

MIGRAINE GENETICS, A FASCINATING STORY TOWARDS BETTER MIGRAINE PROPHYLACTICS

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Migraine is clinically and pathophysiologically a heterogeneous neurovascular disorder. It would probably be better to speak about “*The Migraines*”. Various combinations of genetic and non-genetic susceptibility factors are likely to underlie the different forms. Dissecting the complex genetics of migraine will undoubtedly help to unravel the trigger mechanisms of migraine attacks, ultimately leading to much needed improved prophylactic agents. I apologize to those colleagues whose work could not be mentioned due to space restrictions.

Unravelling the pathogenesis of Familial Hemiplegic Migraine (FHM)

From families to linkage

For most clinicians, genetics remains a “black hole”. So it used to be for me as well... until early 1991. Then, I saw, within the timeframe of two weeks, two different patients from reportedly two separate families with Familial Hemiplegic Migraine (FHM). FHM is a rare, autosomal dominant subtype of migraine with aura associated with hemiparesis. Additional ictal and interictal neurological features may occur as well. There are also sporadic cases (Sporadic Hemiplegic Migraine; SHM). FHM and SHM are frequently misdiagnosed with epilepsy, stroke, encephalitis, or conversion.

The two FHM families were living in a small area in the eastern part of the Netherlands (one of the few parts which is actually above sea level). Two different families, with such a rare disease, in such a small, relatively isolated part of the country? Serendipity? Of course, the two families turned out to be related, making one big family. I discussed this family with Keith Campbell. He kindly “offered” me an extended FHM family that he was seeing at the Mayo Clinic in Rochester. Coincidence? Dr. Caroline Grubben spent over three weeks of travelling through nine different States across the US to clinically characterize this family and to collect blood for DNA. Suddenly we had two extended, multigenerational families with FHM. A “geneticists dream” for linkage analysis. Rune Frants, our geneticist, just had linked another rare neurological disorder called Fascioscapulohumeral Dystrophy (FSHD). He had used microsatellites, a novel method in those days, and was enthusiastic to repeat the same trick for FHM. The Leiden migraine genetics story had started.

Unfortunately, there also is a “geneticists nightmare”, called phenocopies. These are family members who present with the same clinical picture, but actually don’t have the (same) disease. Phenocopies are more common in diseases without objective symptoms and can prove disastrous for linkage analysis. Our FHM families had their fair share of phenocopies and thus took it “just a tiny bit longer” than expected to find the disease locus. Actually, we were scooped. The Leiden team had tried several thousands of, at that time expensive, DNA markers without any success... and then, at the Paris IHS meeting in 1993, the French team lead by Marie-Germaine Bousser and Elizabeth Tournier-Lasserre announced linkage of FHM to chromosome 19p13. They had just linked another neurovascular disorder with migraine, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leuco-encephalopathy (CADASIL), to that chromosomal location. Because of the clinical similarities, they decided to have a go with ... only three (sic!) of their CADASIL markers. Again serendipity. A major disappointment from the Leiden perspective, a major breakthrough for migraine science!

From locus to disease gene

Identifying the actual disease gene can prove challenging, as a locus may still contain many genes. For instance, for Huntington’s Disease it took 10 years from linkage to gene. But we were luckier. First, we could confirm linkage to 19p13 in several of our FHM families (meanwhile we had collected additional families). Then, in early 1996, Roel Ophoff and Gisela Terwindt, by using the combined genetic information from these families, discovered the first gene for FHM: *CACNA1A*. The gene encodes the pore forming, ion-conducting α_{1A} subunit of neuronal P/Q type $Ca_v2.1$ calcium channels. Certain *CACNA1A* mutations may cause FHM1 of various degrees of severity, whilst other mutations are associated with a range of other neurological disorders, including episodic ataxia type 2 (which is often associated with migraine), progressive forms of ataxia, various forms of epilepsy, and fatal mild head trauma-triggered migraine (see below). In the subsequent years, two more genes were identified for FHM: the FHM2 *ATP1A2* gene on chromosome 1q23 by Giorgio Casari’s team in Italy (2003) and the FHM3 *SCN1A* gene on chromosome 2q24 by Martin Dichgans and colleagues in Germany. *ATP1A2* encodes the α subunit of a glial cell Na^+ , K^+ - ATPase pump and *SCN1A* codes for the α subunit of a neuronal voltage-gated sodium ($Na_v1.1$) channel. A prime example of heterogeneity: mutations in three different genes encoding three different proteins all cause FHM. Based on existing linkage data in other families, additional FHM genes are likely to exist.

