimental design, as pertaining to migraine genetics research; and (c) outline possible links between genetic findings and the underlying pathophysiology of migraine.

Syndromic migraines

Migraine can be part of known genetic disorders, displaying multiple manifestations and often involving various organs. The genetic syndromes most frequently associated with migraine are listed in Table 1. These disorders, though rare, may lead to a better comprehension of the mechanisms underlying more common forms of idiopathic migraine.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a genetic Mendelian disorder associated with strokes in adulthood. The onset of the disease is ambiguous and often characterized by migraine episodes associated with neurological symptoms or transient ischemic attacks (TIA), which anticipate strokes. The progression of the disease is a subcortical multi-infarct dementia. CADASIL is caused by mutations in *NOTCH3*, a highly conserved gene located in human chromosome 19q12, encoding for a transmembrane receptor expressed in the smooth muscle cells of small brain vessels [19]. This receptor protein consists in 2321 amino acids with an extracellular domain encompassing 34 EGF-like repeats hosting the vast majority (95 %) of CADASIL mutations described to date [19–24]. The prevalence of *NOTCH3* mutations is approximately 4 per 100,000 adults and is probably underestimated [25, 26]. Mutations lead to a dysfunction of the signaling pathway that physiologically regulates vascular development during embryogenesis and maintains the structural/functional stability of blood vessels in adults [27, 28]. CADASIL may be caused by a vascular dysfunction resulting in increased pressure-induced vascular tone and a relative deficit in vasodilation. A specific characteristic of CADASIL is the accumulation of the NOTCH3 receptor due to its slow clearance, resulting in the formation of granular osmiophilic deposits: the effect on small vessels is decreased cell adhesion, cell loss, deposits, degeneration of smooth muscle cells in the middle layer, and fibrosis [29]. CADASIL migraine occurs typically with aura and its prevalence ranges between 0 and 40 % in studies performed to date [30]. The onset of migraine attacks in CADASIL typically occurs before 26 years of age; it is either concomitant to or can anticipate brain lesions, and females display an earlier onset of MA compared to males [31].

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a disease caused by mutations in several mitochondrial genes, most frequently *MTTL1* encoding for the mitochondrial tRNA for leucine (A to G transition at nucleotide 3243), and is characterized by epileptic seizures, stroke-like episodes, and lactic acidosis [32]. In particular, the typical presentation of MELAS includes epileptic seizures with radiologic evidence of cortical infarcts, often associated with migraine-like headaches; other clinical features include hemiparesis, hemianopsia, cortical blindness, episodic vomiting, and short stature. Systemic manifestations can include cardiac, renal, endocrine, gastrointestinal, and endothelial signs and symptoms. Ragged-red fibers are common, while a symptomatic myopathy is rare. It has been postulated that decreased brain oxidative metabolism may play an important role in the pathogenesis of migraine associated with MELAS [33].

Retinal vasculopathy and cerebral leukodystrophy

Retinal vasculopathy and cerebral leukodystrophy (RVCL) consolidates into a single autosomal dominant disease three neurovascular syndromes previously named hereditary vascular retinopathy (HVR), cerebroretinal vasculopathy (CRV), and hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS) [34]. These syndromes are all caused by mutations in the *TREX1* gene, located on human chromosome 3p21, encoding for former DNase III (i.e., three prime repair exonuclease), an autonomous non-processive 3'–5' DNA-specific exonuclease [35]. This enzyme, localized in the perinuclear region of the cell, plays a fundamental role in granzyme A-mediated cell death and, when mutated, in indirectly activating autoimmunity against undigested dsDNA from dying cells. RVCL-producing *TREX1* mutations delete its carboxyl-terminus, leading to loss of cellular compartmentalization and widespread enzyme distribution in the presence of preserved exonuclease activity [36]. *TREX1* mutations can also cause other disorders including Aicardi-Goutières syndrome, systemic lupus erythematosus, and familial chilblain lupus, through abnormal immune mechanisms [34].

Clinically, HVR is characterized by retinal microangiopathy, with microaneurysms and telangiectatic capillaries [37]. Its onset is typically in early adulthood, with disease progression yielding occlusion of large retinal arteries, proliferative arteriopathy, and avascular areas in the retinal periphery. Migraine is present in 70 % of HVR cases [37, 38]. CRV involves both the retina and the brain [34, 35]. Neuroimaging shows pseudo-tumors appearing as progressive subcortical contrast-enhancing lesions. Progressive visual loss is accompanied by neurological deficits, psychiatric symptoms, migraine, stroke, and death within 10 years of disease onset [34]. Instead,

Table 1 Syndromic migraines in Mendelian disorders

Syndrome	Gene	Chr. region	Gene product	Mutation effects	Migraine subtype	Refs
CADASIL	NOTCH3	19p13.12	Notch homologue protein 3	Damage and apoptosis of vascular smooth muscle cells	MA/MO in 40 % of CADASIL patients	[19–31]
MELAS	MTTL1 and others	mt-DNA	Mitochondrially encoded tRNA leucine 1 (UUA/G)	Impaired mitochondrial functions	MA and other neurological features	[32, 33]
RVCL (HERNS, CRV, HVR)	TREX1	3p21.3	3'-repair exonuclease 1	Brain, skin, and blood vessel damage, due to autoimmunity	MA/MO in up to 70 % of patients	[34–41]
COL4A1-related syndromes	COL4A1	13q34	Collagen type IV, alpha-1 subunit	Small vessel damage	MO in 1 case with porencephaly	[42–46]
FASPS	CSNK1D	17q25	Casein kinase I delta	Reduced CSD threshold, increased spontaneous and evoked calcium signaling in astrocytes	Migraine in 2 families	[47–50]

CADASIL cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, *COL4A1* retinal arteriolar tortuosity and leukoencephalopathy, *CRV* cerebroretinal vasculopathy, *CSD* cortical spreading depression, *FASPS* familial anticipated sleep phase syndrome, *HERNS* hereditary endotheliopathy with retinopathy, nephropathy, and stroke, *HVR* hereditary vascular retinopathy, *MA* migraine with aura, *MELAS* mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes, *MO* migraine without aura, *RVCL* retinal vasculopathy and cerebral leukodystrophy

HERNS is a multi-infartual condition characterized by retinopathy, strokes, and nephropathy [39]. Capillary subendothelial basal membranes display a distinctive multilamination in brain, kidney, gastrointestinal tract, and other tissues [39]. Similarities between CRV and HERNS include pseudo-tumors, retinopathy, migraine-like headaches and neurological complications which may lead to death, while additional features may include psychiatric symptoms, dysarthria, hemiparesis, and apraxia [34, 39]. Finally, also the systemic variant of RVCL, hereditary systemic angiopathy, which shares with the RVCL spectrum many clinical features, including migraine-like headache, progressive neurological signs, and visual disturbances, has recently been shown to stem from *TREX1* mutations, causing a C-terminal frameshift [40, 41].

