Human Studies in the Pathophysiology of Migraine: Genetics and Functional Neuroimaging
F. Michael Cutrer, MD; Jonathan H. Smith, MD

The expansion of technologies available for the study of migraine pathophysiology has evolved greatly over the last 15 years. Two areas of rapid progress are investigations focusing on the genetics of migraine and others utilizing novel functional neuroimaging techniques. Genetic studies are increasingly focusing on sporadic migraine and the utilization of unbiased searches of the human genome to identify novel variants associated with disease susceptibility. At the same time, neuroimaging studies have provided novel insights into the altered neuronal and network dynamics of the migrainous brain. These 2 parallel approaches provide complementary insights into the complexity and heterogeneity of migraine.

Key words: migraine pathophysiology, neuroimaging, genetics in migraine

The study of the pathophysiology of migraine has greatly evolved over the last 15 years. The rapid evolution of investigative techniques that allow us to study migraine with minimal invasiveness in human subjects promises to open new vistas in migraine research. Two areas of rapid progress are investigations focusing on the genetics of migraine and others utilizing novel functional neuroimaging techniques. This review will emphasize these 2 exciting research domains.

GENETICS IN MIGRAINE PATHOPHYSIOLOGY

Early Studies
There is a large body of evidence indicating that migraine is a heritable disorder. Large population-based studies have shown that the risk of migraine in relatives of migraineurs is 3 times that of relatives of non-migraine control subjects. Data from large national twin registries have consistently revealed higher rates of concordance among monozygotic vs dizygotic twins. Two population-based studies have suggested that genetic influences are stronger in migraine with aura (MWA) than in migraine without aura (MWoA). However, subsequent studies have identified candidate gene variants in both MWA and MWoA. Segregation analysis in the common forms of migraine carried out a number of years ago did not identify any single Mendelian pattern, although incomplete penetrance and variable expressivity may to some extent confound such an analysis.

Studying the genetics of migraine is complicated by the fact that it is a complex trait with 2 large subtypes. Both common subtypes, MWA and MWoA share the repeated occurrence of moderate to severe headaches with characteristic features including duration of more than 4 hours, associated light/sound sensitivity, and nausea with or without vomiting. However patients who have MWA also have attacks that include transient episodes of focal neurological dysfunction. Most commonly, the migraine aura consists of 5 to 60 minutes of a characteristic bimodal visual disturbance of shimmering or flashing lights followed by loss of vision in a similar portion of the visual field in both eyes. Less commonly, MWA patients experience language disturbance, unilateral weakness, or episodes of unilateral paresthesias, followed by numbness. At this point in time, it is not clear whether the susceptibility genes for MWA and MWoA are the same or are distinct from each other. Studies of the clinical characteristics of MWA and MWoA report both distinct and shared characteristics, including a similar response to currently available treatment. One study of 210 families with 4 or more migraine-affected members found that 3.2% had experienced aura without headache, 11.1% MWA only, 40.6% had both MWA and MWoA, 23.5% MWoA only, and 20.3% aura-like symptoms that did not meet International Headache Society (IHS) criteria. It is also a common experience in patients with MWA to experience attacks solely with aura during adolescence and into the twenties only to increasingly experience attacks without aura as well as attacks with aura as they move into their thirties and forties. So the categories are not fixed, and in many instances rather changeable. The considerable co-occurrence of MWA and MWoA suggests probable overlap of the 2 syndromes. However, a Danish twin registry study found that the observed numbers of twins with co-occurrence of MWA and MWoA did not differ from what would be expected from chance based on the prevalence in the general population, suggesting that the 2 disorders are genetically distinct and sorted separately. On the other hand, relatively recent studies have reported that loci for both MWA and MWoA coincide in a small area on 4q, suggesting that alteration in a single gene can result in both MWA and MWoA or that the 2 genes are located so near to each other that they may be sorted together. It is also conceivable that certain genes confer susceptibility to the headache only, others to the aura only, and still others predispose to both. Therefore, even with reference...
to this very basic issue, the genetics of migraine is likely to be complex and heterogeneous.

**Familial Hemiplegic Migraine (FHM)**

There have been many linkage studies in migraine. Some of the earliest and most prominent linkage studies have identified gene variants that play a role in the very rare FHM subtype, which is characterized by unilateral headaches, typical aura, and unilateral motor weakness.\(^4\) Mutations in the P/Q type voltage-gated calcium channel gene CACNA1A located at 19p13.1 have been linked with FHM1.\(^{35}\) Twenty-one different missense mutations in this gene have been identified to cause FHM1 thus far.\(^6\) A second FHM locus has been mapped to chromosome 1q21-q23 and over 30 mutations in a gene coding for a subunit of a sodium/potassium pump (ATP1A2) identified.\(^{16,17}\) A third FHM locus has been identified on chromosome 2q24 in a gene (SCN1A) that encodes a neuronal voltage-gated sodium channel subunit.\(^18\) The SCN1A gene has 5 known mutations linked to FHM3.\(^{19-21}\) The fourth and most recent locus linked to FHM resides on chromosome 14 at 14q32.\(^22\) The FHM4 locus was identified in a Spanish pedigree with family members having FHM, MWA, and MWoA. All affected family members, regardless of the migraine type, shared a common 4.15 Mb haplotype, once again suggesting that at least in some families, a single gene alteration may be associated with varied aura status. Analysis of the 3 major candidate genes in the 14q32 region, Solute carrier family 24 member 4 (SLC24A4), ataxin 3 (ATXN3), and inositol-tetrakisphosphate 1-kinase (ITPK1) did not show disease-causing mutations in the affected subjects. Thus far, other attempts to show a connection between FHM genes and MWA or MWoA have not been successful, suggesting a complex and probably heterogeneous genetic basis for the common forms of migraine.\(^23\)

**Candidate Gene Approaches**

Case control studies have shown modest associations with migraine for several candidate genes, but their findings have in many cases not been replicated independently\(^23\) (Table 1).

