REVIEW
Migraine Susceptibility Genes
Rahel Engeli\textsuperscript{1}; Gerd Folkers\textsuperscript{1}; Georg Schönächler\textsuperscript{1}; Reto Agosti\textsuperscript{2}

\textsuperscript{1}Department of Chemistry and Applied BioSciences
Institute of Pharmaceutical Sciences
Professorship of Pharmaceutical Chemistry
Winterthurerstrasse 190
CH-8057 Zürich

\textsuperscript{2}Kopfwehzentrum Hirslanden Zürich
Münchhaldenstrasse 33
CH-8008 Zürich
info@kopfwww.ch
Mechanisms of diseases are never simple. Many systems are involved in migraine attacks. Genetic and environmental predispositions as well as endogenous and exogenous precipitating factors contribute to the expression of migraine. [1]

To investigate the importance of genetic and environmental factors to the etiology of migraine, many different epidemiologic approaches were done: Pedigree studies, family studies, twin studies and adoption studies. A convincing prove of the genetic impact on migraine is the significant higher concordance rate in monozygotic- than in dizygotic twins. In addition, first degree relatives of probands with migraine have, compared to the risk in the general population, increased risks for both migraines with and without aura. All these different study methods propagate a genetic component of the migraine disease. Furthermore, the genetic impact is greater in migraine with aura than in migraine without aura. [2-7] Genetic factors seem to set the individual threshold, and both endogenous and exogenous factors modulate this set point, making migraine a multifactorial disorder. [8]

This Review focuses primarily on the genetic background of migraine. During the past 10 years, significant progress was made in the identification of susceptibility genes in migraine diseases.

**FROM PHENOTYPE TO GENOTYPE**

Due to the absence of any proven biological or radiological marker, the diagnosis of migraine is purely clinical. [9] With the first promulgation of explicit diagnostic criteria for all headache disorders by the International Headache Society in 1988, significant advances were made in describing the epidemiology of migraine. In the past 15 years numerous studies have used IHS criteria and the variation among these studies is considerably less than before 1988. [10] The revised classification of the International Headache Society (IHS-II 2004) distinguishes between migraine without aura (MO), migraine with (typical) aura (MA), familial hemiplegic migraine (FHM), sporadic hemiplegic migraine (SHM) and others. [11] The IHS criteria are based mainly on phenotypic manifestations of migraine. However, this phenotypic view of migraine is not easily brought together with its genotypic background.

There are different ways of combining the phenomenological with the genetic manifestations of migraine:
A “homological” view considers the two disease models as congruent. Based on the same logic, they may explain each other through a simple linkage. In analogy to the properties of light which can be defined by particles (photons) or by waves, phenomenology and genetics are two different approaches to the properties of migraine. The goal is to harmonize these models in a way they can work together and do not contradict each other. Unfortunately, the promising linkage between most migraine phenotypes and genotypes is not a Mendelian inheritance- or expression pattern. In many ambitious efforts, scientists try to discover this missing key by susceptibility genes through linkage studies.

Linkage studies are genetic study designs to identify markers for human diseases without knowledge of an abnormal gene product involved in their etiology. Information on the distance between specific DNA segments, or markers is used to quantify the association between these DNA markers and a particular disease. If a close association is found, probable candidate genes located nearby on that chromosome which may actually cause the disease might be identified. In complex diseases as migraine, the ultimate output of such a gene may be altered by literally thousands of factors that may influence its expression. [12] This
Epigenetic complexity has made the clearing up of susceptibility genes and inheritance patterns a most difficult issue.

A second view on genotype- and phenotype relation may be called the “heterological” view. It bases on the fact that migraine is considered to be a clinically and genetically heterogeneous disorder. [13] Heterogeneity makes an unambiguous linkage between phenomenology and genetics a black box. According to this view, the relation between phenotype and genotype is more complex than one could comprehend it. Due to this missing association, the genetic and phenomenological models are looked at as heterological and therefore not treatable with homological methods. The implications for migraine research will be discussed later on.