From gene mutations to disease mechanisms: transgenic mouse models for migraine

Once a disease gene has been identified, the next challenge is to dissect the functional consequences of the gene mutations. This will lead to a better understanding of the underlying disease mechanisms. $Ca_v2.1$ channels control Ca^{2+} influx and through that neurotransmitter release. In cellular models, FHM1 *CACNA1A* mutations were shown to increase Ca^{2+} influx, predicting increase of neurotransmitter release *in vivo*. In order to characterize the functional changes *in vivo*, Arn van den Maagdenberg generated two knock-in mouse models carrying human FHM1 mutations. The first transgenic FHM1 mouse model carried the FHM1 R192Q mutation, which in humans is associated with mild FHM, as close as possible to common migraine. The second FHM1 mouse model carried the S218L *CACNA1A* mutation, which in humans is associated with probably the most extreme end of the clinical migraine spectrum: fatal mild head trauma-triggered migraine. These unfortunate subjects may die after a trivial head trauma which triggers a migraine attack that is associated with excessive cerebral oedema. We hypothesized that changes that are truly relevant for a predominantly female disease such as migraine, would show a correlation with gender and clinical severity. Although full characterization of both models is still underway, this hypothesis is probably largely correct.

FHM1 R192Q mutations enhanced Ca^{2+} influx, increased basal and evoked release of neurotransmitters, and reduced the triggering threshold for cortical spreading depression (CSD); the effect on CSD was greater in female FHM1 mice and could be reverted by ovariectomy. There was also a gene-dosage relationship: greater abnormalities in homozygous than in heterozygous FHM1 mice. The greatest increase in neurotransmitter release and susceptibility for CSD was found in the "severe" S218L FHM1 model. Remarkably, FHM1 mice also showed migraine-like clinical features such as headache, photophobia, exaggerated adaptation to time zone shifts, and in the S218L model, episodes of hemiparesis!

It is appropriate to emphasise that the characterization of the mouse models would not have been possible without collaborations with electrophysiologists and neurobiologists in Leiden (Jaap Plomp and Joke Meijer), Rotterdam (Chris de Zeeuw), Italy (Daniela Pietrobon and Tommaso Pizzorusso), Boston (Mike Moskowitz and Cenk Ayata), and Montreal (Jeffrey Mogil and Mona Lisa Chanda). Many of these collaborations were funded by the European Commission (EUROHEAD).

Different genes, yet the same disease mechanism?

The studies in the two $\text{Ca}_v2.1$ calcium channel mouse models strongly suggest a pivotal role for CSD due to increased availability of glutamate in the synaptic cleft. This is relevant as CSD is the underlying mechanism for migraine aura and a putative trigger to activate trigeminovascular "headache mechanisms". Is it conceivable that similar mechanisms would play a role in FHM2 and FHM3? An elegant hypothesis states that FHM3 mutations might cause hyperexcitability with increased release of glutamate and that FHM2 mutations reduce the re-uptake of glutamate and K^+ from the synaptic cleft into glial cells. The net result of FHM mutations in all three genes would thus be increased levels of glutamate or K^+ in the synaptic cleft, which would increase the susceptibility to CSD.

Step two: from FHM to common migraine types

Monogenic subtypes as "pathway guides" for common complex diseases

Sceptics will ask: how relevant are the FHM and mouse model findings for the "real thing"? To answer this question, it is important to consider the general research hypothesis that rare monogenic subtypes of common multifactorial disorders may serve as valid models to unravel the genetics and, even more important, the underlying mechanisms for the common complex forms. Examples are Alzheimer's and Parkinson's Disease where genes and, more importantly, mechanisms for rare monogenic subtypes were also found to be involved in the common complex forms. Genes for rare disorders may thus be regarded as "common pathway guides".

FHM as a subtype of migraine with or without aura

A number of clinical and genetic arguments strongly suggest that FHM may indeed serve as a valid model for migraine with and even without aura. Apart from the hemiparesis, the aura and headache features of attacks of FHM, SHM and common migraine are identical and the headaches respond equally well to triptans. The vast majority of FHM and SHM patients also have common non-hemiplegic migraine attacks. Finally, all three forms share similar trigger factors and similar prophylactic agents. FHM mutations are also found in patients with SHM or common non-hemiplegic migraine (incomplete penetrance). This would suggest a clinical and genetic spectrum from FHM to SHM and migraine with and without aura.

