Unraveling genetic mechanisms in headache syndromes

Weller, C.M.

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General introduction and methods

Claudia M. Weller

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General introduction

HEADACHE DISORDERS are called 'primary' when no causal underlying structural lesion is present. The diagnostic criteria of the International Headache Society (IHS) define four groups of primary headaches, that is (1) migraine, (2) tension-type headache, (3) cluster headache and other trigeminal autonomic cephalalgias, and (4) other primary headaches, examples of which are hemicrania continua and hypnic headache.[1] These main categories of primary headaches are thought to consist of multifactorial diseases, meaning that they are likely caused by a combination of multiple environmental and genetic factors. Whereas not much is known about genetic factors in cluster headache and tension-type headache, there is growing body of knowledge on the genetics of migraine and its variants. In this section, first genetic knowledge on migraine and cluster headache will be summarized, followed by a description of our research strategies and populations.

Migraine

Clinical aspects of migraine

Migraine is a paroxysmal neurovascular disorder that is characterized by recurrent attacks of throbbing, unilateral headache of moderate to severe intensity. Attacks are aggravated by physical exercise and last 4-72 hours and are often accompanied by nausea, vomiting, photophobia, and/or phonophobia. One-year migraine prevalence in the general population is 11%, with a clear female preponderance (males 6-8% and females 15-18%).[2-5] Due to the lack of objective reliable biomarkers, such as measurements of specific compounds in blood, migraine diagnoses are currently based on questionnaires and/or interviews using consensus criteria of the International Classification of Headache Disorders (ICHD-3beta) from the International Headache Society (IHS) (table 1).[1] Approximately one-third of migraine patients experience transient focal neurological symptoms, known as migraine auras, which can precede attacks of headache. Auras develop gradually and have duration of 5 to 60 minutes and include (in decreasing order of prevalence) visual, sensory, speech and/or motor symptoms.[6] Based on the presence or absence of the aura phase two main migraine subtypes are distinguished, migraine with aura and migraine without aura, which can co-occur in the same patient.[5,7]

Migraine pathophysiology

The prevailing view is that migraine is a neurovascular disorder.[8] The migraine aura is caused by cortical spreading depression (CSD), a wave of neuronal and glial depolariza-

Table 1 - International headache criteria for migraine without and migraine with aura (ICHD-3beta).

Migraine without aura
A. At least five attacks fulfilling criteria B-D
B. Headache attacks lasting 4 to 72 hours (untreated or unsuccessfully treated)
C. Headache has at least two of the following four characteristics:
1. Unilateral location
2. Pulsating quality
3. Moderate or severe pain intensity
4. Aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D. During headache at least one of the following;
1. Nausea and/or vomiting
2. Photophobia and phonophobia
E. Not better accounted for by another ICHD-3 diagnoses
Migraine with aura
A. At least two attacks fulfilling criteria B-C
B. One or more of the following fully reversible aura symptoms:
1. Visual
2. Sensory
3. Speech and/or language
4. Motor
5. Brainstem
6. Retinal
C. At least two of the following characteristics:
1. At least one aura symptom spreads gradually over ≥5 minutes, and/or two or more symptoms occur in succesion
2. Each individual aura symptom lasts 5-60 minutes
3. At least one aura symptom is unilateral
4. The auro is accompanied, or followed within 60 minutes, by headache
D. Not better accounted for by another ICHD-3 diagnoses, and transient ischemic attack has been excluded

tion that moves slowly over the cortex.[9] Using functional MRI to study the aura in patients with migraine with aura, Hadjikhani and colleagues[10] were able to detect local increases in blood oxygen level-dependent signal that spread through the visual cortex at a rate of approximately 3 mm/min, a speed similar to what is seen when a CSD is evoked in an experimental animal. The headache itself is caused by activation of the trigeminovascular system which consists of the meningeal and superficial cortical blood vessels that are innervated by the trigeminal nerve and that project to the trigeminal nucleus caudalis in the brainstem, which in turn projects to higher-order pain centers (thalamus and cortex) leading to pain.[11]