**Genome-Wide Association Studies (GWAS)**

In contrast to the candidate gene studies carried out since the mid-1990s, the last few years have seen the rise of genetic studies, which assume that polygenic variability with a small effect are of importance in human disease. This approach underlies GWAS, in which large numbers of patients with a given trait such as migraine are assessed by comparing 300,000 to 1,000,000 single nucleotide polymorphisms (SNPs) sampled across their entire genomes to comparable SNPs in unrelated controls subjects who do not have the trait. GWAS has an important advantage. It makes no assumption as to where important variation may exist and increases the likelihood that previously unsuspected variants will be discovered. Early on, there were important discoveries using GWAS in macular degeneration and in Parkinson’s disease.\(^51,52\) Unfortunately, the findings from GWAS studies are sometimes not replicated in different populations, and GWAS studies are very expensive because to detect differences, thousands of case and control subjects must be genotyped in initial and replication phases. Because the case and control subjects in a GWAS are unrelated, there is tremendous genetic variation, and the statistical rigor required to be sure that the differences in the genome are actually related to the trait is so high that only the most common and strongest genetic differences are likely to be considered significant. GWAS is an important technique and is very powerful when there are a relatively small number of variants related to the trait.

Thus far, there have been 3 large GWAS focusing on the common forms of migraine (Table 2). The first of these studies was published in 2010 and reported that the minor allele of the SNP, reference SNP number (rs)1835740 on chromosome 8q22.1, was associated with migraine (\(P = 5.38 \times 10^{-5}\), odds ratio [OR] = 1.23, 95% confidence interval [CI] 1.150-1.324).\(^53\) The finding was based on genotyping of 550,000 SNPs in 2731 migraine cases ascertained from 3 European headache clinics and 10,747 population-matched controls. The association was then replicated in 3202 cases and 40,062 additional population controls for an overall meta-analysis \(P\) value of 1.69 \(\times 10^{-11}\) (OR = 1.18, 95% CI 1.127-1.244). The identified SNP, rs1835740, was found to be located between metadherin (MTDH) (astrocyte elevated gene 1) and encoding plasma glutamate carboxypeptidase (PGCP), both of which are involved in glutamate homeostasis.\(^54,56\) In an expression quantitative trait study in lymphoblastoid cell lines, transcript levels of the MTDH were found to have a significant correlation to rs1835740 (\(P = 3.96 \times 10^{-5}\), permuted threshold for genome-wide significance 7.7 \(\times 10^{-8}\)), suggesting that the SNP might lie in a region involved in the regulation of one or both of these genes. The authors suggest that alteration in the regulation of glutamate, a neurotransmitter long implicated in migraine pathophysiology might explain its association with the disorder.

The second GWAS in migraine was carried out in 5122 female migraineurs compared with 18,108 women not reporting migraine drawn from subjects already genotyped in the Women’s Genome Health Study (WGH5), a large population-based cohort of initially healthy American women.\(^57\) Chasman and colleagues found that the SNP rs2651899 (1p36.32, PR domain containing 16[PRDM16]), rs10166942 (2q37.1, transient receptor potential cation channel, subfamily M, member 8 [TRPM8]), and rs11172113 (12q13.3, low-density lipoprotein receptor-related protein 1 [LRP1]) were associated (\(P < 5 \times 10^{-6}\)) with migraine. These SNPs were significant in a meta-analysis among 3 replication cohorts and met genome-wide significance in a meta-analysis combining the initial discovery and the replication cohorts (rs2651899, OR = 1.11, \(P = 3.8 \times 10^{-3}\); rs10166942, OR = 0.85, \(P = 5.5 \times 10^{-12}\); and rs11172113, OR = 0.90, \(P = 4.3 \times 10^{-9}\)). None of the 3 SNP associations separated MWA from MWoA, nor were any of the associations specific for any

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**Table 1**: Common candidate genes linked to familial hemiplegic migraine (FHM) and hemiplegic migraine with aura (HMA)

- **CACNA1A**: Gene encoding a neuronal voltage-gated sodium channel subunit; 5 known mutations linked to FHM3.
- **SLC24A4**: Solute carrier family 24 member 4; 2 known mutations linked to FHM4.
- **ATXN3**: Ataxin 3; 2 known mutations linked to FHM4.
- **ITPK1**: Inositol-tetrakisphosphate 1-kinase; 1 known mutation linked to FHM4.

