The Genetics of Migraine

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The search for genes involved in the pathophysiology of migraine poses major difficulties. First, there is no objective diagnostic method to assess the status of the individuals studied. Second, migraine is a polygenic multifactorial disorder. Familial hemiplegic migraine (FHM) is the only known autosomal dominant subtype of migraine. In half the families with FHM who have been studied, there are mutations in the calcium-channel gene CACNA1A, located on chromosome 19. In other families, a locus has been mapped on chromosome 1. The role of these loci in typical migraine is still unknown. A susceptibility locus for migraine with aura has been located on chromosome 19 (but is distinct from CACNA1A) and a genome-wide linkage analysis has mapped a susceptibility locus on chromosome 4. Another locus for migraine may be on the X chromosome. Finally, many positive association studies have been published, but few have been replicated.


Migraine is a primary headache disorder consisting of recurrent attacks of disabling pain that last from a few hours to a few days. Typically, patients with migraine are perfectly well between attacks. Migraine is very common, affecting about 15% of the western populations. The mechanisms of migraine are far from being completely understood. Familial aggregation has long been known and was even classically used as a diagnostic criterion. During the past 10 years, the genetics of migraine has become an important research topic with the major goal being the identification of susceptibility genes that may give clues to the mechanisms underlying this disorder and may help to develop new diagnostic and therapeutic strategies.

Why is it so difficult to identify migraine genes?
Genetic studies face difficulties directly related to some of the characteristic features of migraine. First, researchers must establish the clinical status of each person studied—ie, find out whether the individual is affected or not. In the absence of any biological or radiological marker, the diagnosis of migraine is purely clinical. The International Headache Society (IHS) classification (panel), published in 1988, defined migraine with aura as having specific characteristics. However, when dealing with familial migraine, aura is not so characteristic. In fact, when aura is present, it is often not characteristic of migraine and can be more suggestive of a disorder producing secondary headache. For this reason careful diagnostic criteria have been developed for familial hemiplegic migraine (FHM1).

IHS diagnosis criteria for migraine without aura, migraine with aura, and two varieties of migraine with aura (migraine with typical aura and FHM1)

<table>
<thead>
<tr>
<th>Migraine without aura</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least five attacks meeting the criteria below:</td>
</tr>
<tr>
<td>Headache attacks lasting between 4 h and 72 h (untreated or unsuccessfully treated)</td>
</tr>
<tr>
<td>Headache with at least two of the following characteristics:</td>
</tr>
<tr>
<td>Unilateral location</td>
</tr>
<tr>
<td>Pulsating quality</td>
</tr>
<tr>
<td>Moderate or severe intensity</td>
</tr>
<tr>
<td>Aggravation by walking up stairs or similar routine physical activity</td>
</tr>
<tr>
<td>During headache at least one of the following:</td>
</tr>
<tr>
<td>Nausea and/or vomiting</td>
</tr>
<tr>
<td>Photophobia and phonophobia</td>
</tr>
<tr>
<td>At least one of the following:</td>
</tr>
<tr>
<td>History and physical and neurological examinations do not suggest a disorder causing secondary headaches</td>
</tr>
<tr>
<td>History and physical and neurological examinations suggest a disorder causing secondary headaches, but it is ruled out by appropriate investigations</td>
</tr>
<tr>
<td>Such a disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Migraine with aura</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least two attacks meeting the criteria below:</td>
</tr>
<tr>
<td>At least three of the following four characteristics:</td>
</tr>
<tr>
<td>One or more fully reversible aura symptoms indicating focal cerebral cortical and/or brain stem dysfunction</td>
</tr>
<tr>
<td>At least one aura symptom that develops gradually over more than 4 min or two or more symptoms that occur in succession</td>
</tr>
<tr>
<td>No aura symptom lasts more than 60 min; if more than one aura symptom is present, duration is proportionally increased</td>
</tr>
<tr>
<td>Headache follows aura with a free interval of less than 60 mins (it may also begin before or simultaneously with the aura)</td>
</tr>
<tr>
<td>At least one of the following:</td>
</tr>
<tr>
<td>History and physical and neurological examinations do not suggest a disorder causing secondary headaches</td>
</tr>
<tr>
<td>History and physical and neurological examinations suggest a disorder causing secondary headaches, but it is ruled out by appropriate investigations</td>
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<tr>
<td>Such a disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder</td>
</tr>
</tbody>
</table>

Migraine with typical aura
Meets criteria for migraine with aura (above) including all four of the following criteria:
- Homonymous visual disturbances
- Unilateral paresthesias and/or numbness
- Unilateral weakness
- Aphasia or unclassifiable speech difficulty