Migraine is a genetic disorder

Migraine shows strong familial aggregation and is a multifactorial complex genetic disorder.[12-14] Such complex disorders are likely caused by a combination of environmental factors and multiple genetic factors, each with a small effect size meaning that the individual genetic factors increase disease risk only slightly. Population-based family studies

revealed that the relative risk of migraine for a first-degree relative of the index patient is 1.5 to 4 times higher compared to the general population. The risk is highest for patients with migraine with aura, an early age of onset, and high attack severity and disease disability.[12] Twin studies also revealed a higher genetic load in migraine with than migraine without aura.[15,16] A large study of approximately 30,000 twins from six different countries showed that genetic and environmental factors play an almost equal role in migraine susceptibility.[17] Heritability, which is defined as the contribution of genetic factors to susceptibility for a disease, was estimated to range from 34 to 57% in that study. Shared environmental factors seemed to play a minor role, as migraine prevalence was similar in twins raised together and twins raised apart.[18,19]

There is debate whether migraine with and migraine without aura are two separate disease entities or merely different expressions of the same disease. The first view is supported by observations that there is no increased co-occurrence of both types of migraine in Danish twins[20] and the general Danish population.[6,12] In contrast, a study of 210 Finnish migraine families suggested the existence of a migraine continuum with pure migraine with aura and pure migraine without aura on both ends of the spectrum and a combination of both types of attacks in between.[21] The idea that migraine indeed is a continuum is supported by other studies[22,23] as well as by clinical observations that headache characteristics are identical in both migraine with and without aura and that a large number of patients experience both types of attacks.[24]

Approaches to Discover Migraine Genes

Hemiplegic migraine as a monogenic model for common migraine

Identifying genes and biological pathways of a monogenic subtype of a disease is likely to provide useful insight into the pathophysiology of the complex disease form. Gene mutations underlying monogenic disease have a large effect size and are expected to have clear consequences on either the level or the amino acid sequence of the affected protein, which can be investigated in cellular and animal disease model systems. With respect to genetic migraine research, Familial Hemiplegic Migraine (FHM) is a rare monogenic subtype of migraine with aura and insight into its pathophysiology may therefore serve to help unraveling part of the pathophysiology of common forms of migraine as well.

FHM is characterized by a transient hemiparesis during the aura phase, which may last several days. Diagnostic criteria for FHM were determined by the IHS[1] (table 2). Hemiplegic migraine occurring in isolated cases is called Sporadic Hemiplegic Migraine (SHM) and apart from the absence of an affected first-degree relative, diagnostic criteria are identical to those in FHM. Because of its clinical presentation, a significant number

Table 2 - International headache criteria for familial hemiplegic migraine (IHS 2013).

Hemiplegic migraine
A. At least two attacks fulfilling criteria B and C
B. Aura consisting of both of the following:
1. Fully reversible motor weakness
2. Fully reversible visual, sensory and/or speech/language symptoms
C. At least two of the following four characteristics:
1. At least one aura symptoms develops gradually over ≥ 5 minutes, and/or different aura symptoms occur in succession
2. Each individual non-motor aura symptom lasts 5–60 minutes, and motor symptoms last <72 hours
3. At least one aura symptom is unilateral
4. The aura is accompanied, or followed within 60 minutes, by headache
D. Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack and stroke have been excluded.

of patients with hemiplegic migraine initially are diagnosed with stroke or epilepsy. Except for the hemiparesis, the visual and sensory aura symptoms are identical to those seen in migraine with aura, although duration of the aura often is significantly longer than in migraine with aura patients.[25] The headache characteristics in hemiplegic migraine patients and patients with the common forms of migraine are identical. Moreover, the majority of hemiplegic migraine patients experience attacks of common migraine in addition to their hemiplegic attacks.[26] Thus, from a clinical perspective hemiplegic migraine seems to be a valid model to study the common forms of migraine.[7]