In nature, a very famous example of convergent heterogeneity is given: The retina of nocturnal mammals is amazingly similar to the deep-sea fish retina, even though, these eyes have a completely different genetic origin. [14] These organs must have been exposed to a similar selection pressure, so they developed in the same way. Analogously, different migraine genotypes can lead to the very same phenotypic migraine performance. In consequence, the observed convergence in migraine must be due to similar environmental selection pressures. Conversely, identical migraine genotypes can be manifested in different migraine phenotypes (divergence), probably due to different environmental dispositions. Adaptations, due to selection pressure, usually result in an advantaging life disposition. If the convergent heterogeneity in migraine diseases is caused by such a selection, one might argue about possible advantaging dispositions of a migraineur.

- Familial Hemiplegic Migraine

Channelopathy and Heterogeneity

FHM is a rare severe, autosomal dominant subtype of migraine with aura and the only migraine type with a Mendelian mode of inheritance (prevalence 0.001%). In 50% -75% of the families with FHM studied, there are several mutations in the calcium-channel gene CACNA1A on chromosome 19p13. Associated with mutations in the CACNA1A gene are episodic ataxia type 2 (EA-2) and spinocerebellar ataxia (SCA6) FHM with permanent cerebellar signs). [15-18] Some CACNA1A mutations may also cause epilepsy. [19] CACNA1A encodes the Ca2.1 Subunit of brain-specific voltage-gated P/Q-type calcium channels. [18] Approximately 10% -20% of the reported FHM families are linked to chromosome 1q21-23 and 1q31. In the remaining families, linkage to both chromosome 1q21-23, and 19p13 was excluded, suggesting at least a third chromosome for FHM. Chromosome 1q31 may be a good candidate for another neuronal Ca2+ channel alpha-1 subunit, named CACNA1E. This gene needs to be confirmed by further investigations. [20-24] Recently, the 1q23 loci could be assigned to the gene ATP1A2, a Na+/K+-ATPase pump.

The superfamily of voltage-gated ion channels includes the K+, Na+ and Ca2+ cation channels. The channel-macromolecule’s functions include maintenance of electric potential across cell membranes, secretion and signal transduction. Mutations in these voltage-gated ion channel genes have been shown to cause or have been implicated in a number of other disorders: Paralytic disorders of skeletal muscle are mainly caused by mutations in Sodium or Calcium channels. The majority of Long QT-Syndrome, a result of abnormalities of myocardial repolarization, is due to mutations in sodium or potassium channels. [25] Chloride channels play a role in Myotonia Congenita Thomsen and Myotonia Congenita Becker disease. Interestingly, different mutations within the same ion channel gene may cause quite distinct clinical disorders; conversely, mutations in different ion channel genes may result in similar
phenotypes. This reflects the possible convergent or divergent characters of channelopathies [26]

**Na⁺/K⁺-ATPase associated with FHM2**

In very recent surveys mutation in any FHM patients on chromosome 1q23 could be assigned to a defect in ATP1A2 (loss-of-function) that encodes the alpha 2 subunit of the Na⁺/K⁺-ATPase pump. To contrast FHM, caused by mutations in the P/Q-type calcium channels, FHM, due to ATP1A2, is named FHM type 2 (FHM2). It is the first time, mutations of Na⁺/K⁺-ATPase subunits could be associated to a genetic disease like FHM2. The Na⁺/K⁺ pump is present in every human cell and ubiquitous in animals. As a key regulator of cellular ion homeostasis, it is important to several cellular functions. To evaluate the functional consequences of two detected amino-acid replacements (M731T and R689Q) in different areas of the Na⁺/K⁺ pump protein structure, transfection experiments in cells were carried out. Cells transfected with the two mutant isoforms showed rapid mortality typical of cells lacking Na⁺/K⁺ pump activity. The data indicate that both missense mutations are independently sufficient to inhibit Na⁺/K⁺ pump activity, but they do not affect assembly with other subunits or translocation to the cell membrane. A functional haploinsufficiency of Na⁺/K⁺ pump catalytic subunit might therefore be responsible for the FHM2 disorder linked to chromosome 1q23. [27] Thereby, two synergistic events lead to FHM2: An increase in extracellular K⁺ producing a wide cortical depolarization and a local boost in intracellular Na⁺ which promotes an increase in intracellular Ca²⁺ through the Na⁺/Ca²⁺ exchanger. An increase in intracellular Ca²⁺ would resemble the effect of CACNA1A or CACNA1E mutations in FHM1, where a gain-of-function increases the open probability. [28,29]

