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From migraine genes to mechanisms

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Abstract

Migraine is a common multifactorial episodic brain disorder with strong genetic basis. Monogenic subtypes include rare familial hemiplegic migraine, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, familial advanced sleep-phase syndrome (FASPS), and retinal vasculopathy with cerebral leukodystrophy. Functional studies of disease-causing mutations in cellular and/or transgenic models revealed enhanced (glutamatergic) neurotransmission and abnormal vascular function as key migraine mechanisms. Common forms of migraine (both with and without an aura), instead, are thought to have a polygenic makeup. Genome-wide association studies have already identified over a dozen genes involved in neuronal and vascular mechanisms. Here, we review the current state of molecular genetic research in migraine, also with respect to functional and pathway analyses. We will also discuss how novel experimental approaches for the identification and functional characterization of migraine genes, such as next-generation sequencing, induced pluripotent stem cell, and optogenetic technologies will further our understanding of the molecular pathways involved in migraine pathogenesis.

Keywords: Monogenic, Transgenic mice, GWAS, Pain, Pathways

1. Introduction

1.1. Migraine is a genetic disease

Migraine is a common brain disorder that is typically characterized by attacks of severe, unilateral, throbbing headache associated with nausea, vomiting, photophobia, and phonophobia.[53] About 15% of the general population suffer from recurrent migraine attacks, women 3 times more often than men.[57,64] The presence of an aura distinguishes between migraine with and without an aura.[53] Population-based family and twin studies have shown that "the risk of also getting migraine" is increased when a first-degree relative has migraine and revealed that the heritability (ie, the proportion of the phenotypic variance explained by genetic factors) is approximately 50%.[85,102,116] A large Dutch twin study assessed that environmental factors have an almost equally large contribution.[69] In that respect, migraine seems to have a somewhat higher contribution of genetic compared with environmental factors than generally seen with chronic pain syndromes.[72,107]

This review highlights recent advances and possible future developments in the genetics and the unraveling of molecular mechanisms of migraine (see also recent review Ref. 41).

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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1.2. Migraine pathophysiology

Our basic understanding of the neurobiological mechanisms that underlie migraine auras and headaches has advanced greatly in the last decades (for reviews see Refs. 50 and 79; **Fig. 1**). The main findings are briefly described below as they provide essential background knowledge for a discussion on how genetic findings have contributed to the elucidation of aura and pain mechanisms in migraine.

1.2.1. Migraine aura

Up to one-third of patients have migraine attacks that are accompanied by an aura, which consists of transient neurologic symptoms including visual, sensory, motor, or speech disturbances.[53] Auras are likely caused by cortical spreading depression (CSD) events that are characterized by slowly propagating cortical waves of neuronal and glial depolarization that start in the visual cortex and are followed by long-lasting (~1 hour) depression of activity[65,89] (**Fig. 1**). Cortical spreading depression events are believed to originate in metabolically unimpaired tissue upon excessive release of potassium and glutamate into the extracellular space at a level that exceeds the buffering and removal capacity of the tissue.[89] Evidence for the importance of CSD in migraine almost exclusively comes from animal experiments.[23] Some indirect evidence in humans came from functional Magnetic Resonance Imaging (fMRI) showing characteristic blood flow changes[52] and magnetoencephalography (MEG) recordings showing DC-MEG shifts[13] revealing patterns of cortical spreading of signals, which are consistent with patient descriptions of the spread of their aura symptoms.

1.2.2. Headache mechanisms

The pain in migraine is believed to be caused by activation of the trigeminovascular system (TGVS)[73] (**Fig. 1**). The TGVS consists of meningeal and superficial cortical blood vessels that are innervated by sensory afferents from the trigeminal ganglion (TG), which