COL4A1-related syndromes

COL4A1, located on human chromosome 13q34, encodes for the collagen type IV alpha-1 subunit. Mutations in this 52 exon-long gene can result in several autosomal dominant disorders with overlapping features, including perinatal hemorrhage with porencephaly [42–45] and a small vessel disease leading to hemorrhage and hemiparesis with infantile or adult onset [46]. The association of *COL4A1* mutations with migraine is not entirely certain. Although 10 of 52 *COL4A1* mutation carriers reported migraine with or without aura [45], the association is not entirely certain and could still represent a coincidental finding.

Familial anticipated sleep phase syndrome

Familial anticipated sleep phase syndrome (FASPS) is an autosomal dominant disorder characterized by persistent early evening sleep onset and early awakening [47]. Abnormal

circadian rhythmicity results from a mutation in the circadian *hPER2* gene (human period 2, on chromosome 2q37.3), within the casein kinase 1 (CK1)-binding domain of the hPer2 protein [48]. Furthermore, screening two families with MA and FASPS, Brennan et al. [49] have recently identified two missense mutations (T44A and H46R) in the gene encoding casein kinase 1 δ (CK1 δ), resulting in decreased enzyme activity. In addition to playing a pivotal role in the biology of circadian rhythms [50], in vivo and in vitro studies suggest that CK1 δ may contribute to the pathogenesis of migraine. Mice carrying the CK1 δ T44A mutation have a reduced threshold for cortical spreading depression accompanied by increased spontaneous and evoked calcium signaling in astrocytes [50].

Collectively, genetic studies of syndromic forms of migraine provide interesting leads toward the definition of the pathogenic chain of events leading to the more common non-syndromic forms. In particular, CADASIL and RVCL provide further evidence supporting brain vascular tone dysregulation as an important contributor to the pathophysiology of migraine attacks, regardless of whether intrinsically generated or immune-mediated. Instead MELAS and FASPS point toward insufficient oxidative metabolism and abnormal circadian variation in cortical excitability, respectively, as possibly playing a role in specific subgroups of non-syndromic patients.

Monogenic forms of migraine

Familial hemiplegic migraine (FHM), is a rare monogenic subtype of migraine with motor aura (see Box 1 for general

diagnostic criteria) displaying strong familiarity compatible with an autosomal dominant transmission [51]. The overlap between FHM and common idiopathic migraine has thus suggested that the two forms may share common neurobiological underpinnings [52]. Despite its low incidence, FHM may thus represent an important genetic model to understand the molecular pathophysiology of common migraine. Mutation screening has revealed numerous genetic variants able to cause FHM (Table 2). Mutations in two ion channel genes, *CACNA1A* and *SCN1A*, as well as in *ATP1A2* encoding the alpha2 subunit of the Na-K ATPase, cause FHM type 1, 3, and 2, respectively. De novo mutations affecting especially the latter two genes have also been reported in sporadic forms, devoid of a positive family history but clinically superimposable to FHM [53]. Furthermore, the pleiotropic effects especially of *SCN1A* mutations result in a variety of clinical phenotypes, including various forms of epilepsy, autism, and MA/MO without typical FHM-type aura, raising further interest in the pathophysiological overlap between FHM and sporadic forms of migraine [63–65]. Cellular and animal studies have revealed that mutated FHM genes are associated with altered ion homeostasis, cellular excitability, and neurotransmitter release; these abnormalities then predispose to the onset of cortical spreading depression (CSD), the electrophysiological underpinning of aura symptoms paving the path to migraine headache [4].

FHM type 1 is caused by missense mutations in the *CACNA1A* gene on chromosome 19p13 [54]. To date, at least 70 different causal missense mutations have been detected in this gene. Nonsense or missense mutations in *CACNA1A* are typically associated with episodic ataxia type 2 and FHM1, whereas a CAG triplet repeat expansion affecting the C-terminus causes spinocerebellar ataxia type 6, but rare cases with both FHM1 and cerebellar ataxia have also been reported [66, 67]. *CACNA1A* encodes the $\alpha 1A$ subunit of the Cav2.1 (P/Q type) voltage-gated neuronal calcium channel, expressed throughout the central nervous system with highest density in the cerebellum [68, 69]. This subunit is involved in voltage sensitivity and mutations lead to increased Ca^{2+} influx into the

neuron in response to smaller depolarizations. This, in turn, enhances the release of the excitatory neurotransmitter glutamate, because this channel is predominantly expressed pre-synaptically, where it couples membrane depolarization with neurotransmitter release [70]. Ultimately, transgenic mice harboring the human FHM1 R192Q or S218L (KI) mutation exhibit a lower threshold for CSD generation, increased CSD propagation velocity, and CSD extension into subcortical regions [71, 72]. Interestingly, female knock-in mice harboring these human mutations show significantly greater susceptibility to CSD compared to male carriers, which disappears after ovariectomy [73]. The regional distribution and functional role of this subunit explains why some FHM1 patients may also display cerebellar signs and episodic loss of consciousness.

FHM type 2 (FHM2) is associated with mutations in the *ATP1A2* gene, located in human chromosome 1q21-q23 (Table 2). This gene encodes the $\alpha 2$ subunit of the Na^+K^+ pump. FHM2 mutations blunt Na^+K^+ ATPase activity and lower CSD threshold by (a) enhancing extracellular K^+ concentrations and (b) reducing the electrochemical Na^+ gradient required to drive astrocytic glutamate transporters, ultimately slowing the removal of extracellular glutamate and enhancing its concentration [51]. *ATP1A2* mutations have also been very rarely encountered in basilar migraine and in common migraine, as well as in other neurological diseases, such as idiopathic cerebellar syndromes, benign familial infantile convulsions, epilepsy, alternating hemiplegia of childhood, and intellectual disability [55, 56].