**Table 2**: SNPs associated with migraine

<table>
<thead>
<tr>
<th>SNP</th>
<th>Region</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2651899</td>
<td>1p36.32</td>
<td>1.11 (0.90-1.37)</td>
</tr>
<tr>
<td>rs10166942</td>
<td>2q37.1</td>
<td>0.85 (0.71-1.03)</td>
</tr>
<tr>
<td>rs11172113</td>
<td>12q13.3</td>
<td>0.90 (0.79-1.02)</td>
</tr>
</tbody>
</table>

**References**

See text for detailed references.
Table 1.—Candidate Gene Studies in Migraine

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Reference</th>
<th>Migraine Type</th>
<th>SNP ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 p13.3</td>
<td>Glutathione S-transferase (GST)</td>
<td>Kusumi et al, 2003</td>
<td>MWoA</td>
<td>No rs†</td>
</tr>
<tr>
<td>1 p36.3</td>
<td>Methyleneterahydrofolate reductase (MTHFR) C677T</td>
<td>Lea et al, 2004</td>
<td>MWA</td>
<td>No rs†</td>
</tr>
<tr>
<td>4 q31.2</td>
<td>Endothelin type A (ETA-231 A/G)</td>
<td>Tissirio et al, 2001</td>
<td>Not specified</td>
<td>No rs†</td>
</tr>
<tr>
<td>5 q35</td>
<td>DRD1 negative</td>
<td>Corominas et al, 2009</td>
<td>MWA MWoA mix</td>
<td>rs2283265</td>
</tr>
<tr>
<td>11q22</td>
<td>DRD2</td>
<td>Corominas et al, 2009</td>
<td>MWoA</td>
<td>rs40184</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>Tumor necrosis factor α (TNFα)</td>
<td>Rainero et al, 2004</td>
<td>MWoA</td>
<td>No rs†</td>
</tr>
<tr>
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<td>Tumor necrosis factor α (TNFα)</td>
<td>Rainero et al, 2005</td>
<td>MWA</td>
<td>No rs†</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>Tumor necrosis factor α (TNFα)</td>
<td>Colson et al, 2004</td>
<td>MWA and MWoA</td>
<td>rs2228480</td>
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<tr>
<td>6 q25.1</td>
<td>ESR1 (G2014A variant) negative</td>
<td>Oterino et al, 2006</td>
<td>Not specified females only</td>
<td>rs1801132</td>
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<td>MWoA</td>
<td>rs2228480</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>ESR1 negative Progesterone (Alu insertion) negative</td>
<td>Toft et al, 2009</td>
<td>MWA</td>
<td>rs40184</td>
</tr>
<tr>
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<td>MWoA mix</td>
<td>rs2228480</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>ESR1 (PvuII) negative</td>
<td>Peroutka et al, 1997</td>
<td>MWA</td>
<td>rs6275</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>DBH negative</td>
<td>Fernandez et al, 2009</td>
<td>MWA and MWoA</td>
<td>rs1611115</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>DBH negative</td>
<td>Fernandez et al, 2006</td>
<td>MA in males</td>
<td>X63418†</td>
</tr>
<tr>
<td>6 q25.1</td>
<td>DBH negative</td>
<td>Paterna et al, 2005</td>
<td>MWA</td>
<td>No rs†</td>
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<tr>
<td>6 q25.1</td>
<td>DBH negative</td>
<td>Del Zompo et al, 1998</td>
<td>MWA</td>
<td>No rs†</td>
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<td>Peroutka et al, 1997</td>
<td>MWA</td>
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<td>Mochi et al, 2003</td>
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<td>No rs†</td>
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<td>rs1611115</td>
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<td>Fernandez et al, 2006</td>
<td>MA in males</td>
<td>X63418†</td>
</tr>
<tr>
<td>11 q22-23</td>
<td>DRD2 Allele 1 TG dinucleotide non-coding only in &quot;dopaminergic migraineurs&quot; +</td>
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<td>MWA</td>
<td>No rs†</td>
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<tr>
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<td>DRD2 NcoI</td>
<td>Peroutka et al, 1997</td>
<td>MWA</td>
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<tr>
<td>11 p15</td>
<td>Dopamine receptor DRD4</td>
<td>Mochi et al, 2003</td>
<td>MWA</td>
<td>No rs†</td>
</tr>
<tr>
<td>11 p15</td>
<td>Dopamine receptor DRD4</td>
<td>Fernandez et al, 2005</td>
<td>MWA and MWoA</td>
<td>rs1611115</td>
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<tr>
<td>11 p15</td>
<td>Dopamine receptor DRD4</td>
<td>Fernandez et al, 2006</td>
<td>MA in males</td>
<td>X63418†</td>
</tr>
<tr>
<td>22 q11.2</td>
<td>COMT G to A at codon 158 negative</td>
<td>Cevoli et al, 2006</td>
<td>MWA</td>
<td>No rs†</td>
</tr>
<tr>
<td>5 p 15-3</td>
<td>SLC6A3 (DAT) 40 bp tandem repeat in 3′ untranslated region of the DAT gene negative</td>
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<tr>
<td>17 q23.3</td>
<td>ACE ID</td>
<td>Ogilvie et al, 1998</td>
<td>MWA and MWoA</td>
<td>No rs†</td>
</tr>
<tr>
<td>17 q23</td>
<td>ACE</td>
<td>Ogilvie et al, 1994</td>
<td>MWA</td>
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</tr>
<tr>
<td>17 p13.3/2</td>
<td>INSR SNPs</td>
<td>McCormick et al, 2001</td>
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<td>No rs†</td>
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<td>17 q3</td>
<td>INSR</td>
<td>Nedza et al, 2008</td>
<td>MWA MWoA mix</td>
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<tr>
<td>22 q11.2</td>
<td>COMT L allele (Met Met) no L allele</td>
<td>Park et al, 2007</td>
<td>MWA</td>
<td>No rs†</td>
</tr>
<tr>
<td>22 q11.2</td>
<td>COMT G to A at position 1947 +</td>
<td>Emin Erdal et al, 2001</td>
<td>Not specified</td>
<td>Z26491†</td>
</tr>
</tbody>
</table>

†No rs provided in publication.
‡Non SNP variant.