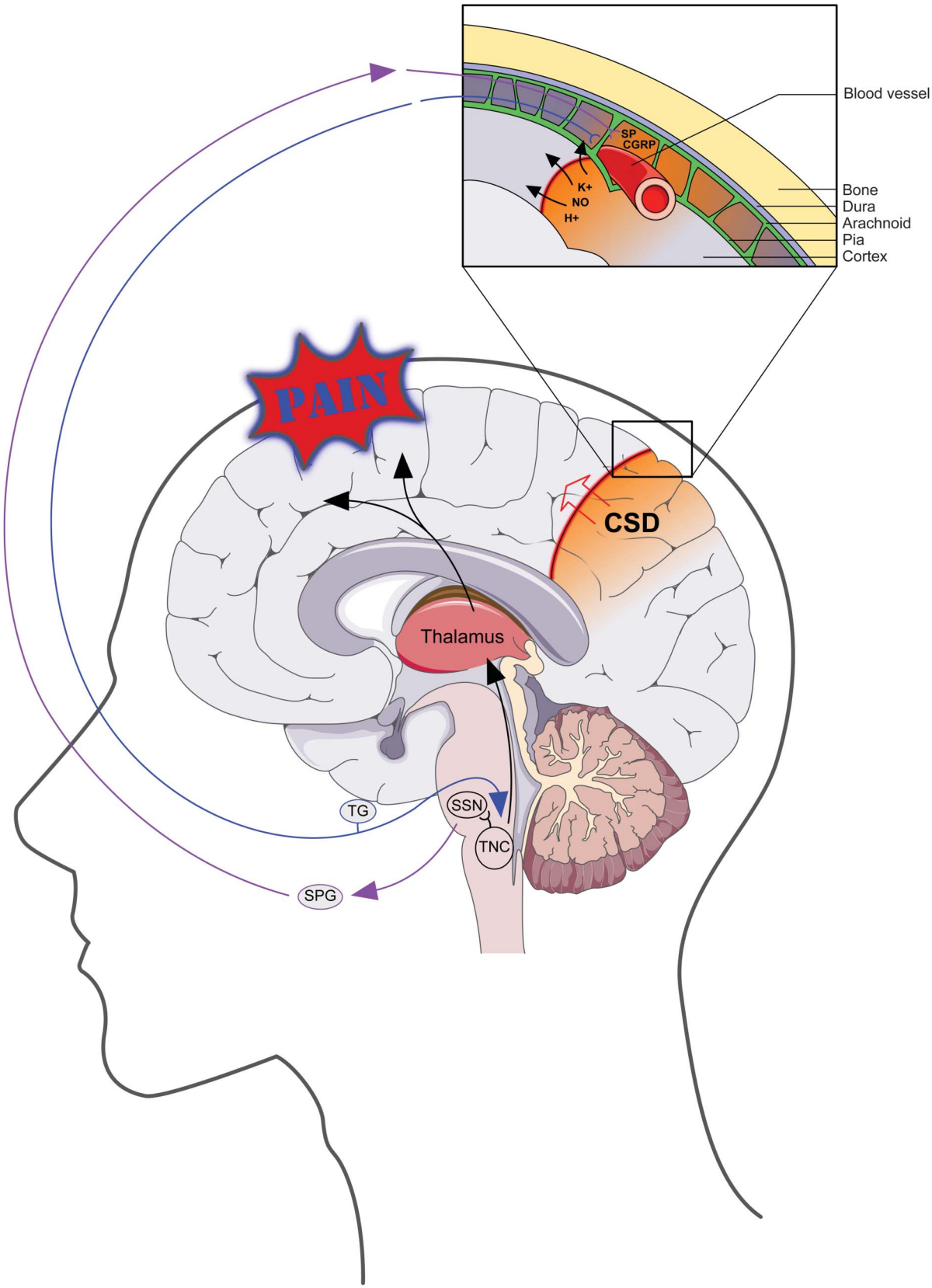

Figure 1. Schematic overview of trigeminovascular pathways and mechanisms underlying migraine aura and headache. Cortical spreading depression (CSD) is a slowly propagating wave of neuronal and glial depolarization that starts in the visual cortex and is accompanied by the release of potentially noxious molecules such as K^+, nitric oxide (NO), and H^+ into the extracellular space. These substances may reach pial, archanoid, and dural surfaces and activate the perivascular sensory afferents from the trigeminal ganglion (TG) neurons that release vasoactive inflammatory mediators such as calcitonin gene–related peptide (CGRP) and substance P (SP) (inset). Signals of activated meningeal nociceptors are relayed through TG nerve processes to trigeminal nucleus caudalis (TNC) neurons and further to thalamic and cortical areas to produce the sensation of pain. From the TNC, collaterals are also send to the parasympathetic superior salivatory nucleus (SSN) that innervates dural vessels through the sphenopalatine ganglion (SPG).

project to the brain stem trigeminal nucleus caudalis. Signals from activated nociceptors on blood vessels are transmitted to trigeminal nucleus caudalis neurons and further relayed to thalamic and cortical areas to produce the sensation of pain[11,50]. Sensitization of the TGVS can occur peripherally at the level of the TG on release of vasoactive mediators such as calcitonin gene–related peptide (CGRP) and substance P by trigeminal perivascular sensory nerve endings that lead to (sterile) neurogenic inflammation in the meninges.[67,68] In addition, or alternatively, activation of macrophages and other nonneuronal cells such as satellite glial cells may contribute to the local increase in vasoactive inflammatory mediators and the generation of headaches.[115]

Evidence for a direct link between an aura and pain mechanisms came from experimental animal studies that have shown release during CSD of various factors such as adenosine 5′-triphospate (ATP), glutamate, and potassium by neurons, glial, and vascular cells, and CGRP and substance P by activated sensory nerve endings, which can activate pain-relevant brain stem regions[12,67,112,113] (**Fig. 1**). It was recently shown that the opening of neuronal Pannexin-1 channels upon CSD can trigger an inflammatory cascade with glial cell responses and subsequent trigeminovascular activation.[60] The accumulating evidence that CSD events are important in migraine has fueled the notion that drugs preventing CSD may be effective in treating migraine attacks.[25,36] In fact, all currently available prophylactic agents, despite coming from different pharmacologic classes, seem to share 1 mechanism of action: blocking CSD.[10] Definite proof, however, that CSD initiates migraine pain mechanisms in patients is lacking. As only part of migraine patients experience auras, additional triggers for TGVS activation such as cortical hyperexcitability and/or brain stem and hypothalamic dysfunction are thought to play a role.[9,50,73,78]