Hemiplegic migraine genes

Genetic studies in FHM resulted in the discovery of three genes.[27] As several FHM families do not have a gene mutation in one of the known genes, likely more FHM genes exist. *CACNA1A*, the first FHM gene (FHM type 1; FHM1) is located on chromosome 19p13 and encodes the α1 pore-forming subunit of $Ca_V2.1$ calcium channels.[28] Some FHM1 mutations lead to pure hemiplegic migraine, whereas other mutations are associated with additional clinical symptoms such as cerebellar ataxia or epilepsy and have been shown to cause gain-of-function of $Ca_V2.1$ channel activity.[27,29] FHM1 is allelic with two other monogenic disorders: episodic ataxia type-2 (EA2)[28], and spinocerebellar ataxia type-6 (SCA6).[30] EA2 is a paroxysmal disorder that is characterized by vertigo, ataxia and nausea and approximately half of the patients suffer from migraine headaches and attacks can be accompanied by hemiplegia. EA2 mutations cause loss-of-function of $Ca_V2.1$ channel activity. SCA6 is a late-onset cerebellar ataxia disorder caused by moderate polyglutamine repeat expansion in the carboxyl-terminal part of the α1 pore-forming subunit.[30] It is still unclear how expansion of the glutamine stretch causes SCA6 and to what extent the pathoanatomical consequences of the different types of *CACNA1A* mutations overlap.

The second FHM gene (FHM2), *ATP1A2*, is located on chromosome 1q23 and encodes the α2 subunit of sodium-potassium pumps.[31] In contrast to FHM1 mutations, hardly

Table 3 - Monogenic Disorders Associated with Migraine.

Syndrome	Symptoms	Migraine subtype	Gene	References
CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy)	Recurrent stroke Cognitive deterioration Psychiatric disease	MA	*NOTCH3* Encodes a cell surface receptor on vascular smooth muscle cells	Joutel et al. 1996[52] Dichgans et al. 1998[51] Liem et al. 2010[53]
RVCL (Retinal Vasculopathy with Cerebral Leukodystrophy)	Retinopathy Cognitive disturbances Depression Raynaud phenomenon Liver and kidney dysfunction	MO	*TREX1* Encodes the major mammalian 3'-5' exonuclease, involved in DNA repair and apoptosis after DNA damage. Evidence is accumulating for additional functions	Grand et al. 1988[132] Jen et al. 1997[133] Terwind et al. 1998[58] Richards et al. 2007[56]
MELAS (Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes)	Stroke-like episodes Encephalopathy with seizures and/or dementia Myopathy (lactic acidosis and/or ragged red fibers (RRF) on muscle biopsy)	MA	Specific mutations in mitochondrial genes	Montagna et al.1988[73]
HIHRATL (Hereditary Infantile Hemiparesis, Retinal Arteriolar Tortuosity and Leukoencephalopathy)	Porencephaly Cerebral and retinal microangiopathy	MA	*COL4A1* Encodes a collagen IV alpha chain in the basement membrane	Gould et al. 2006[61] Vahedi et al. 2007[134]
POLG-related disorders	Ataxia Ophthalmoplegia Epilepsy Liver failure	MA and MO	*POLG* Encodes the mitochondrial DNA polymerase gamma that is important for ATP homeostasis and normal cellular function	Winterthun et al.2005[76] Tzoulis et al. 2006[75]
TGFR2-related disorder	Aortic dissection Joint hypermobility Skin abnormalities Arthralgia	Unspecified	*TGFR2* Encodes the transforming growth factor beta receptor 2, which is involved in regulation of cellular processes and formation of extracellular matrix	Law et al. 2006[63]
Episodic ataxia type 6 (EA6)	Episodic ataxia Epilepsy	Hemiplegic migraine	*SLC1A3* Encodes the excitatory amino acid transporter 1 (EAAT1) which removes glutamate from the synaptic cleft	Jen et al.2005[71]
Proximal renal tubular acidosis (pRTA)	Renal dysfunction Ocular abnormalities	Hemiplegic migraine, MA, MO	*SLC4A4* Encodes a sodium bicarbonate cotransporter involved in regulating intracellular pH	Suzuki et al. 2010[72]
Advanced sleep phase syndrome	Early sleep time Early morning awakening	MA and MO	*CSNK1D* Encodes the casein kinase 1δ that phosphorylates the human circadian clock protein PER2	Brennan et al. 2013[135]