A second study on this issue was able to associate the two identified loss-of-function mutations in the ATP1A2 gene with different phenotypes. The M731T mutation was linked to a family with pure FHM2, while R689Q was identified in a family which FHM2 and benign familial infantile convulsions (BFIC) partially cosegregate. Interestingly non-BFIC Epilepsy has been reported in other families with FHM2. [20,23,24] These findings confirm that migraine and epilepsy, at least in part, have overlapping mechanisms involving dysfunction of ion transport.

The FHM2 causing M731T and R689Q mutations are present in a large intracellular loop that harbors the ATP-binding and hydrolase domains and has been implicated in several functions. Homozygous ATP1A2 knockout animals are severely affected, have reduced contractility of cardiac and skeletal muscles, and die at birth because of failure to breathe. Heterozygous animals, however, grow normally and show increased muscle contractility. [30] A increase of extracellular K⁺, due to a haploinsufficient Na⁺/K⁺ pump, will facilitate cortical spreading depression (CSD), the likely mechanism for MA. [31]

These study supports the concept that dysfunction of ion transportation are important in the pathogenesis of FHM presumably through facilitation of CSD. [19]

<table>
<thead>
<tr>
<th>locus</th>
<th>gene</th>
<th>effect</th>
<th>disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>19p13</td>
<td>CACNA1A</td>
<td>Gain of function intracell. [Ca²⁺] ↑</td>
<td>FHM1 associated with: EA-2 SCA6</td>
</tr>
<tr>
<td>1q31</td>
<td>CACNA1E</td>
<td>Gain of function intracell. [Ca²⁺] ↑</td>
<td>FHM1</td>
</tr>
<tr>
<td>1q23</td>
<td>ATP1A2</td>
<td>Loss of function intracell. [Ca²⁺] ↑</td>
<td>FHM2</td>
</tr>
</tbody>
</table>
• **FHM AS A MODEL FOR MORE PREVALENT MIGRAINE**

So far, many genetic studies on FHM were done, thus it is the only migraine-like disorder with an autosomal dominant disorder, even though FHM appears to be a heterogenic channelopathy. It has been proposed that FHM and MA or MO may have a similar genetic etiology and therefore FHM should provide a useful model for investigating the more prevalent types of typical migraine with a much more complex transmission. However, studies show very contradictory results concerning this issue. The mode of transmission of migraine in families is still unclear, but it is widely believed to be multifactorial and polygenic, while FHM seems to be monogenic. [32] Genetics is done with the hope to discover a major susceptibility gene, which existence cannot be excluded. [33] The identification of susceptibility genes has become a major goal in migraine research during the last decade.

**CACNA1A – a susceptibility gene in MA or MO?**

Several studies aimed the question, whether the CACNA1A gene contributes to more common types of migraine. [34-36] The investigations of CACNA1A mutations in non-FHM migraine-forms lead to conflicting results.

A Danish study found a highly significantly increased risk of MA in probands with FHM and their first degree relatives, while it denies a sharing of genetic mechanisms between FHM and MO. It predicts that the genetic dysfunction causing attacks of FHM also may cause attacks without motor weakness, i.e. typical MA. Yet, the study does not answer if FHM gene mutations can also cause MA in the absence of FHM attacks. [22] A Dutch study confirms the involvement of the CACNA1A region especially in MA. It proposes the P/Q calcium channel alpha1A-subunit gene on chromosome 19p13 as “aura gene” and the involvement of different alleles in FHM and MA, but not MO. [37] An Australian study investigated 82 independent typical migraine pedigrees and a large case control group to determine whether the CACNA1A gene was implicated in typical migraine in the general Caucasian population. In contrast to investigations in FHM-probands, these results did not reveal any mutations within any of the P/Q-calcium-channel-protein coding segments examined. No linkage or association was detected. These findings imply that if the CACNA1A gene is involved in typical migraine, its contribution is very modest and therefore difficult to discern. [38] The absence of CACNA1A-mutations in MA-patients was confirmed by a German investigation. This indicates a distinct molecular basis for FHM and MA. [39] A Finish and an Italian linkage study in families with typical migraine did not find a significant linkage to chromosome 19 either. [40,41] Also the authors of a study with a large dataset of 64 Canadian MA families did not find evidence to support an MA susceptibility gene in the region of 19p13. They are convinced that neither CACNA1A nor the chromosomal region surrounding CACNA1A is linked to MA. [42] In contrast, a research group from USA found a MA susceptibility locus on chromosome 19p13 but which is distinct from the FHM causing CACNA1A gene. With strong statistical evidence they suggest that the markers cosegregating with MA define a locus that is distinct from the FHM gene. [43]