2. Genetics of rare monogenic and common polygenic forms of migraine

2.1. Gene identification in monogenic familial hemiplegic migraine

The classical linkage approach (**Fig. 2**) to identify disease genes in migraine consists of testing several hundreds of polymorphic genetic markers, evenly spread over the genome, for cosegregation with disease in a family-based setting and identifying the causal gene mutation in the "shared genomic region." The approach has been most successful for familial hemiplegic migraine (FHM), a rare monogenic migraine with aura subtype that is characterized by a transient hemiparesis during the aura phase.[53] Three FHM genes have thus been identified; *CACNA1A* (FHM1),[75] *ATP1A2* (FHM2),[27] and *SCN1A* (FHM3)[30] that encode subunits of voltage-gated calcium channels, sodium–potassium ATPases, and voltage-gated sodium channels, respectively. All FHM gene products encode subunits of ion channels or transporters that play an important role in the tripartite synapse (**Fig. 3**), and therefore in neurotransmission.[103] Genotype–phenotype correlation studies revealed that mutation carriers may suffer from a wide variety of associated symptoms that include cerebellar ataxia, seizures, and even mild head trauma–induced edema that can be fatal.[28] Although sporadic hemiplegic migraine patients, ie, patients with no first- or second-degree family members suffering from hemiplegic migraine, have clinically identical attacks as familial cases,[98] only a few carry a mutation in one of the FHM genes.[29,45,94,97] Of the patients with an early age of onset and who exhibit additional neurologic symptoms such as ataxia, epilepsy, or intellectual inabilities,[81] ~75% carry a, in most cases de novo, mutation in *CACNA1A* or *ATP1A2*. Recently, truncating deletions in the *PRRT2* gene, which encodes a proline-rich transmembrane protein, were identified in a few patients with symptoms of migraine or hemiplegic migraine and as a result of which *PRRT2* was put forward as the fourth hemiplegic migraine gene.[82] However, as the same or very similar *PRRT2* deletions were also found in several hundred patients with paroxysmal kinesigenic dyskinesia, benign familial infantile convulsions, and infantile convulsion choreoathetosis, without signs of migraine, the relation between *PRRT2* and migraine seems far from straightforward.[76]

Identification of FHM genes has direct important clinical relevance as identification of the disease-causing mutation can reassure both clinicians and patients of a clinical diagnosis and provides the direct possibility for genetic testing in additional family members.

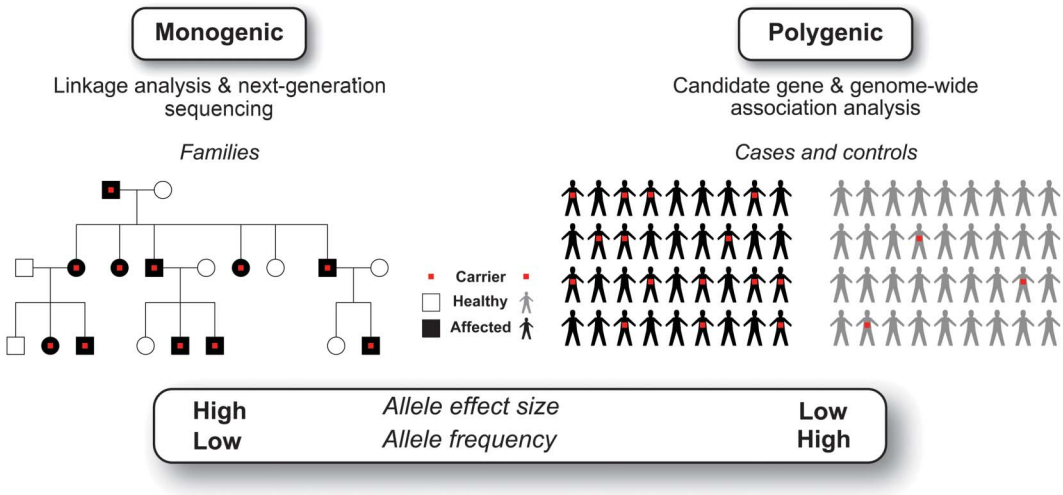

Figure 2. Different categories of genetic variation exist based on the frequency and effect size of the allele. Mendelian monogenic disorders are caused by rare alleles with high effect sizes. Until recently, the classical linkage approach (combined with Sanger sequencing to identify the causal mutation) was the method of choice for disease gene identification, whereas nowadays next-generation sequencing (exome and whole-genome sequencing) is used. For polygenic common forms of migraine, typically common variants with a low effect size are identified. Hypothesis-free genome-wide association studies (GWASs) have been successful in identifying such variants and have replaced the candidate gene association approach.