Familial hemiplegic migraine
Meets criteria for migraine with aura
The aura includes some degree of hemiparesis and may be prolonged
At least one first-degree relative has identical attacks
Genetics of migraine

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distinguishes different varieties of migraine, the two most frequent being migraine without aura and migraine with aura. Attacks of migraine without aura are characterised by moderate to severe head pain which is often unilateral, pulsating, and aggravated by routine physical activity (panel). The headache lasts from 4 h to 72 h and is associated with nausea, vomiting, photophobia, and phonophobia. Attacks of migraine with aura are characterised by transient neurological signs preceding or accompanying the headache phase. These “aura” symptoms develop gradually and last from a few minutes to up to an hour (panel). Visual symptoms, including the classical scintillating scotoma, are the most frequent, followed by sensory disturbances, speech difficulties, and, rarely, motor symptoms. Migraine is characterised by the repetition of such attacks in the absence of any underlying disorder causing secondary headaches. According to the IHS criteria, at least five attacks of migraine without aura or two attacks of migraine with aura are required to classify a person as affected.

Recurrent migraine attacks may be part of the clinical range of several organic cerebral disorders such as arteriovenous malformations, mitochondrial encephalopathies (including mitochondrial encephalopathy with lactic acidosis and stroke-like episodes [MELAS]), antiphospholipid antibody disease, and some cerebral arteriopathies including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Moreover, a migrainous attack may be triggered by various acute vascular events such as cervical-artery dissection, cerebral-venous thrombosis, or cerebral infarction. In patients affected by such disorders, migraine attacks are symptomatic of the underlying problem. However, symptomatic migraine attacks are often difficult to distinguish from those of a primary migraine disorder. Are the mechanisms of symptomatic migraine attacks different from those of primary migraine attacks? Or is it just that patients with these organic cerebral disorders have a lower threshold for developing migraine attacks?

As described above, the IHS classification distinguishes different types of migraine. Migraine without aura is more common than migraine with aura in the general population (6–10% vs 3–6%). Some patients have attacks of only one type—with or without aura—but about a third experience both types of attacks during their life. We do not yet know whether migraine with and without aura are two clinical forms of the same disease, or whether they represent two distinct disorders. There are also other less common types of migraine. Several genetic studies have focused on familial hemiplegic migraine (FHM), a rare type of migraine with aura, because it is the only form in which a monogenic mendelian mode of inheritance has been clearly established. A typical attack is characterised by unilateral motor weakness associated with at least one other aura symptom such as sensory disturbances within the zone of motor deficit, visual symptoms, or aphasia. Bilateral motor and sensory symptoms are reported by about a quarter of patients. The aura phase lasts for 1–2 h and is followed by a migrainous headache. The mean age at onset is lower (at about 11 years) than in other types of migraine. Moreover, severe attacks of confusion or even coma with prolonged hemiplegia, fever, and meningismus are reported by a third of the patients with FHM at least once in their life. Finally, some patients may have permanent neurological signs between attacks, mostly nystagmus and ataxia. These features have led to the distinction between families with pure hemiplegic migraine (80%) and those with hemiplegic migraine and cerebellar symptoms (20%), in which at least one family member has nystagmus or ataxia. FHM is characterised by large clinical variability in the severity and frequency of attacks among individuals belonging to different families but also within the same family. Besides familial cases, some sporadic cases of hemiplegic migraine with cerebellar symptoms have also been reported. Whether FHM has the same pathophysiological mechanisms as other types of migraine with aura is not known.

Classification of individuals as “affected” or “unaffected” for genetic studies does not reflect the important clinical heterogeneity of migraine. Indeed, the frequency and severity of attacks show much variability from one patient to another. Let us consider, for example, a family in which the 40-year-old daughter has had one or two severe attacks of migraine without aura with intractable headache and vomiting every month, lasting 3 days at a time, since the age of 12. The 65-year-old mother has had only two mild and short attacks of migraine with visual aura in her fifties. The daughter is affected by migraine without aura and the mother by migraine with aura, but do they suffer from the same disorder?

Finally, several other factors have to be taken into account in genetic studies of migraine. The high prevalence of migraine (8–25% of the population) could lead, purely by chance, to a significant part of the observed familial aggregation. Women are twice as likely to be affected as men, which also has to be taken into account in segregation studies.

Is migraine a genetic condition?