FHM3 mutations are localized in the *SCN1A* gene, on human chromosome 2q24 [57]. This gene encodes for the $\alpha 1$ subunit of the neuronal voltage-gated sodium ($Na_v1.1$) channel, which is primarily expressed in cortical neurons, where it plays an important role in the generation and propagation of action potentials. *SCN1A* mutations typically lead to hypersynchronous neuronal discharges resulting in seizures, autism, or migraine attacks, with either typical FHM course or appearing as sporadic MA or MO [63–65]. Data derived from a $Na_v1.1$ -knockout mouse model show that a loss of one allele

Table 2 Familial hemiplegic migraine (FHM)

FHM	Gene	Chr. region	Protein product	N. of mutations	Refs
FHM 1	<i>CACNA1A</i>	19p13	Voltage-dependent (P/Q) Ca_v 2.1 channel, $\alpha 1A$ subunit	Over 70 missense mutations with “gain of function” effect	[53, 54]
FHM 2	<i>ATP1A2</i>	1q21-23	Na^+K^+ ATPase, $\alpha 2$ subunit	Over 50 mutations with “loss of function” effect	[53, 55, 56]
FHM3	<i>SCN1A</i>	2q24	Neuronal type I voltage-dependent Na^+ channel, A1 subunit	5 missense mutations	[57, 58]
–	<i>PRRT2</i>	16p11	Axonal proline-rich transmembrane protein 2 involved in exocytosis	Four family members with the same 649dupC mutation	[59]
–	<i>SLC4A4</i>	4q21	Electrogenic $Na^+HCO_3^-$ cotransporter NBCe1	1 homozygous deletion in 2 sisters	[60]
–	<i>SLC1A3</i>	5p13	Glial glutamate transporter EAAT1	1 mutation	[61, 62]

Table 3 Positive association findings with neurological candidate genes

Gene	Locus	Cases	Controls	Ethnicity	n. SNPs analyzed	Associated SNPs/VNTRs	P value	Refs
5-HT-related genes								
DDC	7p12.2	528 (308 MO, 220 MA)	528	Spanish	15	rs2329340 rs11974297 rs2044859 rs11761683	0.0019 (MA)	[77]
MAOA	Xp11.3	528	523	Spanish	2	rs3027400G rs2072743C	0.006 (MO)	[77]
HTR2B	2q37.1	528	523	Spanish	23	rs16827801 rs10194776 rs6323	0.0017 (MO)	[77]
SLC6A4	17q11.2	91	119	Japanese	1	5-HTTLPR S/S	<0.05	[78]
		154 (92MO, 52MA)	105	Italian	–		<0.05 (MA)	[79]
		251	192	German	2	rs1979572	<0.05 (MA)	[80]
Dopamine-related genes								
DBH	9q34	177	182	Australian	1	rs7239728	0.019	[81]
		275	275	Australian	2	rs7239728	0.003 (MA)	[82]
		200	200	Australian	2	rs1611115	0.012	[83]
		300	300			rs1611115	0.031	
		650	2937	German/Br	1	rs2097629	5.57×10^{-8}	[84]
		263	274	Spanish	11	rs1611131	0.04	[85]
		301 (99MA, 202MO)	202	Indian	1	rs72393728	0.027	[86]
		208, 127 (II)	200	Indian	2	rs7239728	<0.05	[87]
SLC6A3	5p15.3	650	2937	German/Br	1	rs40184	6.36×10^{-7}	[84]
DRD2	11q23	650	650	German	1	rs7131056	0.034	[84]
DRD3	3q13.3	263	274	Spanish	10	rs12363125	0.03	[85]
DRD4	11p15.5	194 (93MA, 101MO)	117	Italian	1	rs22832265	0.008	[88]
Glutamate receptors								
GRIA1	5q31.1	250	260	Italian	6	rs2195450	0.00002 (MA)	[89]
GRIA3	Xq25	250	260	Italian	8	rs548294 rs3761555	0.0003 (MO) 0.0001 (MA)	[89]
		472	472	Australian	1	rs3761555	0.008 (MA)	[90]

DBH dopamine beta-hydroxylase, *DDC* dopa decarboxylase, *DRD2* dopamine receptor D2, *DRD3* dopamine receptor D3, *DRD4* dopamine receptor D4, *GRIA1* glutamate receptor, ionotropic AMPA 1, *GRIA3* glutamate receptor, ionotropic AMPA 3, *HTR2B* 5-hydroxytryptamine (serotonin) receptor 2B, *5-HTTLPR* functional serotonin transporter gene promoter, *MAOA* monoamine oxidase A, *SLC6A3* solute carrier family 6 (neurotransmitter transporter dopamine), *SLC6A4* solute carrier family 6 (neurotransmitter transporter), member 4, *II* replication cohort

predominantly decreased activity of GABAergic inhibitory interneurons, without affecting excitatory pyramidal neurons [74]. Indeed, several mutations in the *SCN1A* gene have also been associated with severe childhood epilepsy, such as generalized epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome [75]. In contrast, mutations producing migraine only, such as Q1489K, display ambivalent effects whereby not only the rate of recovery from inactivation is accelerated, as in epileptogenic mutations, but also the transition from closed to the inactivated state of the channel, preventing neuronal discharges from becoming epileptogenic [58].

Other forms of typical FHM due to mutations in novel FHM candidate genes have been recently described (Table 2). Mutations resulting in an FHM phenotype have been identified in the *PRRT2* (proline-rich transmembrane

protein 2) gene, located on human chromosome 16p11 and encoding for an axonal protein associated with the exocytosis protein complex [59]. A mutation in the *SLC4A4* gene, encoding for the $\text{Na}^+\text{-HCO}_3^-$ cotransporter NBCe1, has been identified in two sisters with an FHM phenotype, renal tubular acidosis, and ocular abnormalities [60]. Finally, missense mutations located in the *SLC1A3* gene, encoding the glial glutamate cotransporter EAAT1, have been found to cause episodic ataxia, migraine, seizures, and alternating hemiplegia [61, 62].

In conclusion, familial forms of MA, and in particular FHM, are due to rare inherited or sporadic genetic variants endowed with high penetrance. These mutations disrupt neuronal excitability by affecting the transmembrane electrochemical gradient and/or by enhancing extracellular glutamate concentrations. Each mutation is rare or even private: locus

and allelic heterogeneity is clearly supported by current evidence and further heterogeneity can be anticipated. Importantly, studies investigating the role of FHM genes in common forms of MO have uncovered only few instances where mutations in FHM genes produce familial MO [56, 63]. This leads to conclude that genetic variants in FHM genes do not play widespread causative roles in common forms of MO, seemingly displaying more complex genetic underpinnings [76].