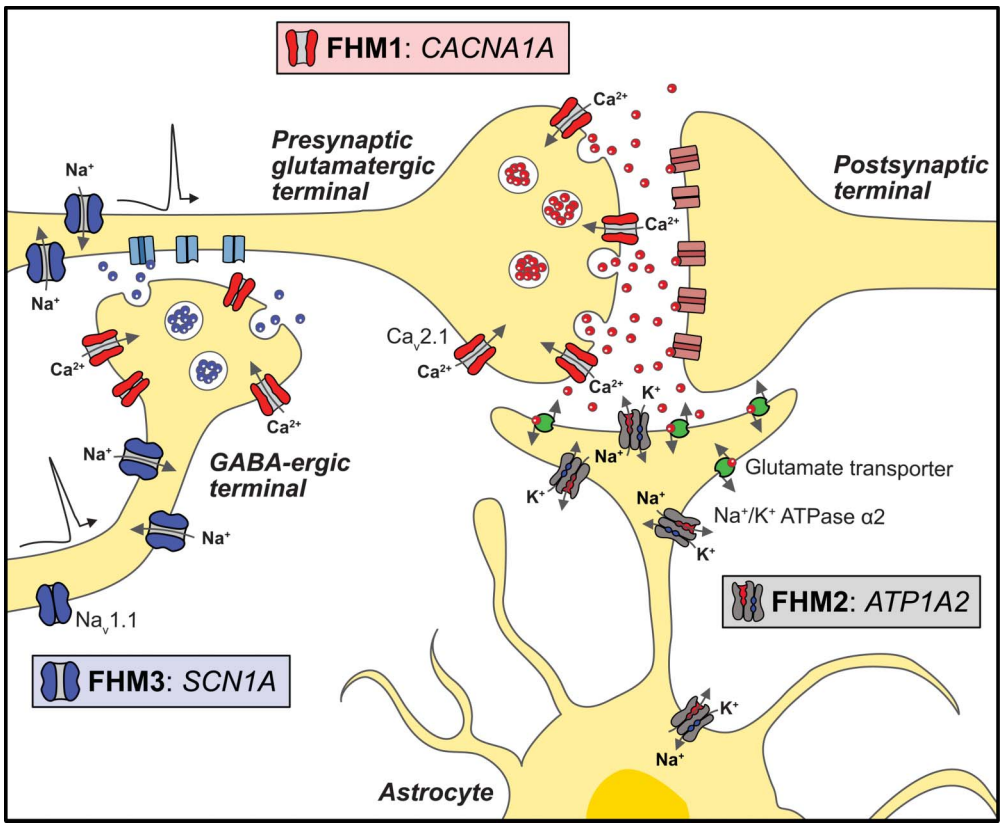

Figure 3. Synaptic function of proteins encoded by FHM1, FHM2, and FHM3 genes in a glutamatergic synapse. FHM1 mutations are located in the *CACNA1A* gene encoding the pore-forming α_{1A} subunit of $Ca_V2.1$ calcium channels (red), which are located in the presynaptic terminal of glutamatergic and GABAergic neurons. When an action potential reaches the presynaptic terminal, $Ca_V2.1$ channels open, allowing Ca^{2+} to enter, triggering vesicle fusion and glutamate release, resulting in subsequent activation of postsynaptic receptors and action potential generation. FHM1 mutations lead to a gain-of-function effect only for $Ca_V2.1$ channels on glutamatergic and not those on GABAergic terminals. FHM2 mutations are found in the *ATP1A2* gene encoding the α2-isoform of the Na^+/K^+-ATPase (gray). This subtype of Na^+/K^+-ATPase is present in the membrane of astrocytes and assists in removing excess K^+ and generating a Na^+ gradient required for uptake of glutamate from the synaptic cleft. FHM3 mutations are found in the *SCN1A* gene encoding $Na_V1.1$ voltage-gated sodium channels (blue) located on inhibitory interneurons. These channels serve to initiate and propagate action potentials. Gain-of-function mutations in $Ca_V2.1$ (FHM1) and loss-of-function mutations in the Na^+/K^+-ATPase (FHM2) and $Na_V1.1$ (FHM3) will each generate a net increase of general excitability.

2.2. Gene identification in other monogenic migraine disorders

Other rare monogenic disorders in which migraine is prevalent may provide useful additional insight in pathophysiologic mechanisms involved in migraine. The clearest example is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is caused by mutations in the *NOTCH3* gene that plays an important role in vascular smooth muscle cells of small blood vessels of the brain.[58] More than one-third of patients with CADASIL have migraine with aura,[31] supporting involvement of a vascular component in migraine pathophysiology. Specific mutations in the *COL4A1* gene cause a vascular disorder with hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy (HIHRATL) with migraine as a prevalent symptom, thus pointing in the same direction.[14,90] A third example is retinal vasculopathy with cerebral leukodystrophy (RVCL), which is caused by truncating mutations in the carboxyl terminus of TREX1, a 3′-5′ exonuclease that is encoded by the *TREX1* gene.[83] Especially, in a large Dutch family, migraine is particularly prevalent in patients with retinal vasculopathy with cerebral leukodystrophy.[95] Finally, all 8 patients, from 2 families who were diagnosed with familial advanced sleep phase syndrome (FASPS) and had a causal missense mutation in the casein kinase 1δ (*CSNK1D*) gene, also suffered from migraine with aura.[15] It was proposed that the mutant gene product CK1D, a known regulator of circadian rhythms, may cause vascular dysfunction through abnormal astrocytic signaling in conjunction with a CSD. Notably, transgenic mice in which a CADASIL or a FASPS mutation was overexpressed showed a reduced threshold for CSD.[15,38] Exactly how mutations in *NOTCH3*, *COL4A1*, *TREX1*, and *CSNK1D* cause vascular phenotypes that might explain the occurrence of migraine in mutation carriers is not yet known.

There is 1 example of a gene identification in seemingly monogenic pure familial migraine. Lafrenière et al.[62] selected *KCNK18*, which encodes the TRESK protein, as their candidate gene for targeted sequencing because it is involved in controlling neuronal excitability and identified a truncating nonfunctional F139WfsX24 mutation that could explain all migraine cases in a multigenerational family with migraine with aura. However, as several rare TRESK variants, including a variant that showed complete loss of function, were also observed in control individuals, it may turn out that *KCNK18* should instead be regarded a genetic modifier of a migraine phenotype and not the direct cause[5] also because no further causal evidence for its involvement came from testing over 600 additional migraine families.[62]

2.3. Candidate gene association studies in common polygenic migraine

A frequently used approach to identify genetic factors for migraine has been the study of DNA variants in candidate genes, which were selected on prior knowledge of migraine pathophysiology (**Fig. 2**). Evidence for causality comes from finding a statistical difference in allele frequency of such a DNA variant when comparing cases and controls. Multiple of these variants, each with a small effect size, combined are thought to confer disease risk. Unfortunately, virtually all migraine studies suffered from small sample sizes, insufficient correction for multiple testing, and most of the studies lacked replication of the association finding in independent populations. Hence, none of the studies provided convincing robust genetic association that survives until today. This is true even for the most promising example, the C677T polymorphism in *MTHFR*, that showed statistical evidence for association of the T-allele with migraine with aura in some studies[84,86] but not in 2 other larger studies.[61,99] Candidate gene association studies also failed to provide genetic evidence for the involvement of FHM genes in common polygenic migraine despite the fact that clinical characteristics between hemiplegic migraine and common forms of migraine overlap.[96] The largest study screened several thousand genetic variants in over 150 ion transport genes in several thousand migraineurs, but the result was negative.[74] This negative outcome may seem paradoxical, at least in light of the (presumed) efficacy in epileptic and migraine patients of antiepileptic drugs acting on neurotransmitter and ion pathways.[70] A possible explanation might be that disease risk in common forms of migraine is conferred by other genes that perhaps exert more subtle regulatory effects on neurotransmitter and ion pathways than those that were found to cause the monogenic forms of the disease.