In conclusion, it seems that the impact of a possible MA susceptibility locus on chromosome 19p13 is low.

---

05.05.2015
The possible second neuronal Ca\textsuperscript{2+} channel alpha-1 subunit gene CACNA1E on chromosome 1q31 that is implicated in FHM1 was investigated by an Australian study. In attempt to substantiate the involvement of 1q31 in migraine, both linkage and association studies were conducted in three large multigenerational Caucasian pedigrees. In one pedigree (MF14) a maximum allele-sharing LOD score of 3.36 was achieved for MA. The Australian group therefore strongly suggests an involvement of chromosome 1q31 in FHM and MA and supports the idea that a common defective gene may be influencing both FHM and typical migraine, specifically MA. Interestingly, in the same pedigree (MF14) linkage of chromosome Xq markers to migraine was previously reported. For ATP1A2, the causing gene of FHM2 on chromosome 1q23, no association with more common types of migraine was found (Data not published).

**FHM INDEPENDENT SUSCEPTIBILITY LOCI FOR MA AND MO**

The researches described so far, were based on FHM as a model for more common migraine types. Genetic screening or linkage analyses were done in areas of known FHM loci. In contrast to this FHM-dependent-procedure, some more resent studies are based on genome wide linkage analysis. Independent of any yet known FHM loci, the whole genome of several migraine populations has been screened for new migraine susceptibility loci.

**Genome wide screens**

**MA susceptibility**

The first genome wide screen being published reports results of 50 multigenerational Finnish families, ascertained for MA and showing intergenerational transmission of migraine with aura. The families were screened for linkage in the 22 autosomes and the X chromosome using 350 polymorphic microsatellite markers, with an average intermarker distance of 11 cM. With parametric two-point linkage analysis, the Californian group found evidence of linkage between MA-phenotype and chromosome 4q24 (marker D4S1647) with a maximum LOD score of 4.20, under the assumption of a dominant mode of inheritance. Multipoint and nonparametric analyses supported this linkage. However, the group observed no other significant linkage in any other chromosomal region.

A second MA susceptibility locus was detected by an English research group. Genome wide screens were undertaken in 43 large multigenerational Canadian MA-families, with an apparent autosomal dominant pattern of transmission. With 395 microsatellite markers parametric linkage analysis revealed a MA-susceptibility locus on 11q24 with a two-point LOD score of 4.2 and a multi-point parametric LOD score of 5.6. Interestingly this genome screen did not provide any evidence to support linkage at the 4q24 locus (LOD score < -7). Again, no support for linkage in other known susceptibility regions was found. The lack of consensus amongst linkage studies may be an indication of the heterogeneity that is inherent for MA.

The 11q24 region harbors several ion channel genes what might supports the theory of the ion-channel-disorder-etiology of MA.