In addition to genetic factors, familial aggregation of a pathological disorder may be due to environmental factors and may also, as previously mentioned, occur purely by chance in very common diseases. A major step in the study of migraine genetics was thus to show the existence of genetic factors by the means of genetic epidemiological surveys. Twin studies have been used to assess the respective roles of genetic and environmental factors. These studies are based on the comparison of concordance rates between monozygotic and dizygotic twins. All twin studies done so far show that migraine has a genetic component in addition to environmental factors. The most recent of these studies was the only one that was carried out in a large population and used the IHS diagnosis criteria. In this Danish twin study including 1013 monozygotic and 1667 dizygotic twin-pairs, the pairwise concordance rate was significantly higher among monozygotic than dizygotic twin pairs for migraine without aura (28 vs 18%, p<0.05) and for migraine with aura (34 vs 12%, p<0.001).


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Family studies provide information about the effect of genetic factors and the potential mode of inheritance. Many studies have produced controversial results owing to methodological biases such as the lack of homogeneous diagnostic criteria before 1988, the lack of separation between migraine without aura and migraine with aura, the use of a questionnaire instead of direct interview of the probands, the lack of a direct interview of the relatives, and the selection of probands from specialised centres.17-41 However, all of these studies concluded that genetic factors are implicated in migraine. The proportion of familial cases was estimated to be between 34% and 90%, and all possible transmission patterns were proposed, apart from X-linked inheritance.

Two family studies addressing all methodological concerns have been published. Russell and Olesen42 studied the relative risk of migraine without aura and migraine with aura in all first-degree relatives and spouses of 44 probands selected out of 4000 representative individuals from the general Danish population. Compared with the general population, first-degree relatives of probands with migraine without aura had a relative risk of 1.86 (95% CI 1.56 to 2.16) of migraine without aura and first-degree relatives of probands with migraine with aura had a relative risk of 3.79 (95% CI 3.21 to 4.38) of migraine with aura.42 Stewart and colleagues43 undertook a population-based study in Maryland (USA) to analyse the relative risk of migraine without aura and migraine with aura in all first-degree relatives of 73 migraineous probands and 72 matched controls. Compared with controls, first-degree relatives of probands with migraine had a relative risk of 1.50. However, this increase in risk was not statistically significant (95% CI 0.94 to 2.40) and no difference was observed when migraine without aura and migraine with aura were analysed separately. When the researchers considered only the 26 probands with important disability due to migraine, they found a 2.17-fold significantly increased risk of migraine in first-degree relatives (95% CI 1.22 to 3.87).43

With regard to the mode of inheritance, the results of a large Danish population-based segregation analysis, including 126 families with migraine without aura and 127 families with migraine with aura, showed that both types of migraine have a non-mendelian multifactorial mode of inheritance.44 Analysis of the Danish twin-study data also favoured a model combining additive genetic and environmental factors.34-36 In addition, analysis of 31 families with a high risk of migraine without aura also suggested multifactorial inheritance.45

Together, these data indicate that both migraine without aura and migraine with aura are complex diseases caused by a combination of genetic and environmental factors. Several studies have suggested that genetic factors may be more important in migraine with aura than in migraine without aura.31,36,42 The possible number of genetic susceptibility loci is unknown. FHM is the only monogenic variety of migraine.12,23

### Strategies in the quest for migraine genes

In non-mendelian diseases, the identification of disease-causing mutations can be achieved by different methods. In families, linkage analysis to candidate genes or markers can be used to map a susceptibility locus, followed by detection of the pathogenetic mutation and positional cloning. Model-free or non-parametric linkage analyses have to be used in migraine because classic parametric linkage analyses require the definition of a precise genetic model, and such a definition is inaccurate in non-mendelian disorders. Association studies, which do not have such difficult methodological considerations, can be performed in homogeneous populations.42 These studies compare the frequency of alleles of a polymorphic genetic marker between patients and healthy controls. In the absence of methodological shortcomings, a significant difference means that the polymorphism is located within the susceptibility gene or is in linkage disequilibrium with the susceptibility gene. However, association studies do not provide any indication of the normal function of the tested gene or of its abnormal function in the pathophysiology of the disease being studied.46

By contrast, genetics provides powerful tools for the identification of genes causing mendelian disorders. Several research teams have thus focused their efforts on the localisation and identification of the genes involved in FHM, since these genes may also be good candidates for the much more frequent migraine types, migraine with aura and migraine without aura.