ACE = angiotensin converting enzyme; Alu; COMT = catechol-O-methyltransferase; DAT = dopamine transporter gene; DBH = dopamine beta-hydroxylase; DRD1 = dopamine receptor D1; DRD2 = dopamine receptor D2; DRD3 = dopamine receptor D3; DRD4 = dopamine receptor D4; DRD5 = dopamine receptor D5; ESR1 = estrogen receptor 1; HLA-DRB1 = major histocompatibility complex, class II, DR beta 1; ID = insertion deletion; INSR = insulin receptor; PROGINS; PvuII; MWA = migraine with aura; MWoA = migraine without aura; NcoI; rs = reference SNP number; SLC6A3 = solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; SLC6A4 = solute carrier family 6 (neurotransmitter transporter, serotonin), member 4; SNP = single nucleotide polymorphism; VTNR = vitronectin receptor.
clinical migraine features. It is interesting to note that in 2 of the 3 replicating SNPs, rs10166942 and rs11172113, the ORs are less than 1, indicating that the variant allele was more associated with the non-migraine controls than the migraine cases. Nevertheless, these findings point to loci that may be important in migraine susceptibility. All 3 of the SNPs identified in this study were in or near transcribed regions of known genes. The SNP rs2651899 located at 1p36.32 was within the first intron of PRDM16, a gene that contains 2 arrays of C2H2 zinc finger domain repeats, which are often linked to transcriptional activity. Another SNP associated with migraine, rs10166942 at 2q37.1, was located 950 bp 5′ to the transcription start site for TRPM8 in a block of moderate linkage disequilibrium spanning about 168 kb and extending through TRPM8. TRPM8, a gene that is expressed in sensory neurons and dorsal root ganglion neurons, encodes a sensor for cold and cold-induced burning neuropathic pain. Based on subgenome-wide significance, TRPM8 was previously identified as a potential candidate gene for association with migraine in the International Headache Genetics Consortium cohort studied in the earlier GWAS. The third associated SNP, rs11172113, located at 12q13.3 maps to the first intron of LRP1 in a gene-rich region. LRP1 is expressed in many tissues including brain and vasculature, modulates synaptic transmission, and serves as a sensor of the extracellular environment. The fact that LRP1 protein and N-methyl-D-aspartic acid (glutamate) receptors are co-localized on neurons and interact would make sense in the context of findings from the earlier GWAS in migraine that reported genetic variants (MTDH and PGCP) implicated in glutamate homeostasis. However, in this GWAS based on the WGHS cohort, the SNP rs1835740 at 8q22.2 which was implicated in the first GWAS was neither associated with overall migraine (P = .22) nor MWA or MWoA separately.

The most recent migraine GWAS reported additional SNPs associated with MWoA based on 2326 clinic-based German and Dutch individuals with MWoA and 4580 population-matched controls. Two SNPs out of 12 candidate loci identified were replicated in a second cohort of 2508 individuals with MWoA and population 2652 controls. The first replicated SNP (rs3790459) was at 1q22 in myocyte enhancer factor 2D gene (MEF2D; replication \( P = 4.9 \times 10^{-4} \); combined \( P = 7.06 \times 10^{-3} \), OR = 1.20), and the second (rs7640543) was located at 3p24 near the transforming growth factor beta receptor 2 gene (TGFB2; replication \( P = 1.0 \times 10^{-6} \); combined \( P = 1.17 \times 10^{-4} \), OR = 1.19).

The replicated SNP on 1q22 is located in the MEF2D gene. The protein product of this gene is a transcription factor highly expressed in the brain, where it regulates neuronal differentiation and neuronal activity through restriction of the number of excitatory synapses. The latter function might be most pertinent to migraine given the implication that decreased restriction of excitatory synapses due to altered function in the MEF2D protein might render the brain more excitable. The replicated SNP at 3p24 is located near (about 200 kb) the TGFB2 gene, which encoded a serine-threonine kinase involved in cellular differentiation, proliferation, and creation of the extracellular matrix. It is interesting to note, as the authors point out, that a missense mutation within the TGFB2 was associated with both familial aortic dissection and migrainous headaches in mutation carriers in a large multigenerational family.

It is also of note that 2 SNPs associated with migraine in the WGHS cohort, rs10166942 (2q37.1, TRPM8) and rs11172113 (12q13.3, LRP1), were replicated in this GWAS.

Other Recent Genetic Investigations of Migraine of Particular Note

Another very interesting investigative approach was taken in a genetic study of migraine published in 2010 by Lafreniere and colleagues, which linked a dominant-negative mutation in the TWIK-related spinal cord potassium channel (TRESK) to MWA.