Note: MA = migraine with aura; MO = migraine without aura.

any recurrent mutations were reported for *ATP1A2.*[32,33] Another striking difference with FHM1 mutations is that almost all *ATP1A2* mutations are associated with pure hemiplegic migraine, that is without any additional clinical symptoms,[31,34-37] although some mutations are associated with epileptic seizures,[35,38] benign familial childhood convulsions,[39] febrile seizures[40] and mental retardation.[35] FHM2 mutations cause loss-of-function of the Na^+,K^+ ATPase.[27,29]

The third FHM gene (FHM3), *SCN1A*, is located on chromosome 2q24 and encodes the α1 subunit of neuronal $Na_V1.1$ sodium channels.[41] *SCN1A* mutations seem to account for only a small proportion of FHM families. Interestingly, the *SCN1A* gene is a well-known epilepsy gene with many mutations causing monogenic forms of childhood epilepsy, i.e. Dravet syndrome (also known as severe myoclonic epilepsy of infancy (SMEI)) or generalized epilepsy with febrile seizures (GEFS+).[42] At the start of the research described in this thesis, only five FHM3 mutations had been reported,[41,43-46] although from the clinical description in patients it is far from certain that one of them, mutation T1174S, is an FHM3 mutation as patients with the mutation may not have hemiplegic migraine.[45] Mutations L1649Q and Q1489K are associated with pure familial hemiplegic migraine,[43] whereas mutations Q1489H, and L263V have been associated with childhood epilepsy and generalized tonic-clonic seizures[41,44,46] in addition to FHM, at least in some of the mutation carriers. Some patients with FHM3 mutations Q1489H and F1499L were also reported to suffer from 'elicited repetitive daily blindness' (ERDB), which occurred apart from their hemiplegic migraine attacks.[46,47] FHM3 mutations seem to cause either gain or loss of function of $Na_V1.1$ channel activity.[27]

The first screens of FHM genes in hemiplegic migraine patients without a positive family history of other patients with hemiplegic migraine (so-called sporadic hemiplegic migraine = SHM) revealed mutations, predominantly in *ATP1A2*, in only a small proportion of patients.[48-50] Riant and coworkers, however, recently identified mutations in *CACNA1A* and *ATP1A2* in 23 out of 25 SHM patients with an age-of-onset before 16 years, of which most had additional symptoms such as epilepsy, learning difficulties, cerebellar ataxia and/or coma.[33] Three-quarter of the mutations had occurred *de novo* and mutation carriers thus represent the first patients of new FHM families. The question remains what is causing SHM in the patients that do not have an FHM gene mutation, which is a rather large proportion of patients in most studies.[48-50] Possibilities are that either other FHM genes may cause hemiplegic migraine in those patients or that SHM (especially when the phenotype is not severe) is due to a combination of multiple low-risk genetic variants, similar to what is predicted to occur in common forms of migraine. Support for the latter hypothesis comes from the observation that migraine with aura is frequent in families of SHM patients.[24] Also it would fit a view of migraine being a spectrum of disorders.

Investigating Monogenic Disorders in which Migraine is a Part of the Phenotype

Migraine can also be part of non-FHM monogenic disorders, which may be a useful source for identifying genes that may shed light on the pathophysiological mechanisms involved in migraine. In fact, the number of examples that are relevant to migraine is increasing (table 3).