### Genetic heterogeneity of FHM

FHM is genetically heterogeneous.12,14,25,26,47-49 Joutel and co-workers47 localised the first gene on the short arm of chromosome 19 in 1993. In 1996, Ophoff and colleagues12 identified this gene as CACNA1A, which encodes the α1A subunit of the neuronal P/Q type voltage-gated calcium-ion channel. CACNA1A is implicated in about 50% of families with FHM and in all families with FHM and permanent cerebellar symptoms.14,16,27,26 In 1997, a second gene was mapped to chromosome 1q; significant linkage with markers located on 1q31 was found in a large American family47 and significant linkage to more centromeric markers located at 1q21-q33 was found in three French families.49 From present evidence, we do not know whether one gene or two distinct genes for FHM are located on chromosome 1q. The chromosome 1 locus is implicated in a few families (10–20%) of those affected by pure FHM.49 Finally, 30–40% of families show no linkage to these first two loci, which suggests the existence of at least one other FHM gene.49

CACNA1A, α1A subunit, and P/Q-type channels

CACNA1A, the only known FHM gene, encodes the main subunit of the P/Q type voltage-gated calcium channel.12 These channels are large multimeric proteins located within the cell membrane; in the centre is a pore that opens in response to membrane depolarisation, letting calcium ions flow into the cell.50,51 In neurons, they have a major role in the control of neuronal excitability and neurotransmitter release. Each channel is formed by a main α1 subunit associated with four auxiliary subunits (α2, β, γ, and δ).52-53 The α1 subunit forms the ionic pore that includes the structures that bring about voltage sensitivity and ionic...
distinct types of calcium currents: the slow inactivating results of alternative splicing, peripheral neurons at the neuromuscular junction.50,54 As a channels, which are expressed in central neurons and in subunit is the main subunit of P/Q type neuronal calcium selectivity. All α subunits have the same structure (figure). There are at least ten genes (CACNA1A, B, C, D, E, F, G, H, I, and S) coding for distinct α subunits.60-62 Depending on the α1 subunit type, a calcium channel has distinct pharmacological and kinetic properties generating different types of calcium currents: L, N, P/Q, R, or T.63,64 The α1A subunit is the main subunit of P/Q type neuronal calcium channels, which are expressed in central neurons and in peripheral neurons at the neuromuscular junction.63,65 As a result of alternative splicing, α1A subunits can generate two distinct types of calcium currents: the slow inactivating P-type currents that are the major calcium currents in cerebellar Purkinje cells, and the fast inactivating Q-type currents that play a major part in the control of neurotransmitter release.66

CACNA1A is a large gene containing 47 exons.62 Exon 47 contains a polymorphic CAG repeat, which is predicted to code for a polyglutamine stretch in a subset of the known human transcripts.67 Thus far, 15 CACNA1A mutations have been identified in 31 families with FHM (24 affected by FHM with cerebellar symptoms and seven by pure FHM) and in two sporadic cases affected by hemiplegic migraine with cerebellar symptoms (table 1).57,62,68-71 Five of these mutations are recurrent—ie, they have been detected in two or more unrelated families.57,62,68-71 The commonest mutation, T666M, has been detected in 12 unrelated families and in one sporadic case.57,62,68-71 All 15 mutations are missense mutations, causing a substitution of one amino acid of the 2550 that constitute the predicted protein. The mutations are located in important functional domains of the subunit, near the ionic pore or within the voltage sensor (figure). Nine of the 15 mutations, including the five recurrent ones, are associated with hemiplegic migraine with cerebellar symptoms. Different CACNA1A mutations have been identified in two other autosomal dominant neurological disorders: episodic ataxia type 2 (EA2)12 and spinocerebellar ataxia type 6 (SCA6).14 EA2, like FHM, is a paroxysmal neurological disorder causing recurrent attacks of major instability with lack of coordination. Attacks are often provoked by exercise or emotion and last between 15 min and a few hours. Acetazolamide is effective in preventing attacks in most patients. During attack-free intervals, clinical examination commonly discloses permanent cerebellar signs (nystagmus and/or ataxia). Most of the mutations identified in patients with EA2 are predicted to result in a truncation of the putative protein.12,14 SCA6 is a progressive cerebellar disease