Other monogenic subtypes of migraine

Common types of migraine can also be part of monogenic neurovascular syndromes such as CADASIL (*NOTCH3* mutations), Retinal Arteriolar Tortuosity and Leucoencephalopathy (*COL4A1* mutations), Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL: *TREX1* mutations) and Familial Advanced Sleep Phase Syndrome (FASPS: CK1 δ -T44A mutation). The functional characterization of the disease gene mutations is underway.

Migraine as a multifactorial disorder with complex genetics

In the early nineties, Michael Bjorn Russell, Jes Olesen (Copenhagen) and later others, convincingly showed that migraine with and without aura are multifactorial disorders. Complex genetic factors are estimated to account for up to 61% of the heritability, whereas non-genetic environmental factors may contribute for up to 39%. Although some believe that migraine with and without aura are different diseases, it is more likely that both are different clinical expressions of the same disorder. A wide range of candidate genes and loci have since been associated to migraine with or without aura, but for most findings replication is lacking. The most promising and multiply replicated genetic associations include the *MTHFR* gene and loci at 4q24 and 5q21. The latter loci were found in unbiased, hypothesis-free genome wide analyses (GWA), using various endophenotyping and novel trait component analyses in Finland (Aarno Palotie) and Australia (Dale Nyholt).

Common migraines as genetic ionopathies

FHM and SHM are clearly due to disturbances of ion transportation across cell membranes. The evidence that common migraines might also be ionopathies is so far only circumstantial, but accumulating. First, migraine shares strikingly similar clinical characteristics with established channelopathies such as FHM and SHM (see above) and episodic neuromuscular disorders. These include an episodic clinical presentation, a similar distribution for attack duration and frequency, similar trigger factors for attacks such as emotion, stress, food, alcohol and weather changes, and a similar gender-related expression with an onset mostly around puberty and an amelioration after age 40. Secondly, FHM gene mutations have been found in patients with common non-hemiplegic migraine types and linkage and association studies suggest a role of FHM genes in at least some common migraineurs. Thirdly, a clinical neurophysiological study from Jean Schoenen's group in Liege revealed sub-clinical cerebellar dysfunction in migraineurs suggesting dysfunction of cerebellar P/Q-type $\text{Ca}_v2.1$ channels. Finally, Peter Goadsby's group in London and others showed that P/Q-type $\text{Ca}_v2.1$ channels within the

brainstem may modulate putative migraine mechanisms such as neurogenic inflammation, release of CGRP, and trigeminal firing and nociceptive transmission.

From disease mechanisms to prophylactic treatments

Many people may experience just one or two aura's or migraine attacks throughout life. The attack itself may thus not be abnormal; rather the *repeated occurrence* of attacks is abnormal. There is growing evidence that the *disease* migraine (i.e. getting recurrent attacks) might be due to a genetically determined reduced threshold for migraine triggers. Attacks may then occur: i) when migraine triggers are particularly strong or frequent; ii) when there is a temporarily further reduction of the threshold due to endogenous factors such as menstruation, sleep deprivation, or (relaxation after) stress, that can facilitate the triggering of an attack; or iii) when there is a temporal coincidence of both triggering and facilitating factors. Prevention of attacks may thus be achieved by increasing the trigger threshold. To this end, one need to understand the mechanism involved in setting and modulating the trigger threshold. The findings described above would suggest that reduction of the trigger threshold for CSD is important in FHM and possibly also in at least some "common migraineurs". Agents that increase the trigger threshold for CSD are attractive candidates for novel migraine prophylactics. Indeed, existing migraine prophylactics, although from different pharmacological classes, share the ability to inhibit CSD and tonabersat, a representative of a novel class of drugs known to block CSD, showed promising results in a prophylactic proof-of-concept migraine trial.

Conclusions and the future

The quest for migraine genes has resulted in a number of interesting genes and, even more important, novel pathways for how migraine attacks might be triggered. The ultimate result will be novel, specific, better tolerated, and more effective prophylactic agents to prevent migraine attacks. An additional outcome might be more objective tests for diagnosing migraine sub-types with the possible exciting benefit of "gene profile guided" personalized treatment. It is quite likely that novel, unbiased hypothesis-free GWA and "omics" approaches may result in even more and thus far unsuspected migraine genes and pathways, similar to what is currently occurring for a wide range of other complex disorders.