2.4. Genome-wide association studies in common polygenic migraine

The last few years, genome-wide association studies (GWASs) have become the standard approach to identify genetic factors underlying complex disorders (**Fig. 2**). In GWASs, hundreds of thousands of single-nucleotide polymorphisms (SNPs) that are distributed over the genome are tested for association with a (disease) trait in a hypothesis-free manner. The allele frequency distribution between several thousand cases and (properly matched) controls is compared for each SNP. To sufficiently correct for multiple testing, only P values below 5×10^{-8} are considered genome-wide significant. The findings of GWASs are generally speaking statistically very robust, also because initial association findings need to be confirmed in independent case and control cohorts within the same study. Several GWASs have been performed for migraine. Two studies investigated European clinic-based patients, ie, patients who seek medical attention in specialized headache clinics, diagnosed with migraine with aura[6] or migraine without aura.[44] These studies identified 1 locus (DNA variant) for migraine with aura that pinpoints the *MTDH* gene and an additional 6 loci for migraine without aura with *MEF2D*, *TGFBR2*, *PHACTR1*, *ASTN*, *TRPM8*, and *LRP1* as the likely susceptibility genes. Combining findings of a large population-based GWASs[24] and a subsequent meta-analysis of 29 cohorts[7] yielded 13 migraine susceptibility loci. The genes assigned to these loci seem to mainly affect neuronal pathways (in case of *MTDH*, *LRP1*, *PRDM16*, *MEF2D*, *ASTN2*, *PHACTR1*, *FHL5*, *MMP16*), metalloproteinases (in case of *MMP16*, *TSPAN2*, *AJAP1*), and vascular pathways (in case of *PHACTR1*, *TGFBR2*, *C7orf10*) (**Fig. 4**).

The genetic associations that have been detected by migraine GWASs are statistically very robust (with successful replication in

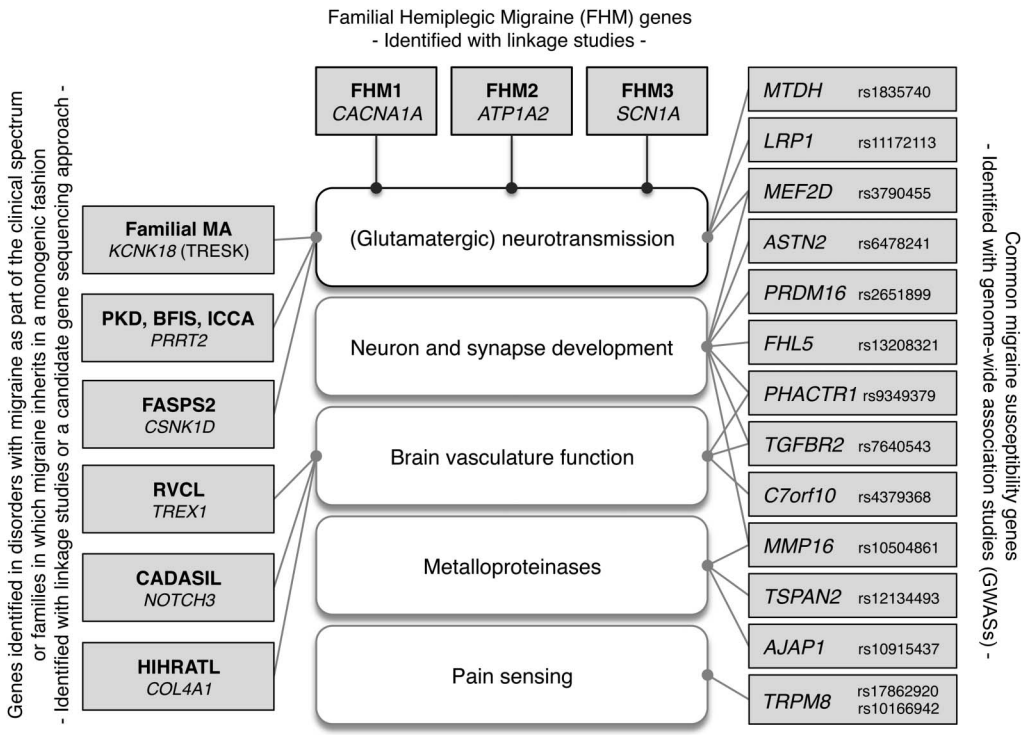

Figure 4. Genes and pathways involved in familial hemiplegic migraine (FHM), common forms of migraine, and other disorders in which migraine is prominent. Arrows connect genes to the presumed function of that gene.

many independent cohorts). Several of the GWAS hits have already been replicated in small follow-up studies,[4,39,40,49,80] although not in all,[88] that investigated various ethnic groups around the world. Without exception, GWAS hits are associated with small (0.8-1.2) odds ratios, which make disease risk ascertainment based on the presence of associated variants for clinical practice impractical, or even impossible. Moreover, the fact that GWAS hits are generally not in coding regions, but more often located outside known gene boundaries, hampers a straightforward analysis of their functional consequences. Ultimately though, such future analyses are expected to impact patients as they can potentially highlight novel migraine mechanisms that are useful for the development of better migraine therapies.