Oligogenic/polygenic forms of migraine

Candidate gene studies

Candidate gene approaches have been widely used to study migraine genetics. Replication studies have been performed with conflicting or inconclusive results for a significant number of these genes. This can be due to several reasons, such as low statistical power, interethnic differences in linkage disequilibrium, genetic, and phenotypic heterogeneity between

independent case–control cohorts. Nonetheless, candidate gene studies remain of interest, as they can unveil the contribution of common genetic variants to a complex phenotype in specific ethnic groups and especially in genetic isolates (see “Methodological Issues in Migraine Genetics Research” below). Within this framework, positive findings are bound not to be replicated in all ethnic groups but are nonetheless relevant if at least some replications occur and functional allelic dimorphism is proven *in vitro* and/or *in vivo*. Candidate genes have been previously clustered around four functional gene families, namely neurological, vascular, hormonal, and inflammatory genes [76]. Tables 3, 4, 5, and 6 list positive case–control studies supporting the association between migraine and candidate genes yielding positive results in at least two independent studies. Negative studies are not listed.

(I) *Neurological genes*. This category broadly includes candidate genes encoding: (a) ion channels (calcium channel, voltage-dependent, P/Q type, alpha 1A subunit [CACNA1A], voltage-potassium intermediate/small conductance calcium-activated channel, subfamily N, member

Table 4 Positive association findings with vascular candidate genes

Gene	Locus	Cases	Controls	Ethnicity	n. SNPs analyzed	Associated SNPs/polymorphisms	P value	Refs
ACE	17q23.3	191	201	Italian	1	rs4646994	<0.05	[91]
		176	248	Japanese	1	rs4646994	<0.01 (MA)	[92]
		150	150	Indian	1	rs4646994	0.04 (MA)	[93]
MTHFR	1p36.3	74	261	Japanese	1	rs1801133	<0.01	[94]
		102	136	Turkish	2	rs1801133	0.015	[95]
		78MA,152MO	204	Spanish	1	rs1801133	0.006 (MA vs MO)	[96]
		270	270	Australian	1	rs1801133	0.017 (MA)	[97]
		91	119	Japanese	1	rs1801133	<0.05	[78]
		124	1725	British	1	rs1801133	<0.05 (MA)	[98]
		151	137	Chinese	1	rs1801133	0.003 (MO)	[99]
		150	107	Turkish	1	rs1801133	<0.001	[100]
NOTCH3	19p13.12	97	97	German	2	rs1043994	0.005	[101]
		275	275	Australian	2	rs3815188	0.002 (MO)	[102]
		300 (II)	300 (II)		2	rs1043994	0.001 (MA)	
						rs3815188	0.06 (MO)	
EDNRA	4q31.22	850	890	Finnish	13	rs1043994	0.003 (MA)	
		648 (II)	651 (II)	German	1	rs2048894	0.015 (MA)	[103]
		217+179TTH	217	Indian	1	rs2048894	0.010 (MA)	
		77MA; 111MO	287	Portuguese	3	rs702757-rs5333	0.002 (MA/MO)	[104]
		140		French	5	c.-231A>G	<0.05	[105]
NOS2	17q11.2	504	512	Chinese	2	PNRP	<0.05	[106]
		200 (52MA, 148MO)	142	Brazilian	1	rs2779249	0.007	[107]
NOS3	12q14	156	125	Italian	1	rs2297518	<0.05	[108]
						rs1799983	<0.05 (MA)	[109]

ACE angiotensin I-converting enzyme, EDNRA endothelin receptor type A, MA migraine with Aura, MO migraine without Aura, MTHFR methylenetetrahydrofolate reductase, NOTCH3 neurogenic locus notch homologue protein 3, PNRP pentanucleotide repeat polymorphism, TTH tension-type headache, II replication cohort

Table 5 Positive association findings with hormonal candidate genes

Gene	Locus	Cases	Controls	Ethnicity	n. SNPs analyzed	Associated SNPs/polymorphisms	P value	Refs
ESR1	6q25.1	224	224	Australian	1	rs2228480	0.003	131
		260 (II)	260 (II)				8×10^{-6}	
		240	160	Spanish	1	rs1801132	0.008 (females)	132
		356 (198MA, 158MO)	374	Spanish	1	rs1801132	0.004	133
		217MA, 179TTH	217	Indian	1	rs2234693	0.002 MA	134
		207	200	Indian	4	rs2234693	$P_{\text{cor}}0.01$	135
ESR2		356 (198MA, 158MO)	374	Spanish	1	rs4986938	0.004	133
FSHR	2p21-p16	356 (198MA, 158MO)	374	Spanish	1	rs6166	0.004 MA (females)	133
CYP19A1	15q21	207	200	Indian	1	rs10046	$P_{\text{cor}}0.01$	135
		127 (II)						
PGR	11q22	275	275	Australian	1	PGR PROGIN insert	0.002	136
		300 (II)	300		1		0.003	

CYP19A1 cytochrome P450, family 19, subfamily A, polypeptide 1 gene, *ESR1* estrogen receptor 1 gene, *ESR2* estrogen receptor 2 gene, *FSHR* follicle stimulating hormone receptor gene, *PGR* progesterone receptor gene, *TTH* tension-type headache, *II* replication cohort

3 [KCCN3]), (b) Na^+/K^+ -ATPase subunits, (c) molecules involved in the synthesis, release, and binding of neuropeptides (calcitonin gene-related peptide) or neurotransmitters (glutamate, GABA, dopamine, serotonin) relevant to neuronal excitation and/or to nociception. Some case–control association studies have yielded positive results, as listed in Table 3 [77–90], although most studies have been negative especially for the

former two gene families. Nonetheless, a thorough screening of 150 brain-expressed genes involved in ion homeostasis (channels, transporters, exchangers, and accessory subunits) identified three genes encoding potassium channels associated with migraine, namely *KCNK18*, *KCNG4*, and *KCNAB3* [121]. *KCNK18* is especially interesting, in view of its expression in the trigeminal and dorsal root ganglia, as well as its