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**Table 2.—GWAS in Migraine**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Reference</th>
<th>Migraine Type</th>
<th>SNP</th>
</tr>
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<tr>
<td>8q22.1</td>
<td>MTDH</td>
<td>Antril et al, 2010</td>
<td>MWA</td>
<td>rs1835740</td>
</tr>
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<td>PRDM16</td>
<td>Chasman et al, 2011</td>
<td>Not specified</td>
<td>rs2651899</td>
</tr>
<tr>
<td>2q37.1</td>
<td>TRPM8</td>
<td>Chasman et al, 2011</td>
<td>Not specified</td>
<td>rs10166942</td>
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<tr>
<td>12q13.3</td>
<td>LRP1</td>
<td>Freilinger et al, 2012</td>
<td>MWoA</td>
<td>rs11172113</td>
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<tr>
<td>1q22</td>
<td>MEF2D</td>
<td>Freilinger et al, 2012</td>
<td>MWoA</td>
<td>rs3790459</td>
</tr>
<tr>
<td>3p24</td>
<td>TGFB2</td>
<td>Freilinger et al, 2012</td>
<td>MWoA</td>
<td>rs7640543</td>
</tr>
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</table>

GWAS = genome-wide association studies; LRP1 = low-density lipoprotein receptor-related protein 1; MEF2D = myocyte enhancer factor 2D; MTDH = metadherin; MWA = migraine with aura; MWoA = migraine without aura; PRDM16 = PR domain containing 16; rs = reference SNP number; SNP = single nucleotide polymorphism; TGFB2 = transforming growth factor beta receptor 2; TRPM8 = transient receptor potential cation channel, subfamily M, member 8.
in a large pedigree.\textsuperscript{67} The TRESK two-pore domain potassium channel (K2P) channel was identified as a potential target for study in migraine genetics because of its role in pain modulation\textsuperscript{68} and because TRESK is activated by halothane,\textsuperscript{69} which has been reported to inhibit cortical spreading depression (CSD).\textsuperscript{70} a phenomenon linked to migraine visual aura in humans.\textsuperscript{71} In this study, the entire potassium channel, subfamily K, member 18 (KCNK18) gene which codes for the TRESK K2P channel was sequenced in 110 unrelated migraine proband subjects. This revealed 5 mutations, one of which, F139WfsX24, was a 2-bp deletion predicted to cause a frame-shift that resulted in premature truncation of the protein produces at 162 residues. This KCNK18 mutation was not present in any of the 80 population controls that were sequenced or in additional sequencing in a separate large Australian cohort of 511 migraine probands and 505 ethnicity-matched, non-migraine controls. However, the F139WfsX24 mutation was identified in a large multigenerational family in which MWA was inherited in an autosomal dominant, fully penetrant fashion. Genotyping in 15 additional samples from the family showed that the F139WfsX24 frameshift mutation segregated perfectly with the 8 affected members of the family but was absent in the 8 non-affected members.

After analysis of 141 SNPs genotyped in the family, a broad area of linkage was identified on chromosome 10, which was overlay 10q25.2-3. Subsequent haplotype mapping defined a crucial linkage region of 13.0 cM which contained 52 known genes but only 1 ion channel gene. The sole ion channel gene was KCNK18. \textit{In situ} hybridization in adult mice localized highest expression of the KCNK18 messenger RNA to the trigeminal ganglion. In human tissue, immunohistochemistry revealed strong staining of TRESK in the cell bodies of trigeminal ganglion cells. Electrophysiological studies of the TRESK mutation in Xenopus oocytes showed the normal, large, outwardly rectifying whole-cell K\textsuperscript{+} currents present in wild-type oocytes were absent in the oocytes expressing the F139WfsX24 mutation. There was a dose-dependent reduction of the wild-type current amplitude in the heterozygotes vs the mutant homozygotes. Subsequent functional studies in a TRESK-knockout mouse have shown lowering of TRESK channel activity \textit{in vivo} results in altered neuronal activity hinting at its role in migraine. The authors also speculate that the presence of aura in all the affected family members suggest that TRESK alteration may also be involved in the pathogenesis of aura through lowering thresholds for CSD.

Another recent notable study examining ion channel alteration in migraine was undertaken in a very interesting population. In this study, SNP genotyping throughout the human potassium channel gene potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 (KCNN3) was carried out in an isolated population on Norwalk Island who were descendants of the mutinous crew of the famous 18th century British navy ship, the HMS Bounty.\textsuperscript{72} This studied the identified 4 SNPs (rs4845663, rs7532286, rs6426929, and rs1218551) to be significantly associated with the minor allele occurring in the non-migraine-affected subjects, suggesting that the KCNN3 variant conferred protection from migraine susceptibility. Alteration in the KCNN3 gene has been suggested to aid the initiation of CSD.\textsuperscript{73}

Next we turn to the possible functional consequences of the genetic variation underlying migraine as detected by the evolving techniques of functional neuroimaging. These techniques highlight the complexity of both the pain- and non-pain-related components of the migraine syndrome.

**NEUROIMAGING-BASED INVESTIGATIONS OF MIGRAINE PATHOPHYSIOLOGY**

Migraine may clinically exist as either an episodic or chronic neurologic disorder, of which headache is only 1 feature. Pain processing in the central nervous system is complex and involves a synthesis of sensory-discriminative, emotional-affective, and cognitive-evaluative components represented in a neural network termed the “pain-matrix.”\textsuperscript{74} Further, migraine may be associated with aura referable to all lobes of the brain, in addition to autonomic symptoms. Therefore, neuroimaging biomarkers in migraine need to capture functional information elements across multiple neuroanatomic regions.