**Table 1. CACNA1A mutations identified in FHM**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Reference (or case)</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>α1A subunit domain</th>
<th>Cerebellar symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>S218L</td>
<td>62</td>
<td>5</td>
<td>TCG→TG</td>
<td>IS4–SS</td>
<td>+</td>
</tr>
<tr>
<td>R583Q</td>
<td>57</td>
<td>13</td>
<td>CGA→CAA</td>
<td>IS4</td>
<td>+</td>
</tr>
<tr>
<td>T666M</td>
<td>12</td>
<td>16</td>
<td>ACG→ATG</td>
<td>II P loop</td>
<td>+</td>
</tr>
<tr>
<td>D715E</td>
<td>16</td>
<td>1</td>
<td>GAC→GAG</td>
<td>IS6</td>
<td>+</td>
</tr>
<tr>
<td>Y1385C</td>
<td>27</td>
<td>26</td>
<td>TAC→TGG</td>
<td>IS5</td>
<td>+</td>
</tr>
<tr>
<td>R1668W</td>
<td>16</td>
<td>32</td>
<td>CGG→TGG</td>
<td>VS4</td>
<td>+</td>
</tr>
<tr>
<td>L1682P</td>
<td>61</td>
<td>32</td>
<td>CTN→CCN</td>
<td>VS4</td>
<td>+</td>
</tr>
<tr>
<td>W1684R</td>
<td>16</td>
<td>32</td>
<td>TGG→CGG</td>
<td>VS4–SS</td>
<td>+</td>
</tr>
<tr>
<td>I1811L</td>
<td>12</td>
<td>26</td>
<td>ATG→CTC</td>
<td>VS6</td>
<td>+</td>
</tr>
<tr>
<td>R192Q</td>
<td>12</td>
<td>4</td>
<td>CGA→CAA</td>
<td>IS4</td>
<td>-</td>
</tr>
<tr>
<td>R199K</td>
<td>16</td>
<td>4</td>
<td>AGG→AAG</td>
<td>IS4</td>
<td>-</td>
</tr>
<tr>
<td>V714A</td>
<td>12</td>
<td>17</td>
<td>GTC→TGG</td>
<td>IS5</td>
<td>-</td>
</tr>
<tr>
<td>K1336E</td>
<td>16</td>
<td>25</td>
<td>AAA→GAA</td>
<td>IS3–SS</td>
<td>-</td>
</tr>
<tr>
<td>V1457L</td>
<td>58</td>
<td>27</td>
<td>GTC→TTG</td>
<td>II P loop</td>
<td>-</td>
</tr>
<tr>
<td>R1668W</td>
<td>16</td>
<td>32</td>
<td>CGG→TGG</td>
<td>VS4</td>
<td>-</td>
</tr>
<tr>
<td>V1696I</td>
<td>16</td>
<td>33</td>
<td>GTC→ATC</td>
<td>VS5</td>
<td>-</td>
</tr>
</tbody>
</table>
characterised by an adult-onset statokinetic ataxia caused by small expansions of the CAG repeat contained within exon 47 of CACNA1A.54,64

Consequences of CACNA1A mutations
Several methods can be used to understand the mechanisms leading from the different CACNA1A mutations to the observed phenotypes.

Seven CACNA1A mutations causing FHM have been studied electrophysiologically by comparison of the calcium currents between cells expressing the wild-type gene and cells expressing the mutant gene.60–62 All the mutations modify the density and the gating properties of P/Q-type currents. FHM mutations are thus “gain of function” mutations with the abnormal protein producing altered calcium currents. No difference was observed between the mutations causing pure FHM and the mutations causing FHM with cerebellar symptoms. However, the conditions in vitro are unlikely to reflect the complexity of the situation in vivo. On the other hand, EA2 mutations are “loss of function” mutations with the mutated protein incapable of generating any current when expressed in heterologous cells.63–65

Knockout mice that do not carry the Cacna1a gene are born with a severe ataxia and die within a few days.72 Mice carrying different mutations within Cacna1a display distinct phenotypes called tottering,73 leaner,73 or rocker,74 which are characterised by various paroxysmal manifestations (absence epilepsy and motor attacks) and are in all cases associated with a permanent cerebellar ataxia of variable severity. Tottering mice show abnormal control of acetylcholine release at the neuromuscular junction.74 Moreover, the different mutants display several anatomical abnormalities, including abnormal arborisation of Purkinje cells, abnormal migration of granular cells, and abnormalities of the locus coeruleus.75,76 These abnormalities suggest that P/Q-type channels may be implicated not only in the control of neurotransmitter release but also in neuronal development.

Genotype–phenotype correlations in FHM
FHM is characterised by striking clinical variability. Age at onset, frequency, duration, and features of attacks may vary from one patient to another, even among affected members from a given family who all carry the same mutation in the same gene.60–63 This variability suggests complex interactions between the consequences of the mutation and environmental factors or modifying genetic factors.