3. Functional studies of gene mutations in monogenic FHM

3.1. Functional characterization of FHM mutations in cellular models

The large effect sizes of gene mutations in monogenic FHM make an investigation of their functional consequences in cellular and transgenic animal models feasible. Most cellular studies showed that FHM1 mutations exert gain-of-function effects by shifting the voltage-dependence of neuronal $Ca_V2.1$ channels toward more negative membrane potentials while enhancing the open probability of a channel.[77] Few studies instead report loss-of-function effects.[17,92] Inhibitory G-protein modulation of FHM1-mutated $Ca_V2.1$ channels was found reduced for all 6 mutations studied predicting a prolonged opening of the channels, thus favoring an overall gain-of-function effect of FHM1 mutations.[47,48,87,109] A loss of glial Na^+/K^+ ATPase function seems the most likely mechanism for FHM2 mutations.[93] Finally, a loss-of-function effect for $Na_V1.1$ sodium channels has been reported for several FHM3 mutations and was thought to impact primarily inhibitory neurons. A gain-of-function effect on excitatory neurons was proposed for FHM3 mutation L263V that was found associated, at least in 1 family, with a combined seizure FHM phenotype in most mutation carriers.[18,59] This view, however, is challenged by recent observations that a neuronal gain-of-function effect of FHM3 mutations involves self-limited hyperexcitability, as a possible protection against epileptic activity.[20] Notably, FHM3 mutations that seemed to exert a loss-of-function phenotype when studied in a nonneuronal cell type may, in fact, act as gain-of-function mutations in GABAergic neurons.[21] Future studies using knock-in (KI) mouse models with human pathogenic FHM3 mutations need to reveal how mutations cause disease when expressed in their natural cellular environment. All in all, the cellular studies of FHM mutations predict increased neurotransmitter and potassium ion levels in the synaptic cleft, especially after high-intensity neuronal firing, which would facilitate CSD and thereby could explain the devastating migraine aura symptoms of patients with FHM.

3.2. Functional characterization of FHM mutations in transgenic mouse models

In vivo consequences of FHM mutations at the organism level have been investigated using transgenic KI mouse models in which human pathogenic mutations were introduced using a gene-targeting approach in either the endogenous *Cacna1a* or *Atp1a2* gene.[66,104,105] The 2 FHM1 KI mouse models express gain-of-function missense mutations R192Q or S218L in the *Cacna1a* gene.[104,105] In line with the severe clinical phenotype in patients with the S218L mutation,[91] only S218L mice exhibit the complex phenotype of cerebellar ataxia, susceptibility to seizures, and delayed cerebral edema after minor head trauma.[46,105] Mice with the milder R192Q mutation show no overt phenotype.[104] The FHM2 KI mouse model expresses the loss-of-function W887R missense mutation in *Atp1a2*.[66] Only the heterozygous mice are viable as homozygous FHM2 mutant mice die at birth, identical to the fate of homozygous *Atp1a2* knockout mice.[56] No FHM3 KI mouse model has been generated yet.

3.2.1. FHM1 mice: abnormal neurotransmission and increased susceptibility to cortical spreading depression

At the neurobiological level, mice of both the R192Q and the S218L mutant strains revealed increased neuronal calcium influx and (cortical) excitatory, but not inhibitory, neurotransmitter release and an increased susceptibility to CSD.[34,35,37,100,104–106] When comparing both strains, features were more prominent in the severe S218L mutant mice and/or more pronounced in female mutant mice (in line with the female preponderance in migraineurs).

A study of calyx of Held brain stem neurons suggests that the specific $Ca_V2.1$ channel characteristics of the severe S218L mutation result in an increased basal intracellular Ca^{2+} concentration.[32] Investigating cerebellar neurons of these mice supports the concept that the channels are in a basally facilitated state,[2] which would fit with the severe phenotype in mice and, by extrapolation, patients with the S218L mutation. However, additional studies in FHM1 mice of various types of neurons suggest that characteristics of an FHM1 mutation may be specific to the neuron type[42,55] and not easily extrapolated to other preparations. Regardless, it is tempting to speculate that relatively minor disturbances in the brains of FHM1 mice (and patients) bring about the observed phenotypes.

3.2.2. Abnormal behavioral pain responses and a molecular pain phenotype in FHM1 mice

Behavioral analyses in FHM1 mutant mice revealed evidence for signs of *spontaneous* unilateral head pain indicative of trigeminal pain that included excessive head grooming with excess oculotemporal strokes on 1 side of the body and increased blink rates with 1 eye closed. Notably, total grooming and closure of both eyes during blinking did not show a genotypic difference. Some of the behaviors were more frequent and pronounced in the severe FHM1 S218L strain.[22] The abnormal behavior was normalized by administrating morphine or serotonergic antimigraine drugs and seemed novelty-induced and/or restraint stress–induced as the abnormal behavior was decreased or absent on retesting the mice.[22] Signs of spontaneous head pain in FHM1 mice were also observed using the mouse grimace scale that objectively measures abnormal facial pain expression in mice.[63] Also, in that paradigm, antimigraine drugs seem effective.