As will be discussed, neuroimaging research has driven major advances in our understanding of many important aspects of migraine pathophysiology, including generation, chronification, consequences, and treatment.\textsuperscript{75,76} In this review, we will discuss investigations utilizing various functional imaging modalities, although it should be emphasized that these are simply different approaches to understanding a unified pathophysiology. Only human imaging studies have been included in this review.

**Functional Neuroimaging in Acute Migraine**

Migraine sufferers, clinicians, and researchers alike have long suspected that dynamic extracranial and intracranial arterial changes are associated with migraine attacks. However, debate over whether changes in blood flow represent a primary pathogenic event (“the vasogenic theory”) or a secondary epiphenomenon related to neuronal dysfunction (“the neurogenic theory”) was considered to be highly speculative until the emergence of empirical neuroimaging data in the 1980s. Supporters of the vasogenic theory held that migraine aura resulted from transient vasoconstriction, headache from rebound meningeal vasodilation, and resultant mechanical activation of perivascular nociceptors. Remarkably, this debate continues, with a recent study utilizing high-resolution magnetic resonance angiography (MRA) to study calcitonin gene-related peptide-induced migraine-like headaches.\textsuperscript{77} The authors observed unilateral middle meningeal artery (MMA) and middle cerebral artery (MCA) dilatation ipsilateral to headache, and bilateral dilatation in bilateral headache. Further, successful treatment with
sumatriptan resulted in vasoconstriction of the MMA, but not the MCA. Conversely, Schoonman and colleagues did not identify any changes in vascular caliber (either intracranial or meningeal) using 3 Tesla MRA during the study of nitroglycerin-induced migraine-like attacks. For a comprehensive historical review of early observations, see Tfelt-Hansen and Koehler.

Functional imaging techniques are able to capture dynamic, real-time data regarding cerebral blood flow, neuronal activity, and metabolism. The earliest functional imaging studies in migraine utilized intra-arterial 133Xenon (133Xe) to study regional cerebral blood flow (rCBF) during aura occurring in the setting of carotid angiography. Oleen and his co-investigators demonstrated reductions in rCBF, predominantly in the parieto-occipital regions of the brain, which tended to spread anteriorly. Reduction in rCBF to the frontal cortex was also noted in some cases. This “spreading oligemia” which was coincident with aura symptoms persisted for up to 1 hour, after which the rCBF would normalize or remain diminished. Three critical observations emerged from these studies: (1) the magnitude of rCBF reduction was felt to be insufficient to result in neuronal ischemia; (2) the observed anterior spread of the oligemia did not respect neurovascular boundaries; and (3) headache developed in some patients while blood flow was still decreased (and remitted in others during persistent hyperemia). The observations were hypothesized to be better explained by neuronal dysfunction, possibly on the basis of the experimental entity of CSD. The findings of course were highly controversial, and Compton-scattered radiation, a measurement artifact, was cited to account for both an underestimation of rCBF decrement and the spread of oligemia across neurovascular borders.

Further investigations with both perfusion-weighted imaging (PWI) and single photon emission computed tomography (SPECT) were able to confirm these earlier observations. Importantly, neither of these techniques is vulnerable to scatter artifacts. PWI is a gadolinium-based functional magnetic resonance imaging (fMRI) technique sensitive to microvascular changes (capillary/arteriolar), with higher spatial resolution than radionuclide-based techniques. Visual aura was initially investigated with PWI in 4 patients during 5 spontaneous attacks. Hemodynamic changes, consistent with earlier 133Xe-based studies, were documented in the occipital cortex contralateral to the affected visual hemifield. In a larger follow-up study, 28 spontaneous migraine episodes were studied, half of which were represented by cases of MWoA. Notably, during MWoA, hemodynamic changes were not observed. In a single patient, who experienced both MWA and MWoA, perfusion deficits were only observed during reported visual aura. A lack of flow abnormalities in patients without aura and regional hypoperfusion corresponding to aura symptomology has also been demonstrated in reports utilizing SPECT imaging. However, utilizing other imaging techniques (ie, positron emission tomography [PET]), other authors have appreciated similar posterior hypoperfusion in spontaneous MWoA.

More recent reports have documented reversible perfusion deficits despite protracted hemiplegic migraine with both PWI and SPECT, including concordance of both in a single case of persistent aura. The reduction in blood flow, as measured from SPECT data, also falls below the ischemic threshold. Diffusion-weighted imaging measures the net translational movement of water and is highly sensitive in the early detection of cerebral ischemia. However, diffusion-weighted changes are typically not reported in the context of persistent migraine aura, despite protracted symptom duration. The exceptions to this include experimental CSD and a minority of reports of prolonged hemiplegia in patients with genetically defined syndromes. Further, migrainous infarction is recognized as a rare complication of migraine, the definition of which requires “a typical attack of migrainous aura in a patient with a history of migraine with aura and evidence of cerebral ischemia proven by neuroimaging.” While the directionality of the relationship between ischemia and aura is controversial, a recent large series did not identify any cases with overlapping ischemic lesions in different vascular territories, arguing against a CSD-induced ischemic mechanism. Furthermore, in contrast to patients with persistent aura without infarction, patients with migrainous infarction have normal (31)P-magnetic resonance spectroscopy (MRS) of cortical metabolism, suggesting that migrainous infarct has a unique pathophysiology.