Several studies have shown that the various FHM genotypes have a role in producing this clinical variability. In a study of five families, the clinical features of 46 patients from three families with linkage to chromosome 19 were compared with those of 20 patients from two unlinked families.15 Patients from the families with chromosome 19 linkage had a higher frequency of attacks with loss of consciousness (39% vs 15%) and head-trauma triggered attacks (70% vs 40%) than unlinked families.15 In a study of 17 families, the clinical and genetic features of FHM were compared between three groups of families: ten with linkage to chromosome 19 (94 patients), three with linkage to chromosome 1 (24 patients), and four unlinked families (24 patients).16 Two major differences were shown: first, the penetrance of FHM was lower in chromosome-1-linked families than in the other group and second, permanent cerebellar symptoms were present in a subset of chromosome-19-linked families and absent in all the other family groups.25

The detailed clinical analysis of a large series of 117 individuals with CACNA1A mutations causing FHM has allowed better delineation of the clinical range of this type of migraine with aura.16 FHM caused by CACNA1A mutations is characterised by a high penetrance of hemiplegic migraine attacks (86%), in which the motor aura is always associated with sensory, language, or visual aura symptoms. However, in contrast to other varieties of migraine with aura in which visual symptoms are present in about 99% of patients, a quarter of patients with FHM do not have visual symptoms.23 FHM is also characterised by the frequency of bilateral motor signs during aura (about 30% of patients), severe attacks with impairment of consciousness (30% of patients), an early age at onset (close to 11 years), and by the importance of minor head trauma as a triggering factor. Finally, permanent cerebellar signs are found in about 80% of patients with mutations belonging to families who have hemiplegic migraine with cerebellar symptoms. Altogether, these characteristics suggest that FHM due to CACNA1A mutations is a very unusual variety of migraine with aura.

In addition, striking correlations have been found between the genotype and phenotype. The frequency of symptoms was studied in 85 patients with the three most frequent mutations linked to FHM with cerebellar signs: T666M (55 patients), R583Q (16 patients), and D715E (14 patients).26 Patients with the T666M mutation had the highest penetrance of hemiplegic migraine (98%), severe attacks with coma (50%), and nystagmus (86%). Patients with the R583Q mutation had the highest penetrance of gait ataxia (81%) in the absence of any nystagmus, and those with the D715E mutation had the lowest penetrance of hemiplegic migraine attacks (64%).26

The various studies of CACNA1A mutations in FHM have provided several important insights. First, the mutations causing FHM with cerebellar symptoms are distinct from those causing pure FHM, and all recurrent mutations cause FHM with cerebellar symptoms.26–28 This genotype–phenotype correlation strongly suggests that mutations causing FHM with cerebellar symptoms have particular pathogenetic consequences in cerebellar neurons, whereas mutations causing pure FHM do not have such deleterious effects. Second, other important genotype–phenotype correlations have been demonstrated, suggesting that the existence of distinct CACNA1A mutations may explain, at least partly, the clinical variability of FHM.16,29 Finally, mutations have been detected in two sporadic cases, including a de novo mutation; thus, sporadic hemiplegic migraine is due to CACNA1A mutations in at least some cases.16,27
Are FHM genes implicated in migraine without aura and/or migraine with aura?

CACNA1A is the first gene for which an involvement in a subtype of migraine has been demonstrated. CACNA1A and the other FHM genes represent good candidate genes for migraine with aura and migraine without aura. Indeed, several clinical features support the hypothesis that migraine without aura and migraine with aura could also be neurologically channelopathies, the main factor being paroxysmal presentation with attacks lasting a few hours to a few days. Several groups have tried to implicate CACNA1A or other ion channel genes indirectly in migraine with aura and migraine without aura. Subtle subclinical cerebellar abnormalities have been discovered by the analysis of hand reaching movements in patients with migraine. This impairment was more pronounced in patients with aura than those without. By means of single-fibre electromyography, subclinical abnormalities of neuromuscular transmission have been shown in a subset of patients with migraine with aura. Single-endplate abnormalities were significantly more frequent in a group of patients affected by migraine with prolonged aura than in a group of healthy volunteers. These studies do not show that CACNA1A is involved in migraine without aura or migraine with aura, but they do provide important information about the existence of mild subclinical neuronal abnormalities in a subgroup of patients with migraine that could be used as objective markers to constitute homogeneous groups of patients for genetic studies.