Table 6 Positive association findings with inflammatory candidate genes

Gene	Locus	Cases	Controls	Ethnicity	n. SNPs analyzed	Associated SNPs/polymorphisms	P value	Refs
TNFA	6p21.3	299	306	Italian	1	rs1800629	<0.001 (MO)	[110]
		221 (MO)	183	Iranian	1	rs1800629	<0.0001	[111]
		216	216	Indian	1	rs1800629	<0.006 (MA)	[112]
		67 (MO)	96	Turkish	1	rs1800629	0.004 (MO)	[113]
		203	202	Turkish	1	rs1800629	<0.0001	[114]
TNFB/LTA	6p21.3	79 (47MO+32MA)	101	Italian	1	TNFB*2al.	0.004 (MO)	[115]
		439	382	South Korea	15	LTA-294C	0.005	[116]
		299	278	Italian (Sardinian)	1	rs909253	0.018	[117]
		91	119	Japanese	1	rs909253	<0.05	[78]
TNFRSF1B	1p36.22	416	415	Chinese Han	1	rs5745946	0.004	[118]
HLA-DRB1	6p21.3	255 (41MA, 214MO)	325	Italian	–	DRB1*16al.	0.043 (MO)	[119]
IL1 β	2q14	67 (MO)	96	Turkish	1	rs1143634	0.004	[113]
COX-2	1q25.2	144	123	Turkish	2	rs20417	0.0001	[120]

COX-2 cyclooxygenase-2, *HLA-DRB1* major histocompatibility complex, class II, DR Beta 1, *LTA* lymphotoxin alpha, *MA* migraine with Aura, *MO* migraine without Aura, *TNFA* tumor necrosis factor α gene, *TNFB* tumor necrosis factor β gene, *TNFRSF1B* tumor necrosis factor receptor superfamily, member 1B

relationship with MA also detected through linkage (see below).

- (II) *Vascular genes.* Association studies of genes involved in blood pressure regulation, endothelial cell function, vasoconstriction, and vasodilation have provided more consistent positive results (Table 4) [91–109, 122–129]. Many vascular genes associated with migraine also confer risk for stroke and heart disease [125–127]. This is especially important for MA, which displays a significant comorbidity with stroke and depression [15, 16]. Common functional variants in several vascular genes may predispose to migraine, while also influencing type and frequency of the attacks [127]:
- (a) Angiotensin converting enzyme (ACE) plays a key role in the maintenance of blood pressure and vessel wall tension. The D-D (“deletion-deletion”) common variant located in the ACE gene (human chr. 17q23) increases ACE enzymatic activity, as well as the frequency and duration of MA attacks [91–93];
 - (b) An association between methylenetetrahydrofolate reductase (MTHFR) gene variants and migraine has also been found in several studies [128]. MTHFR is a key component of the remethylation of homocysteine to methionine, as it catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Rare loss-of-function mutations in the *MTHFR* gene, located in human chromosome 1p36.22, can lead to hyperhomocysteinemia due to decreased enzyme activity. Several studies involving different ethnic groups [78, 93–100, 122–124] and several recent meta-analyses [98, 123, 124, 129] provide converging evidence of contributions by the T677 *MTHFR* allele at SNP rs1801133 to migraine, more consistently for carriers of the TT genotype and relative to MA than to MO. Nonetheless, lack of association with *MTHFR* gene variants has also been reported, which may be related to advancing age and selective survival [122];
 - (c) *NOTCH3* encodes for a transmembrane receptor regulating vascular development and differentiation during embryogenesis, as well as contributing to vascular integrity in adults [27, 28]. In addition to rare *NOTCH3* mutations producing MA within the context of CADASIL [19–24], also common variants are significantly associated with migraine [101, 102]. Hence, *NOTCH3* may play a broader role also in the pathogenesis of common migraine, well beyond rare forms associated with CADASIL;
 - (d) Endothelial genes assessed for association with migraine encode for endothelin-1 (*EDN1*), endothelin receptor type A and B (*EDNRA* and *EDNRB*), inducible NO synthase (*NOS2*), endothelial NO synthase (*NOS3*), and vascular endothelial growth factor (*VEGF*) [103–109, 125]. Endothelin-1, encoded by the *EDN1* gene (human chr 6p24), is involved in blood pressure regulation and produces vasoconstriction. EDN1 has two receptors, A and B, encoded by the *EDNRA* and *EDNRB* genes located on human chr 4q31 and 13q22, respectively. Several studies have found an association between *EDNRA* alleles and migraine (Table 4), including one study involving an experimental and a replica sample of Finnish and German migraineurs, respectively, reporting a nominal association with MA at *EDNRA* SNP rs2048894, especially with age at onset <20 years [103].
- (III) *Hormonal genes.* Genes governing estrogen and progesterone metabolism would be predicted to at least partly explain the sex-biased distribution of affection status, especially considering menstrual migraine [130]. However, results from genetic association studies are conflicting, although several positive case-control studies were published [131–136], as well as a pedigree-based association performed in the genetic isolate of Norfolk Island, a population with high prevalence of migraine descended from a small number of Isle of Man “Bounty Mutineer” and Tahitian founders [137]. In the latter study, three estrogen receptor 1 (*ESR1*) haplotypes were significantly associated with the disorder ($P < 0.05$ or 0.01). In addition to *ESR1*, six other hormonal genes have been investigated: estrogen receptor 2 (*ESR2*), progesterone receptor (*PGR*), androgen receptor (*AR*), follicle stimulating hormone receptor (*FSHR*), nuclear receptor interacting protein 1 (*NRIP1*), and cytochrome P450, family 19, subfamily A, polypeptide 1 (*CYP19A1*). However, a meta-analysis of eight genetic association studies investigating these genes suggests an association only with the *ESR1* c.594G>A and c.325C>G polymorphisms, a finding that does not differ between MA and MO [9] (Table 5).
- (IV) *Inflammatory genes.* Animal and human studies suggest that inflammation and components of the immune system may play an important role in the pathogenesis of migraine. In the case of CSD, for example, this process triggers local neurogenic inflammation, with activation of mast cells and macrophages accompanied by the release of proinflammatory cytokines, ultimately resulting in the sensitization of meningeal nociceptive nerve endings [138]. Among several genes implicated in neurogenic inflammation, results support a positive association especially for tumor necrosis factor alpha (TNF- α) [110–120] (Table 6).