Blood oxygen level-dependent (BOLD) imaging may be utilized to generate functional brain activation maps, utilizing changes in flow-related deoxyhemoglobin concentrations and corresponding MRI signal changes. In the earliest investigation utilizing BOLD, visually triggered attacks were studied in 10 patients with aura and 2 without. In 5 patients, development of headache or visual changes (not classical aura) was preceded by an initial activation pattern and subsequent spread of suppression at a rate suggestive of a CSD mechanism, 3 to 6 mm per minute. In a separate study, also utilizing BOLD, a subject was studied with reliably exercise-triggered MWA. This individual underwent imaging before, during, and after aura, including the transition to the headache phase. Concordant with onset of visual aura, suppressed activation was seen initially in extrastriate cortex area V3a, with subsequent contiguous spread (rate of 3.5 mm per minute) to eventually involve primary and association visual cortices. These BOLD imaging studies strongly argued for a CSD-like mechanism as the substrate of human migraine visual aura, given the precise correlation of BOLD events in the visual cortex and the reported aura percept.

In 1995, Weiller and colleagues reported PET findings in 9 patients with spontaneous MWoA. Interestingly, a modest increase (11%) in rCBF was observed in the medial brainstem contralateral to the headache, in addition to cingulate, auditory, and visual association cortices. These changes were not present in
the headache free interval; however, the brainstem activation persisted immediately following relief of headache with sumatriptan. Brainstem activation was purported to be inherent to the migraine attack and to act as a “generator” for migraine attack. Similar findings were replicated in a subsequent case report in a patient with both cluster and migraine headaches, suggesting that functional imaging could provide a reliable biomarker of distinct clinical disorders. Along these lines, in another study, brainstem activation was not seen with PET imaging following forehead capsaicin injection. Afriadi and colleagues were able to further validate the finding of brainstem activation in 5 additional cases of spontaneous migraine using a higher resolution PET. The authors found activation of the ipsilateral dorsal pons, in addition to an area of deactivation in the contralateral pons. Activation of the contralateral anterior and posterior cingulate, cerebellum, thalamus, insula, prefrontal cortex, and temporal lobes were also seen.

In a subsequent series utilizing BOLD, 75% of patients (both MWA and MWOA) developed symptoms during visually triggered migraine. In these patients, baseline T2*−weighted magnetic resonance signal intensities increases in the red nucleus and substantia nigra prior to the onset of symptoms or signal changes in the occipital cortex. Red nucleus activation was bilateral in the majority of patients, and signal increase in the substantia nigra was bilateral in 50% of patients. In a minority of patients, signal increases were also appreciated in other brainstem structures, including the locus ceruleus, periaqueductal gray, central midbrain, pontine tegmentum, and medial lemniscus. These structures are thought to be important in modulation of nociceptive transmission and vascular control. The dorsolateral pons and cerebellum were also implicated in a series of 24 patients who underwent PET imaging during glyceryl trinitrate-triggered migraine attacks. In this study, lateralization of dorsal pontine activation was ipsilateral to the headache (both right- and left-sided groups) and bilateral in patients reporting bilateral headache. Thus, lateralization of brainstem activity was associated with pain lateralization.

In addition to reproducing earlier findings of cortical and brainstem activation, a later study of PET in 7 patients with spontaneous migraine demonstrated hypothalamic activation. This activity, like that seen in the midbrain and pons, persisted following successful headache treatment. In addition, activation of basal ganglia and various cortical regions, including frontal, cingulate, temporal, parieto-occipital, and insular, have all been observed.

Activation of these brain regions may relate to the generation of premonitory symptoms, in addition to cognitive-evaluative and emotional-affective processing of pain signals.

Intriguingly, MRI-BOLD of patients experiencing extra-cephalic allodynia during a migraine attack is associated with activation of the posterior thalamus. These findings were consistent with animal data presented in the same manuscript, demonstrating sensitization of thalamic trigeminovascular neurons to innocuous stimulation of extracephalic skin following chemical stimulation of the dura. In addition, this same thalamic region is thought to be pivotal in the pathophysiology of photophobia, where retinothalamic projections may influence trigeminovascular neurons. In a human PET study of photophobia during spontaneous migraine, low-intensity photostimulation activated visual cortex during acute migraine, but not during an attack-free interval. Finally, in a BOLD study of spontaneous migraine, exposure to an olfactory stimulus resulted in limbic and rostral pontine activation.

**Functional Neuroimaging in the Attack-Free State**

Functional studies implicate dysfunction at the levels of brainstem, hypothalamus, thalamus, basal ganglia, and cerebral cortex even during the attack-free (“interictal”) state. Stankewitz and colleagues studied nociceptive responses to intranasal administration of gaseous ammonia in 20 migraine patients during their interictal periods. If a patient experienced a migraine within 72 hours after scanning, they were considered to have been “preictal.” BOLD signal changes in previously described cortical and subcortical regions did not differ between interictal patients and matched controls. Interestingly, control patients showed greater activation of the spinal trigeminal nucleus than did interictal migraineurs. Further, the intensity of BOLD responses in the spinal trigeminal nuclei significantly predicted the number of days until a forthcoming attack. In the preictal and ictal states, the BOLD signal was seen to become rapidly downregulated. Thus, while dorsal pontine activation is linked to migraine ictus, the gradient-like increase in activity in the spinal trigeminal nucleus appears to be important in raising the susceptibility to migraine long before the attack onset.