Evidence for a photophobia phenotype in FHM1 mice came from the observation that mutant mice tended to avoid the brightly illuminated "safe" closed arms in a modified version of the elevated plus maze without showing signs of anxiety in the normal version of the maze.[22] Testing mutant mice of the milder R192Q strain for a "battery" of standard algesiometric assays showed essentially unchanged sensitivity to various exogenously applied noxious (thermal, mechanical, and chemical) stimuli.[22]

Molecular and cellular studies of trigeminal ganglia from FHM1 mice revealed a molecular pain phenotype, namely an abnormally

increased ATP-gated purinergic P2X3 receptor activity in the TG that is brought about by an altered phosphorylation state of intracellular protein domains.[71] In fact, enhanced neuronal Ca^{2+} influx by FHM1 mutations seems to result in constitutively upregulated P2X3 receptors. Mutant TG sensory neurons exhibit an increased release of soluble mediators such as CGRP, BDNF, and TNF-α, already at baseline, so potentiation of P2X3 receptors by, for instance, exogenous CGRP or TNF-α is no longer possible.[54] The combination of these features seems to contribute to a constitutive local inflammatory phenotype that is seen in TG of FHM1 mice with abnormal cytokine and chemokine profiles and the presence of activated macrophages.[43] Coculturing of TG neurons and satellite glial cells revealed that increased baseline CGRP release, in addition to the potentiation of neuronal P2X3 receptors, also causes potentiation of glial P2Y receptor function with subsequent glial activation.[19] The proinflammatory state of mutant TGs with constitutively active purinergic receptors in neurons and glial cells may facilitate transduction of pain signals. Such a state is in line with evidence of migraine-relevant neuroinflammation in the meninges with inflammatory cells releasing pain mediators such as TNF-α that are relevant to chronic pain.[114] It is tempting to speculate that the complex basal inflammation phenotype in TG of FHM1 mice might transform acute trigeminal pain, coming from pain signals initiated by a pannexin-activated inflammatory cascade on CSD,[60] into long-lasting headaches. If true also at the in vivo level, it would validate the FHM1 mouse model as a highly relevant animal model for screening migraine-relevant drugs, including drugs that counteract the action of CGRP and inflammation.

4. Future directions in genetic migraine research

This section highlights some of the novel methodologies that can be used to identify migraine genes and to further understand their functional consequences and the molecular pathways that they are involved in.

4.1. From genome-wide association study hits to causal variants and pathways

Major hurdles still need to be taken before the molecular pathways are unraveled that link to the associated SNP variants, ie, GWAS hits. One major hurdle is that most GWAS hits lie in intronic or intergenic regions and therefore are more likely influencing (in a subtle manner) gene regulation instead of directly affecting protein function (as is the case with many monogenic mutations). A second even bigger hurdle is that GWAS hits consist of SNPs that only "tag" the disease locus implying that the identified associated SNP is not the disease-causing variant but in fact is in linkage disequilibrium with the disease-causing variant. Fine-scale mapping of a locus is needed to identify the truly functional variant(s), ie, those with the greatest effect sizes and lowest *P* values, underlying observed GWAS signals. This would require massive research efforts of large-scale targeted sequencing of the locus to ensure that all variants are captured, although this can to large extent be overcome by imputation with 1000 Genomes Project data,[1] and subsequent genotyping of rare variants (using specifically designed chip arrays) in very large accurately phenotyped cohorts. Although being tried for some diseases, such an approach is regarded unpractical at the moment.[33] More feasible are pathway-based analyses that