Linkage studies

Many classical linkage studies have been performed on migraine using either a genome-wide approach or targeting specific regions using microsatellite markers (139–161; Table 7). Results have been highly conflicting, and very few linkage peaks have been replicated in two independent samples (Table 7). This relative lack of success can be attributed to several causes, including locus heterogeneity, reduced penetrance, the existence of phenocopies, the separation/clustering together of MA and MO phenotypes which may represent in some families different clinical expressions of the same underlying mutation, while identical mutations may yield distinct clinical phenotypes in other families. Nonetheless, greater success has been achieved in linkage studies of FHM (162–169), which have detected several loci involved in these monogenic forms, as

described above (Table 8). Some studies applying linkage to single large pedigrees, rather than combining several multiply affected families evidently afflicted by underlying heterogeneity, have also been successful. For example, a frameshift mutation in the *KCNK18* gene was found to cosegregate with MA in a large pedigree from Ontario [170]. *KCNK18* encodes for the TRESK pore potassium channel, highly expressed in trigeminal and dorsal root ganglia during embryogenesis: the truncated TRESK protein produces a dominant-negative effect blocking K^+ flow through TRESK and yielding abnormal neuronal excitability [170, 171]. TRESK may play a broader role in common forms of migraine, since *KCNK18* gene variants were found associated with migraine also in a case-control study (see above) [121]. Interestingly, the loss-of-function missense mutation C110R, found in sporadic cases, has also been detected among controls, underscoring

Table 7 Overview of the linkage studies of migraine with or without aura

Locus	Migraine subtype	N. of families	Ethnicity	Experimental method	Refs
1p36	MA	64	Canadian	Loci-specific microsatellite markers	[139]
1q21-23	MA	21	Caucasian	Loci-specific microsatellite markers	[140]
	MA and MO, LCA	756	Australian twins	Genome-wide scan	[141]
1q31	MA and MO	1	US (German descent)	Genome-wide scan	[142]
1q31	MA and MO	82	Australian	Loci-specific microsatellite markers	[143]
1q (ATP1A2 gene)	MA and MO, LCA	105	Dutch	Genome-wide scan	[144]
3qter	MA and MO, LCA	92	Australian	Genome-wide scan	[145]
4q21	MO	103	Icelandic	Genome-wide scan	[146]
4q24	MA, TCA	50	Finnish	Genome-wide scan	[147]
					[148]
5q21	MA and MO, LCA	756	Australian twins	Genome-wide scan	[141]
6p12.2-p21.1	MA and MO	1	Swedish	Genome-wide scan	[149]
	MA and MO, LCA	756	Australian	Genome-wide scan	[141]
6p12	MA and MO	134	Spanish	Loci-specific microsatellite markers	[150]
9q21-q22	MA	36	Finnish	Genome-wide scan	[151]
10q22-q23	MA and MO, TCA	210	Finnish/Australian	Genome-wide scan	[152]
11q23	MO	50	Sardinia, Italy	Loci-specific SNP markers	[153]
11q24	MA	43	Canadian	Genome-wide scan	[154]
12p13	MA	36	Finnish	Genome-wide scan	[151]
13q14-q21 (HTR2A gene)	MA and MO	3	Australian	Loci-specific microsatellite markers	[155]
13	MA and MO, LCA	105	Dutch	Genome-wide scan	[144]
14q21.2-q22.3	MO	1	Italian	Genome-wide scan	[156]
15q11-q13	MA	19	Italian	Loci-specific microsatellite markers	[157]
18p11	MA and MO, LCA	92	Australian	Genome-wide scan	[145]
19p13	MA and MO	28	German	Loci-specific microsatellite markers	[158]
19p13	MA	1	Australian	Loci-specific microsatellite markers	[159]
20	MA and MO, LCA	105	Dutch	Genome-wide scan	[144]
Xq	MA and MO	2	Australian	Loci-specific microsatellite markers	[160]
Xq27					[161]

MA migraine with aura, MO migraine without aura, LCA latent class analysis, TCA trait component analysis

Table 8 Summary of linkage studies in familial hemiplegic migraine (FHM)

Locus	N. of families	Ethnicity	Experimental method	Refs
1q21-q23	1	French	Loci-specific microsatellite markers	[162]
1q21-q23	1	Italian	Loci-specific microsatellite markers	[163]
1q23	2	Italian	Loci-specific microsatellite markers	[164]
1q23	1	Irish	Loci-specific microsatellite markers	[165]
2q24	2	German	Genome-wide scan	[166]
14q32	1	Spanish	Loci-specific microsatellite markers	[167]
19p	2	French	Loci-specific microsatellite markers	[168]
19p	1	British	Loci-specific microsatellite markers	[169]
19p	44	Danish	Genome-wide scan	[52]

the need for a multiple-hit model to explain TRESK contributions at least in some patients with MA [172].

To overcome the limitations of classical linkage in the presence of genetic and clinical heterogeneity and to target more homogenous subgroups of patients, latent class analysis (LCA) and trait component analysis (LTC) methods have been used in some migraine studies with promising results [141, 144, 148, 152].

Genome-wide association studies

To this date, six original GWAS of migraine with no selection based on specific comorbidities have been performed:

- 1) The first GWAS of migraine was produced by the International Headache Genetics Consortium (IHGC) in 2010. Anttila and colleagues [173] carried out a two-stage association study in six clinic-based and one population-based European migraine samples. In the discovery stage, 2748 cases with MA, recruited from headache clinics in Finland, Germany, and Denmark, were compared with 10,747 population-matched controls. Out of 429,912 markers genotyped, only rs1835740 reached genome-wide significance, while 11 additional SNPs reached suggestive significance. This association was then replicated in 3202 cases and 40,062 controls, and combined results indicated an 18 % increase in migraine risk conferred by the minor allele at rs1835740 (OR=1.18, 95 %; $P=1.69 \times 10^{-11}$), with a stronger effect in the MA than in MO. This SNP is located on human chr 8q22.1, between the metadherin gene (*MTDH*), also known as astrocyte elevated gene 1, and the plasma glutamate carboxypeptidase Q gene (*CPQ*), also known as plasma glutamate carboxypeptidase (*PGCP*). Although the proximity of an association signal to a gene in GWAS does not necessarily implicate that gene in the pathophysiology of the disease, interestingly, these genes are both involved in glutamate homeostasis. In cultured astrocytes, metadherin down-regulates EAAT2, the major glutamate transporter in the brain: this in turn is predicted to increase glutamate concentrations in the synaptic cleft and/or delay glutamate removal, thus lowering the threshold for cortical spreading depression [174, 175], which plays a major role in the pathophysiology of MA, as discussed above.
- 2) A population-based GWAS was then performed using a total of 23,330 women, divided in 5122 cases and 18,108 controls [176]. No SNP reached genome-wide significance in the discovery step. Among the seven loci showing suggestive significance ($P < 5 \times 10^{-6}$), SNPs tested in three independent replication cohorts provided significant results at SNPs located in or near the *TRPM8*, *LRP1*, and *PRDM16* genes. *TRPM8* is expressed in sensory neurons and dorsal root ganglion neurons and encodes a sensor for cold and cold-induced pain [177]. The role of *TRPM8* in animal models of neuropathic pain further supports a functional link with migraine, which shares several commonalities with this pain syndrome [178]. *LRP1*, expressed in brain and in many other tissues [179], modulates synaptic transmission and interacts with glutamatergic NMDA receptors. Finally, *PRDM16* is a pleiotropic gene important for craniofacial development, grown fat determination, and for the proliferation of cardiomyocyte, neural, and leukocyte precursor cells [180]. Interestingly, the mouse homologue *Prdm16* has been shown to act as a negative regulator of TGF- β , encoded by *TGFBR2*, another migraine candidate gene [181]. An association with *PRDM16*, but not with *LRP1* and *TRPM8*, has been recently replicated in a Chinese Han cohort [182]. Conversely, an association with *LRP1* and *TRPM8* has been detected in a Danish and Icelandic sample including 2523 cases and 38,170 controls, while a meta-analysis confirmed an association at all three loci [183].
- 3) Lighthart et al. [184] performed a GWAS and meta-analysis using six European cohorts from the Dutch Icelandic migraine genetics consortium for a total of 2446 cases and 8534 controls. No SNP reached genome-wide significance, while 32 SNPs reached suggestive evidence ($P < 1 \times 10^{-5}$). The lowest *P* value was