The involvement of CACNA1A in the more frequent types of migraine has been analysed by linkage and association studies with contradictory results. None of the positive studies provided a direct analysis of the CACNA1A gene to detect pathogenetic mutations. As a consequence, these studies showed only that a susceptibility locus for migraine with or without aura might be located nearby or within the 19p13 region, without direct evidence that CACNA1A was the susceptibility gene. Nyholt and colleagues published a linkage study in a large family affected by migraine with aura showing positive linkage results with 19p13 markers. Subsequently, the same group reported that sequencing of the 47 exons of CACNA1A in two patients from this family did not reveal any mutations. In a second step, they conducted a linkage analysis in 82 pedigrees and a large case-control association study but did not detect any linkage or association between CACNA1A polymorphisms and migraine in these groups.

Jones and colleagues analysed families affected by migraine with aura for cosegregation of the disease with six chromosome-19p13 markers. Positive lod scores were obtained by use of multipoint model-free linkage analysis as well as a dominant affected-only model with all six tested markers. The maximum lod score (model-free lod=4.28) was obtained close to marker D19S592, which is located about 10 cM telomeric to CACNA1A. This study reinforces the positive results previously obtained by several groups and is consistent with the presence of a susceptibility locus for migraine on 19p close to, but distinct from, the CACNA1A gene.

On the basis of these studies, CACNA1A is most likely not a major susceptibility gene for migraine with aura and migraine without aura. Some explanations for this conclusion are as follows. First, as discussed above, FHM resulting from CACNA1A mutations differs from typical migraine in its unusual genetic and clinical features, including a high penetrance of hemiplegic migraine attacks and a frequent association with permanent clinically obvious cerebellar symptoms. Second, CACNA1A is implicated in less than 40% of families with pure FHM. The genes causing pure FHM (ie, without cerebellar signs) may be better candidate genes for migraine with aura and migraine without aura. However, none of the previously cited studies have definitively ruled out the possibility that CACNA1A may contribute to migraine with aura, at least in a small subset of patients and families. Three of the 16 families with migraine with aura studied by Jones and colleagues had higher lod scores with markers from the CACNA1A locus than with markers located telomeric to CACNA1A. These families may therefore show linkage to CACNA1A. Further data are needed to clarify the relations between CACNA1A and migraine with aura.

Genes encoding ion-channel subunits are major candidates for other FHM genes and remain good candidates for migraine with aura and migraine without aura. The chromosome 1q FHM gene (or genes) remains unidentified. Moreover, nothing is known about the involvement of the chromosome 1q FHM locus in migraine with aura and migraine without aura, and a third FHM gene has yet to be mapped.

Are other loci or genes implicated in migraine with or without aura?

Nyholt and colleagues have analysed X-chromosome markers in three large Australian pedigrees. They found positive non-parametric lod scores with Xq24–28 markers in two of these families. According to these researchers, this locus may be involved in the higher female prevalence of this disorder. Further studies, including a much larger panel of families, are necessary to confirm the role of an X-chromosome locus in migraine susceptibility.

As previously discussed, Jones and colleagues have shown that chromosome 19p13 contains a susceptibility locus for migraine with aura that seems to be distinct from the FHM locus. Subsequently, the same group found a significant association between migraine and five different single-nucleotide polymorphisms within the insulin receptor gene (INSR) located on 19p13. This association was found in two large case-control populations collected independently, including a total of 827 cases and 765 controls. Positive association was mainly accounted for by the results obtained in patients with migraine with aura and in one of the two populations. The five single-nucleotide-polymorphism alleles associated with migraine had no effect on INSR transcription, translation, protein expression, and INSR-mediated functions. This study did not exclude the possibility that the five polymorphisms in INSR could be in linkage disequilibrium with another gene close to INSR. The nature of INSR gene involvement in migraine remains to be
elucidated and present evidence does not allow us to consider INSR as a migraine gene.

Recently Wessman and colleagues, published the first genome-wide linkage analysis in migraine with aura, which led to the mapping of a susceptibility locus on chromosome 4q24. This large study included 50 Finnish families with vertical transmission of migraine with aura consistent with dominant inheritance. The Finnish population is genetically homogeneous owing to a small number of founders. The researchers analysed only families affected by migraine with aura because of data suggesting that genetic factors may be more important in migraine with aura than in migraine without aura. Moreover, only individuals affected by migraine with aura were considered to avoid heterogeneity and eventual phenocopies that might have been introduced by inclusion of migraine without aura. Statistically significant linkage was found with the marker D4S1647 by parametric two-point linkage analysis on the assumption of a dominant mode of transmission, as well as multipoint parametric and non-parametric analyses. The chromosome 4q24 locus was the only one to give significant linkage results in this panel of families. In addition, non-significant nominal evidence of linkage (ie, positive lod scores <3, p>0.001) was found with other markers, including markers on 19p13.2, 1q42.2, and Xp (but not Xq). The 19p13 marker that gave a positive lod score is located telomeric to CACNA1A, and the 1q42 marker that gave a positive lod score is located telomeric to both FHM loci (1q21–23 and 1q31). The results of this study strongly suggest a susceptibility locus for migraine with aura on 4q24.