Heightened cortical excitability is considered to be a hallmark interictal finding in migraine patients. PET has been used to study the cortical, interictal responses of 7 patients to light stimulation with and without concomitant trigeminal pain. Light activated the bilateral visual cortices of migraine patients but not controls, and this response was potentiated by concomitant trigeminal pain. Light activated the bilateral visual cortices of migraine patients but not controls, and this response was potentiated by concomitant trigeminal pain. Intriguingly, MRS have suggested an impairment of neuronal oxidative metabolism in both the interictal and ictal states. Both phosphocreatine (PCr) and adenosine triphosphate concentrations have been shown to be decreased without...
depletion of inorganic phosphate (Pi), suggesting underlying mitochondrial dysfunction. Visual cortex metabolites were studied in patients both with and without aura following photic stimulation in the interictal period. Patients with aura were noted to have a more consistent decrease of the N-acetylaspartate signal as compared with those without aura, despite comparable baseline MRS evaluations, pointing toward diminished mitochondrial functioning in individuals with MWA. Furthermore, clinical characteristics of the aura have been associated with the cortical PCR/Pi ratio, the latter showing an inverse relationship with aura duration and varying with aura phenotype (lower in hemiplegic migraine vs non-motor aura). Functional Neuroimaging in Chronic Migraine

In contrast to the numerous functional imaging studies of episodic migraine, there are relatively few investigations of individuals with chronic migraine. Chronic pain (in general and including migraine) has been associated with disruption of functional connectivity in the resting state network, including the pain matrix. A surprising observation has been that spontaneous pain in distinct chronic pain conditions (osteoarthritis, pelvic pain, etc.) is associated with unique patterns of brain activation. In chronic back pain, with a spontaneous rise in pain, regions typically associated with acute pain (insula, mid-anterior cingulate) are only very briefly activated, followed by sustained activation of the medial prefrontal cortex (emotional suffering). Despite specific differences between pain types, the transition from acute to chronic pain is generally associated with a shift toward the activation of limbic and paralimbic regions.

Maleki and colleagues have studied responses to experimental pain in patients with either high- or low-frequency attack rates of migraine. A differential response pattern was observed between the 2 groups, with responses to pain in the high-frequency group being lower in the caudate, putamen, and pallidum. In a study of chronic migraine, 8 patients who had been responsive to suboccipital neurostimulation were studied with PET to understand regional activation patterns. These patients typically experienced a peak of their headaches within 20 minutes of turning off the stimulator. Significant changes in rCBF, correlating with pain scores, were seen in the dorsal rostral pons, anterior cingulate cortex, and left pulvinar. In another important study, PET correlates of cortical hyperexcitability in chronic migraine were examined. It was observed that cortical hyperexcitability is statistically increased even further than that seen in episodic migraine patients. Activation of the dorsal pons and right temporal cortex was seen, with concurrent decreased metabolism of the caudate nucleus, medial frontal, bilateral medial parietal, and somatosensory cortices. The authors propose that dysfunctional inhibitory pathways, resulting in circular cortical-subcortical activation, underlie the observed cortical hyperexcitability.

Functional neuroimaging has also been applied to individuals with medication-overuse headache. Fumal and colleagues carried out PET imaging in individuals with chronic migraine and medication-overuse headache. Interestingly, 3 weeks after withdrawal of analgesics, persistent hypometabolism of the orbitofrontal cortex, a region also relevant for drug dependence, was seen. Metabolic changes in all other brain regions were reversible. The findings therefore demonstrate a possible neurologic predisposition to analgesic overuse in this patient population. Reversible hypometabolism, especially in the lateral pain system, has been replicated in subsequent imaging studies following analgesic withdrawal.

Overview of Findings From Neuroimaging in Migraine

In summary, major advances in our understanding of migraine pathophysiology have emerged from neuroimaging research. There is strong fMRI evidence to support CSD as the neuronal mechanism underlying migraine aura. However, there continues to be conflicting data regarding the role of meningeal dilatation as an important component of migraine pain. The existence of a “brainstem generator” specific to migraine in the rostral pons remains a matter of debate. Neuroimaging data have been hypothesis generating, revealing cortical hyperexcitability, deranged neuronal metabolism, and altered functional activation patterns and connectivity in migraine patients. As migraine frequency and disease duration increase, the burden of these changes increase, with alterations seen in critical components of the pain-processing system. Many of these changes have been shown to be reversible with treatment, with residual changes perhaps reflecting an underlying predisposition to migraine (brainstem), and/or analgesic overuse (orbitofrontal cortex).

CONCLUSION

The rise and rapid expansion of the technologies allowing for genetic- and neuroimaging-based investigations of migraine in humans and the data that they have yielded thus far foster optimism for our attempts to better understand migraine. It is conceivable that in the not too distant future there will be application of the 2 complimentary approaches onto the same study populations with highly informative results as to the functional consequences of specific genetic variation. The investigation of migraine simultaneously in 3 dimensions: clinical phenotype, genetic/epigenetic variation and neuroimaging consequence may ultimately be required to better understand and more effectively treat this highly prevalent, pathophysiologically complex and heterogeneous disorder.

References


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