produced by a variant located in the nerve growth factor receptor (*NGFR*) gene. However, none of these SNPs reached significance in independent replica samples from the Netherlands and Australia. Metanalysis of the full data set provided weak evidence of association for the metadherin *MTDH* gene ($P < 0.05$).

- 4) Freilinger et al. [185], with the aim to identify SNPs associated with MO, performed a GWAS including 2326 cases and 4580 controls from German and Dutch populations. In the discovery step, significant genome-wide association was reached by one locus located in chr. 1q22 ($P < 5 \times 10^{-8}$), while eleven additional loci provided suggestive evidence of association ($P < 1 \times 10^{-5}$). Eighteen SNPs from these 12 loci were then analyzed in four independent European replication samples consisting of 2508 cases and 2652 controls. Results showed a convincing replication at the 1q22 (*MEF2D*) and the 3p24 (near *TGFBR2*) loci, and suggestive evidence of replication for SNPs located in or near the *PHACTR1* and *ASTN2* genes, while previously identified loci (*TRPM8* and *LRP1*) were also found associated. *MEF2D* is a transcription factor highly expressed in the brain, where it regulates neuronal differentiation and restricts excitatory synapses [186, 187]. This poses *MEF2D* as a strong candidate gene for migraine, given the involvement of glutamatergic neurotransmission in CSD and migraine pathogenesis [175], as well as possibly increased plasma levels of glutamate in individuals with migraine [188]. *TGFBR2* encodes for transforming growth factor- β (TGF- β), involved in the regulation of cell proliferation, differentiation, and in extracellular matrix production [189]. A missense mutation has been associated with migraine headaches in 11 of 14 mutation carriers in a large pedigree [190].
- 5) Cox et al. [191] recently performed a pedigree-based GWA study of an isolated population of Norfolk Island with a high prevalence of migraine (25.5 %). They found an association with rs4807347 located in the finger protein 555 (*ZNF555*) gene which was replicated in an independent case–control sample albeit with opposite alleles, as well as association with SNPs located in the *ADARB2*, *GRM7*, and *HTR7* genes, mostly related to the serotonergic system. This association is apparently specific to this isolated population but might bring insights to future research.
- 6) A recent large meta-analysis of 29 population- and clinic-based samples including a total of 23,285 individuals with migraine and 95,425 matched controls identified 142 SNPs located in 12 different loci significantly associated with the phenotype, with 8 loci containing SNPs located in known transcripts [192]. Five loci were not previously associated with migraine (*AJAP1*, *TSPAN*, *FHL5*, *C7orf10*, and *MMP16*), although the *TSPAN* SNP is close

to a previously associated SNP located near *TSPAN-2* [145]. The remaining loci confirmed previous reports (*PRDM16*, *MED2D*, *TRPM8*, *TGFBR2*, *PHACTR1*, *ASTN2*, and *LRP1*). The most significant P values were observed in the *LRP1* locus ($P = 2.69 \times 10^{-19}$). Finally, an eQTL analysis performed on 394 brain tissue specimens revealed 5 additional loci potentially implicated in migraine susceptibility (*APOA1BP*, *FUT9*, *STAT6*, *ATPB5*, and *TBC1D7*). Results of this study analyzed by network analysis have been suggestive for a possible involvement of the identified gene into a common functional glutamate pathway [192].

Common variants identified by GWAS collectively contribute to migraine pathogenesis each exerting a small effect size, with ORs below 1.3 as typically occurs in complex disorders [192]. This contribution is detectable in common forms of polygenic migraine with multiple epistatic “gene \times gene” and “gene \times environment” interactions, whereas highly penetrant rare variants, such as those responsible for FHM, are able to produce sporadic and familial migraine without requiring contributions by common variants, which may at most modulate clinical features in monogenic forms. Despite the small effect size exerted by each single polymorphism, common variants unveiled by GWAS have proven very valuable by underscoring glutamatergic roles in migraine, likely underlying cortical spreading depression and the sensitization of nociceptive nerve endings, along with contributions by NGF and 5-HT signaling, as well as neuroimmune interactions.

Methodological issues in migraine genetics research

Genetic studies performed to date provide converging evidence supporting the existence of two broad migraine categories, caused either by (a) rare genetic variants with high penetrance, inherited, or de novo or by (b) a myriad of common variants, typically inherited, each providing small contributions to the overall disease risk, and collectively endowed with incomplete penetrance, hence requiring multiple gene-gene and gene-environment interactions as well as epigenetic influences in order to produce full-blown pathology. In simple terms, rare variants could be defined as “causal”, while common variants would be “predisposing”. These two categories do not overlap with current clinical categorizations, such as MA vs MO, although MA appears more frequently explained by rare causal variants compared to MO, which instead may more often fall into the latter category. Interestingly, several lines of evidence from twin and family studies already did support this prediction: for example, the presence of MA in the proband was found associated with a mean relative risk (RR) of 3.79 for MA in his/her first-degree relatives, as

compared to 1.02 for MO; conversely, a diagnosis of MO conferred a RR of 1.86 and 1.44 for MO and MA, respectively [193]. It was reassuring to see this prediction confirmed by recent GWAS studies, identifying several common variants for MO, but none for MA [192]. Unfortunately, both migraines due to rare and to common variants display great heterogeneity, although in different ways: migraine forms due to rare variants are highly heterogeneous, with each rare variant causing the disorder in few families worldwide; migraine forms due to common variants are also highly heterogeneous, with multiple different combinations of common variants enhancing migraine risk in different individuals and ethnic groups, in turn, exposed to only partly overlapping environmental factors.