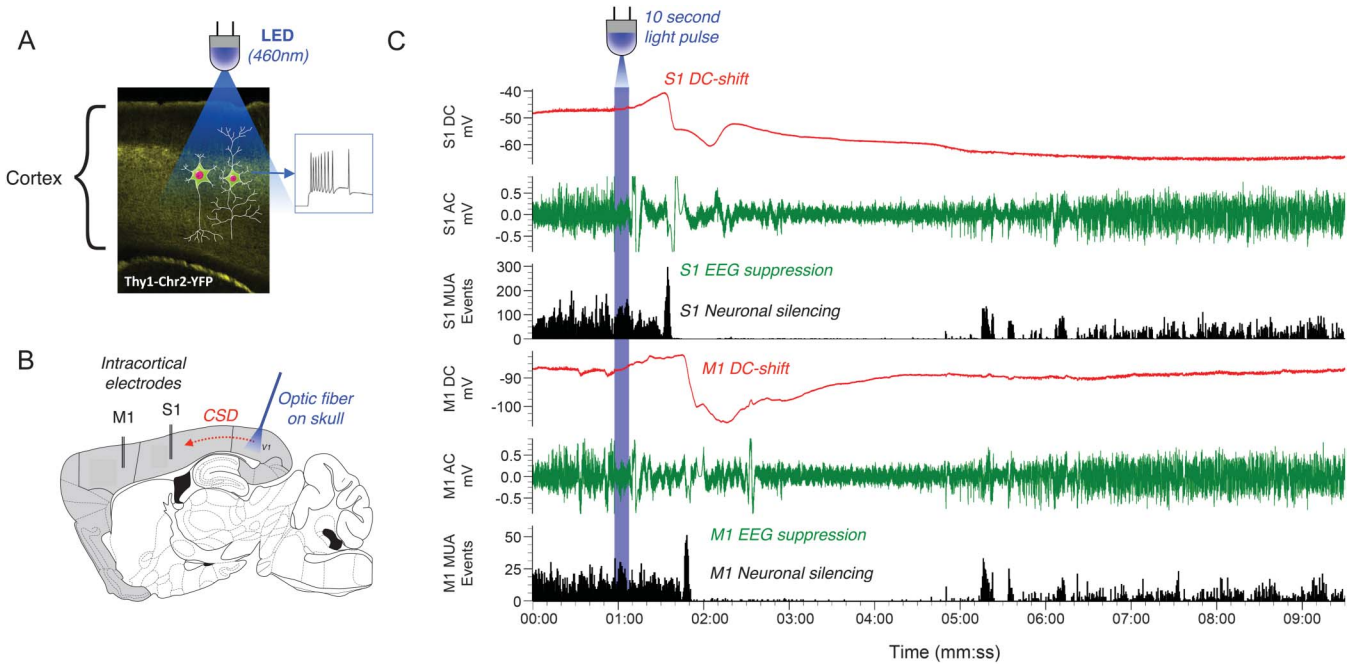

Figure 5. Optogenetic induction of cortical spreading depression (CSD) in freely behaving mice. (A) Thy1-Chr2-YFP transgenic mice express the light-sensitive cation-channel channelrhodopsin-2 (ChR2) in cortical neurons.[8] The fused yellow-fluorescent protein (YFP) causes yellow fluorescence and is used as a marker for ChR2 expression. Illumination with blue light (460 nm) opens ChR2 channels, thereby depolarizing and exciting the neurons, causing action potential firing. (B) Under isoflurane anesthesia (2%), 2 pairs of intracortical electrodes were implanted in the somatosensory (S1) and motor (M1) cortex. An optic fiber (400-μm diameter) that terminated on the skull bone overlaying the visual cortex (V1) was attached to the skull together with the intracortical electrodes using dental cement. (C) After a 7-day recovery period, animals were connected to an electrophysiologic recording setup that allowed for multiple day recordings of DC-EEG (DC; 0-500 Hz), AC-EEG (AC; 0.1-500 Hz), and multiunit activity (MUA; spikes per seconds in the 500-5000 Hz frequency band). (A) Ten-second illumination of the visual cortex with blue light induces a CSD that is visible on the S1 and M1 electrodes as a transient DC shift, coinciding with suppression of EEG amplitude and peak MUA followed by temporary neuronal silencing as evident from suppressed MUA. The CSD wave first reached the S1 electrode before reaching the M1 electrode.

examine the GWAS signals for a group of genes that are involved in the same biological process, which can be used to prioritize genes from regions identified by GWASs or to explore pathways affected by multiple GWAS hits.[108] Finally, mining databases, eg, gene expression databases, data from the ENCODE and the NIH Roadmap Epigenomics Roadmap projects, may yield relevant functional information on GWAS hits. A final major hurdle is to understand the biology behind GWAS hits by investigating the function of identified SNPs and associated genes for their effects on disease-relevant pathways by modulating their expression in cellular and/or animal (eg, zebra fish or mouse) models. Novel technological possibilities that can be exploited for the development of more sophisticated cellular models now include the use of (neuronally differentiated) human-induced pluripotent stem cells, as was proposed for schizophrenia[16] and successfully tested for age-related macular degeneration.[110] Novel genome-editing technologies that make use of transcription activator–like effector nucleases (TALEN) or clustered regularly interspaced short palindromic repeats that rely on RNA-guided DNA endonuclease Cas9 (CRISP/Cas) in mammalian cells will surely be exploited for functional studies based on GWAS findings to generate cellular[51] and animal models[111] in a more efficient manner.

4.2. Next-generation sequencing in gene identification

Recent breakthroughs in massive parallel DNA sequencing, ie, next-generation sequencing (NGS), allow for cost-efficient sequencing of all protein-coding regions (exome sequencing) or even the complete genome (whole-genome sequencing) in a single experiment. The NGS will aid the identification of genetic factors in monogenic migraine disorders, such as in FHM families in which no mutations have been identified yet. Sophisticated procedures need to be applied for data pooling, bioinformatic filtering, and variant prioritization methods to uncover causal mutations among the vast amount of DNA variants, given the fact that phenocopies and reduced penetrance are not uncommon. An even bigger challenge will be to successfully apply NGS to complex disorders and identify causal gene variants with a moderate effect size, ie, larger than those of GWA studies but smaller than those in monogenic diseases.

4.3. Functional evaluation of migraine pathophysiology by optogenetics technology

To gain more detailed mechanistic insight in the neuron-type or brain region–specific contribution to migraine pathophysiology, the advent of optogenetic technology[101] seems particularly instrumental. Optogenetics allows for noninvasive stimulation of brain cells in brain areas of interest with unprecedented advantage over conventional stimulation methods.[3] In the field of pain, optogenetic tools have been applied for remote activation of nociceptive pathways by optically controlling nociception and central sensitization in freely behaving mice.[26] In that study, transgenic mice with conditional expression of light-sensitive channelrhodopsin-2 (ChR2) channels in $Na_V1.8$-positive sensory neurons showed robust nociceptive behavior in reaction to blue light illumination (which acts on ChR2-containing neurons and depolarizes them) on the skin that caused remote stimulation of peptidergic and nonpeptidergic nociceptive fibers. In the field of migraine research, optogenetic technology will likely also have an important impact. Using transgenic animals in which ChR2 channel expression was driven by the neuronal Thy1 promoter, which allows for wide expression in the brain including cortex,[8] it was possible to noninvasively trigger CSD events in freely behaving mice just by shining blue light through the intact skull while recording changes in DC-EEG and neuronal multiunit activity signals that acted as witnesses of the CSD (**Fig. 5**).

5. Conclusions

Genetic research of the last 2 decades has led to the identification of various migraine genes. Gene discoveries in monogenetic migraine types, among which FHM and several disorders in which migraine is a prominent part of the phenotype, highlighted the importance of ion channels and transporters, abnormal neurotransmission, and vascular dysfunction in migraine pathophysiology. The GWAS methodology made possible the first genetic breakthroughs in the common forms of migraine with over a dozen DNA variants also hinting toward neuronal and vascular pathways. Still, knowledge of migraine mechanisms that is based on genetic discoveries mainly comes from investigating transgenic mouse models with pathogenic human mutations from monogenic disorders. However, novel technologies are emerging that may help harvesting from recent genetic discoveries in GWASs, which is needed to have a more complete understanding of the disease mechanisms in migraine.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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