**MA and MO susceptibility**

A Swedish group performed a genome wide screen in 52 individuals belonging to a large four-generation migraine pedigree from northern Sweden. No difference in penetrance between MA and MO or between men and women was assumed. Among the assessed
probands both MA and MO was present and not handled differently. Four hundred polymorphic markers were applied. The results of the study give evidence of linkage of MA and MO with chromosome 6p12.2-p21.1 (marker D6S452) with a maximum two-point LOD score of 5.41 under a dominant model. The authors suggest one or more common genetic factors in both MA and MO in the discussed Swedish family. They report no linkage to other known susceptibility loci. [48]

**MO susceptibility**

An Italian study reports linkage data from a genome wide screen of the 22 autosomes in a large Italian pedigree in which MO segregates as an autosomal dominant trait. Any association between MO and FHM or MA was excluded. Using 482 polymorphic microsatellite markers, evidence of linkage between the MO phenotype and chromosome 14q21.2-q22.3 (flanked by markers D14S1027 and D14S980) was obtained with a multiparametric maximum LOD score of 5.25. [49]

<table>
<thead>
<tr>
<th>Study</th>
<th>year</th>
<th>Sample source</th>
<th>#Makers</th>
<th>Chromosome LOD Scores*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>population</td>
<td>type</td>
<td>4q24</td>
</tr>
<tr>
<td>Wessman et al. [46]</td>
<td>2002</td>
<td>Finnish</td>
<td>MA</td>
<td>50 families, 43 individuals</td>
</tr>
<tr>
<td>Cader et al. [47]</td>
<td>2003</td>
<td>Canadian</td>
<td>MA</td>
<td>43 families, 420 individuals</td>
</tr>
<tr>
<td>Carlsson et al. [48]</td>
<td>2002</td>
<td>Swedish</td>
<td>MO</td>
<td>1 family, 52 individuals</td>
</tr>
<tr>
<td>Soragna et al. [49]</td>
<td>2003</td>
<td>Italian</td>
<td>MO</td>
<td>1 family, 52 individuals</td>
</tr>
</tbody>
</table>

*two point LOD score

**Female prevalence- X-linkage?**

For both, MO and MA there is an unequal sex distribution. An X-linked dominant pattern of inheritance in typical migraine may cause the increased female prevalence. In such a case there would be no male-to-male transmission of migraine but affected males would transmit to all daughters and therefore support the female preponderance of 2:1 to 3:1. According to this hypothesis the apparent male-to-male transmission could only occur in non-X-linked pedigrees due to heterogeneity. In fact, an X chromosomal susceptibility gene could give explanation for the higher prevalence in women than in men and the increased risk of migraine in female relatives of male probands. [50]

The results of a linkage analysis with 28 microsatellite markers spanning the entire X chromosome, with an average separation of 6.9cM, indicated the presence of at least two genes involved in migraine. [45]

The involvement of an X chromosomal component in migraine could be in the form of a causal X chromosome defect or an interacting X chromosomal component. Another study, investigating two large multigenerational pedigrees (MF7 and MF14), was able to reduce the potential location of a migraine susceptibility gene to three distinct regions towards the telomere of Xq (Xq24-q28). [44]

Besides an X chromosomal defect, also a Y chromosomal protection gene would support the observed effect of female preponderance. This hypothesis has to be proved.
• THE “HETEROLOGICAL” VIEW

Despite considerable efforts by clinicians and scientists around the world, the “genetic” cause of the common forms of migraine and other types of headache remain largely unknown. It is univocal that only approximately 50% of migraine susceptibility can be attributed to multiple, additive genes, with the remaining component attributable to what appears to be a wide variety of environmental factors. Therefore, in most migraine types the genetic contribution to clinical susceptibility seems to be relatively minor and certainly multigenic.

Due to these observations, the ongoing search for the genetic basis of migraine susceptibility would make a daunting task. During the last decade, the linkage between phenomenology and genetics in migraine remained a black box. The “homological model” seems to be difficult to translate into practice, due to the polygenic and heterogenic conditions.

An example of a more heterological view is the critical voice of Steven J. Peroutka. Peroutka, suggest to review the lessons learned from more successful molecular genetic analyses of other common human disorders. He proposes the example of cardiovascular disease, another common public health problem for which genetic and environmental determinants coexist. In contrast to migraine susceptibility research, molecular genetic research in cardiovascular disease has been able to focus successfully on very specific biochemical pathways. Molecular genetic analyses indicate that the interaction of more than 100 genes is likely to be involved in determining an individual’s inherent “susceptibility to cardiovascular disease. The search of these relevant “susceptibility” genes in cardiovascular disease has been significantly facilitated by the knowledge of the specific biological pathways involved in lipid metabolism.