Experimental design must match this level of complexity for studies to provide meaningful results. Rare variants can hardly be tackled using classical linkage by combining multiple pedigrees as rare variants are so heterogeneous, while there is much greater probability of success for studies involving single large pedigrees with high recurrence rates. In this regard, the possibility that rare variants responsible for migraine in some family members may yield other disease phenotypes, perhaps among those so often comorbid with migraine, in other family members should be considered [15, 16]. Next-generation sequencing (NGS) technologies foster rare variants discovery through unbiased approaches, including whole-exome sequencing (WES) and whole-genome sequencing (WGS), whereas array-CGH can detect copy number variants (CNVs), microdeletions and microduplications acting either as rare or as common variants. Surprisingly, to our knowledge, no array-CGH study of migraine has been published to this date. WES has already been used in many monogenic disorders and also to detect mutations in complex disorders, such as autism [194]. Limitations of this technique include not only its cost and incomplete exome coverage but also the possibility that causal variants in non-coding regions are missed. WGS overcomes this limitation, at the expense of huge data production [195]. Multifactorial forms of migraine, such as most common forms of MO, can be explored by case-control or family-based association, using either a candidate gene or an unbiased GWAS approach. Within the framework of the ongoing debate on the role and meaning of candidate gene studies in the GWAS era, it is important to acknowledge the relative strengths and weaknesses of these approaches. GWAS needs extremely large sample sizes to control for multiple testing, and this typically requires the collaborative merging of pre-existing samples recruited in different countries. Hence, GWAS is a powerful and unbiased tool able to detect relevant common variation, provided it is not ethnic-specific and it is endowed with a sizable effect as compared to other common variants. Since much common variation is indeed ethnic-specific both due to disease pathophysiology and to linkage

disequilibrium patterns, GWAS can discover the most consistent variants but by no means exhaust genetic contributions to common disorders. This can instead be sought also by using candidate gene approaches, which are clearly more at risk of false positives until at least some replication occurs and, more importantly, allelic variants are proven to exert functional roles. The efficacy of both candidate and unbiased association strategies can be further improved applying a qualitative diagnostic system and by implementing endophenotypes, familial, and heritable quantitative intermediate phenotypes associated with a complex disease and able to identify subgroups of patients possibly sharing a homogeneous pathophysiological basis [196, 197]. Biomarkers and endophenotypes applied in migraine research have been recently reviewed [198]. Additional endophenotypes may be represented by frequently comorbid disorders [15, 16], whereby the process leading to migraine may differ from non-comorbid forms [199]. A phenotyping approach involving endophenotypes, while requiring greater care in statistically controlling for multiple testing, has already yielded valuable results also in linkage analysis through LCA and TCA, as discussed above.

Finally, it is highly unlikely that genetic markers will be able to explain large portions of phenotypic variance in many common forms of migraine. A combination of epigenomic, transcriptomic, proteomic, and metabolomic biomarkers assessed in first-onset cases prior to starting chronic pharmacological treatment and followed longitudinally for migraine subtype characterization may provide, in combination with common genetic variants, greater amounts of clinically useful information [197]. To this date, few studies have identified biochemical biomarkers of potential clinical significance, such as the neuropeptide calcitonin gene-related peptide (CGRP) [198]. This path of investigation remains entirely to be explored. Additional areas where genetic investigations will undoubtedly play a major role, but complementary approaches will likely be necessary, include the underpinnings of treatment response, the sex bias observed in migraine, and the overlap between migraine and comorbid conditions.

Conclusions

Genetic studies have provided pivotal insights into the molecular mechanisms underlying migraine. Cellular and animal models of monogenic forms have helped in understanding the pathophysiology of migraine, which essentially leans upon three main domains, a “neural” domain (i.e., CSD-like neural activity patterns), a “vascular” domain, (intracranial vasospasm followed by excessive vasodilatation of intra and extracranial blood vessels), and a “nociceptive” domain (sensitization of perivascular trigeminal nociceptors involving inflammatory mediators, growth factors, and neuropeptides, such as CGRP) [4]. The relevance of these three

pathophysiological domains has received further support by the results of many candidate gene association studies and linkage studies, as well as more recently by GWAS. Hence, despite great genetic heterogeneity, also in migraine as in other complex neurological and psychiatric disorders, pathway analysis holds promise to converge upon a relatively limited number of functional pathways starting from mutations and polymorphisms located in many different loci.

The success of migraine genetic investigations will largely rely upon their capacity on one hand to apply the methodological approaches most apt to respond to each specific experimental question, on the other hand, on their capacity to integrate multiple levels of phenotypic, functional, and genetic information, in accordance with the complexity of the disorder itself. This should ultimately lead not only to the identification of novel genes associated with migraine but more importantly with a better definition of the pathophysiological mechanisms underlying specific forms of migraine in specific patients. New reliable and valid animal models will be especially useful in studying not only monogenic forms but also the cross-talk between genetic vulnerability and epigenetic contributions in common forms of migraine. Environmental factors, such as early and recent life events, hormones, and inflammation, can indeed act upon a genetically vulnerable background to trigger the onset and determine the progression of the disease [13]. This is likely mediated through modifications of the epigenetic profiles of migraine-relevant genes [14]. Animal models will be especially helpful, because epigenetics is cell- and tissue-specific; it may thus be especially challenging in humans to move from peripheral epigenetic biomarkers to relevant chromatin signatures located in cells directly involved in migraine pathogenesis. An example is medication overuse headache, which implies both genetic vulnerability to migraine and triptan-induced chromatin changes affecting the trigeminovascular system [200]. These animal models, combining genetic and epigenetic components, will be instrumental in leading to more effective pharmacological treatments for the most common forms of this debilitating disorder.

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