Lea and colleagues recently published a linkage study in a large family affected by typical migraine. They reported significant linkage using a chromosome 1q31 marker located within the locus for FHM. These researchers also conducted a linkage analysis of 82 pedigrees and a case-control association study, which produced positive linkage results (maximum lod score of 1.24, p=0.008) and significant association results (p=0.010). These observations will need to be confirmed by other researchers but they reinforce the hypothesis that pure FHM may be better candidate genes for migraine with aura than in migraine without aura. Moreover, only individuals affected by migraine with aura were considered to avoid heterogeneity and eventual phenocopies that might have been introduced by inclusion of migraine without aura. Statistically significant linkage was found with the marker D4S1647 by parametric two-point linkage analysis on the assumption of a dominant mode of transmission, as well as multipoint parametric and non-parametric analyses. The chromosome 4q24 locus was the only one to give significant linkage results in this panel of families. In addition, non-significant nominal evidence of linkage (ie, positive lod scores <3, p>0.001) was found with other markers, including markers on 19p13.2, 1q42.2, and Xp (but not Xq). The 19p13 marker that gave a positive lod score is located telomeric to CACNA1A, and the 1q42 marker that gave a positive lod score is located telomeric to both FHM loci (1q21–23 and 1q31). The results of this study strongly suggest a susceptibility locus for migraine with aura on 4q24.

Finally, many association studies have been performed in migraine. Positive associations have been found between migraine and polymorphisms within genes encoding the dopamine D2 receptor, the human serotonin transporter, catechol-O-methyltransferase, endothelin type A, dopamine β-hydroxylase, and 5,10-methylene-tetrahydrofolate reductase. These various candidate polymorphisms have been chosen on the basis of the hypothesis that the genes containing them encode proteins that are thought to be involved in migraine. However, further studies are needed to find out whether the alleles associated with migraine have any biological effect. Moreover, most of these studies are single reports and await replication.

**Summary**

Migraine is a complex polygenic multifactorial disorder in which genetic factors interact with environmental factors. Several studies have suggested that genetic factors may be more important in migraine with aura than in migraine without aura. The number of different susceptibility loci and genes is still unknown (table 2). FHM, which is the only known monogenic variety of migraine, is caused by mutations in the calcium channel gene CACNA1A located on chromosome 19p13 in about 50% of families. In those families, FHM is thus a neuronal channelopathy. However, CACNA1A is most likely not a major susceptibility gene for the other types of migraine. Other FHM genes, responsible for pure FHM, may be better candidate genes for migraine with aura and migraine without aura. Recent data suggest that a susceptibility locus for migraine with aura is located on 19p13, telomeric to CACNA1A. Another susceptibility locus for migraine with aura has been mapped on 4q24 by a genome-wide linkage analysis in 50 Finnish families. Finally, a migraine susceptibility locus may reside on Xq. Several associations have been found between polymorphisms within various candidate genes and migraine, but the significance of these findings is still unclear. Future objectives in migraine genetics are: to identify other FHM genes and to analyse their role in migraine with aura and migraine without aura; to identify the susceptibility gene or genes for migraine with aura located within the 19p13 locus and to find out whether CACNA1A is implicated; and to replicate the 4q24 significant linkage results in migraine with aura in a non-Finnish population and identify the underlying susceptibility gene.

**Table 2. Susceptibility loci identified in FHM and in migraine with aura**

<table>
<thead>
<tr>
<th>Migraine subtype</th>
<th>Chromosome</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHM with cerebellar symptoms</td>
<td>19p13</td>
<td>CACNA1A</td>
</tr>
<tr>
<td>Pure FHM</td>
<td>19p13</td>
<td>CACNA1A</td>
</tr>
<tr>
<td>Pure FHM</td>
<td>1q21–23</td>
<td>Unknown</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>1q31</td>
<td>Unknown</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>19p13.2</td>
<td>Distinct from CACNA1A? INS?</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>4q24</td>
<td>Unknown</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>Xq</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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AD wrote this review with assistance from MGB. ETL and MGB critically evaluated the review at its various stages.

**Conflict of interest**

We have no conflict of interest.

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