According to the cardiovascular-disease-example, Peroutka considers migraine as likely to be influenced by a very large number of genes that however contribute only few to an individual’s migraine susceptibility. He proposes that a focused candidate gene analysis of critical biological pathways will be more fruitful than searching the entire genome for a “migraine susceptibility gene”. [51]

As proposed by Peroutka, Mochi et al. investigated migraine susceptibility gene, starting from a general biochemical pathway- the lipoprotein metabolism: [52]

Vascular co-morbidity- LDL Polymorphism

Based on the hypothesis that genes involved in cholesterol regulation and coagulation make interesting candidates for migraine vascular co-morbidity, the Low Density Lipoprotein Receptor (LDLR) was investigated for migraine susceptibility. LDLR, the major gene involved in cholesterol metabolism, localized in 19p13.2, is peculiar in that it is a mosaic of exons shared with other genes and encodes proteins homologous to complement factors, plasma proteases of the blood clotting system and the precursors for epidermal growth factor (EGF). [53] Many polymorphisms are described along the LDLR gene. Some of them are considered to be nonfunctional because they seem not to affect plasma cholesterol levels in the general population. However, where LDLR exons share homology with clotting and coagulation proteins, the peculiar structure of the LDLR gene may activate these polymorphisms in functions other than cholesterol binding and internalization.

In an Italian survey two very informative LDLR gene polymorphisms (G142A transition and (TA)n repeat) were chosen and an association study between these polymorphisms and MA (140 probands) and MO (220 probands) was performed. While the genotyping frequencies at G142A showed no significant differences between the control group and MA or MO, (TA)n polymorphisms were much frequenter in MO than in MA or CONTR. These results could be
taken to implicate LDLR in the genetic predisposition to MO. The mechanisms of liability of the LDLR gene to MO remain unknown at least in the analyzed population. [52]

Summary
Migraine is clinically divided into different forms: Migraine without aura (MO), migraine with aura (MA), Familial Hemiplegic Migraine (FHM) and others. This division is not easy comprehensible on the molecular basis. Genetics should solve the interrelations between the different disease manifestations. Many genetic approaches were done to reveal the mystery of migraine diseases.

FHM is the only migraine form with a Mendelian pattern of inheritance. This rare disease could be partly assigned to mutations in a brain specific Ca\(^{2+}\) -channel (CACNA1A and CACNA1E) and the Na\(^+/K^+\)-Atpase (ATP1A2). These mutations in ion channel subunits give indication of FHM as a channelopathy. It is also learned that different mutations in different channels can lead to the same disease manifestation or conversely. FHM is a heterogeneous disorder of which not all possible genes have been detected. Due to its simple inheritance pattern, FHM served as model for more common migraines. Mutations found in FHM patients were investigated for their susceptibility to MA or MO. Conversely MA and MO patients were screened for the mutations involved in FHM. While the first method suggested a common genetic cause for both FHM and typical migraine, the latter technique disagreed on the same issues. The involvement of chromosome 1q31 (CACNA1E) in migraine is the only FHM-locus-involvement that has not yet been contradicted. These contradictory results may indicate an only small contribution of FHM genes in more common migraine.

Independent genome wide screens in migraineurs detected many susceptibility loci for the disease. Nameable mutations were found on chromosomes 11q24, 4q24, 6p, and 14q. However, most of them exclude each other.

Due to the significantly higher prevalence in women than in men, a migraine susceptibility locus was proposed on the X chromosome. A possible locus is placed on chromosome Xq24-q28. Instead of a dominant migraine-causing mutation on the X chromosome, also a migraine protection gene on the Y chromosome is imaginable as explanation for the female preponderance.

An interesting detection is the possible migraine susceptibility to a LDL-receptor polymorphism. This mutation in the main responsible gene for cholesterol metabolism might give an important hint to the vascular-co morbidity in migraine.

Peroutka’s quite critical voice requests to learn from more successful molecular genetic analyses of other common human disorders, like the cardiovascular disease. Instead of migraine susceptibility research, the focus should be more on specific biochemical pathways that might be involved in migraine.