Migraine as part of the phenotype of monogenic vasculopathies

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a monogenic syndrome characterized by recurrent stroke, cognitive deterioration, and psychiatric disease.[51] CADASIL is caused by mutations in the *NOTCH3* gene,[52] which encodes a cell surface receptor that is expressed in vascular smooth muscle cells. About one-third of CADASIL patients also suffer from migraine, predominantly migraine with aura, often with migraine as the presenting clinical symptom.[53] Whether there is a genetic link between migraine and Notch3 is still under debate, also because genetic association studies investigating *NOTCH3* polymorphisms gave conflicting results.[54,55]

Retinal Vasculopathy with Cerebral Leukodystrophy (soon to be renamed to Cerebral Hereditary Angiopathy with Vascular Retinopathy and Impaired Organ Function caused by TREX1 Mutations (abbreviated as CHARIOT)) is a monogenic vascular syndrome caused by mutations in the *TREX1* gene[56] that encodes the major 3'-5'-mammalian exonuclease, an enzyme thought to be involved in clearing cytosolic DNA.[57] RVCL patients suffer from a number of features that can include pronounced retinopathy, kidney and liver dysfunction, Raynaud phenomenon and various neurological features such as cognitive disturbances, depression and migraine.[58] In advanced stages of the disease, brain imaging shows characteristic contrast-enhancing white matter lesions.[58] A small genetic family-based study seemed to suggest a potential role for the RVCL gene as a susceptibility gene in migraine and Raynaud phenomenon.[59]

An increased prevalence of migraine with aura was reported in a rare angiopathy that can be described as Heriditary Infantile Hemiparesis, Retinal Arteriolar Tortuosity, and Leucoencephalopathy (or its acronym HIHRATL) with clinical symptoms of porencephaly and cerebral and retinal microangiopathy, hemiparesis, and stroke.[60] The causal gene is *COL4A1*, which encodes type IV collagen, an integral component of the vascular basement membrane.[61] The association adds to growing evidence for a link between migraine and early-onset cerebral angiopathies that is remarkable, but the mechanisms underlying this association are still poorly understood.[62] An additional piece of information that links affected blood vessels with migraine comes from a genetic study in a large pedigree in which patients suffer from familial aortic dissection and several other blood vessel

abnormalities.[63] Ten out of 14 carriers of the R460H mutation in the transforming growth factor factor-β receptor 2 (*TGFBR2*) gene also suffer from migraine.

In particular it needs to be further investigated whether endothelial dysfunction, which was also observed for migraine in several studies,[64-67] may underlie the extensive vasculopathy seen with CADASIL and RVCL. Given the high occurrence of migraine with aura in CADASIL patients, increased susceptibility for CSD may well explain the link with migraine. For RVCL this explanation seems unlikely, since the disease seems to be linked with both migraine without and with aura. Interestingly, prevalence of cardiovascular disease is increased in common forms of migraine, especially in migraine with aura patients. The basis for the comorbidity of migraine and cardiovascular disease is yet unknown, but these findings support the notion that shared pathophysiological processes could be involved.[68,69]

Other monogenic disorders in which migraine is part of the phenotype

There are several other monogenic disorders in which migraine can be prominent (table 3). First of all, in Familial Advanced Sleep Phase Syndrome (FASPS) there is a severe disruption of the sleep-wake cycle and other circadian rhythms. The disease is caused by missense mutations in the *CSNK1D* gene encoding Casein Kinase 1d (CK1δ), which is involved in the phosphorylation of the circadian clock protein Per2.[70] In two independent families a pathogenic *CSNK1D* mutation co-segregated with both FASPS and migraine with aura. Second, a complex monogenic phenotype of episodic ataxia, hemiplegic migraine, and seizures was reported for a 10-year-old boy with a P290R mutation in *SLC1A3* that encodes the excitatory amino acid transporter 1 (EAAT1), which removes glutamate from the synaptic cleft.[71] The missense mutation causes a dramatic loss in glutamate uptake in a cellular assay. Third, Suzuki and co-workers[72] reported various homozygous mutations in the Na^+-HCO_3^- co-transporter NBCe1 in patients with proximal renal tubular acidosis and ocular anomalies and, in addition, various clinical presentations of migraine, that is migraine without or with aura, hemiplegic migraine, and even episodic ataxia. Although the mutations themselves clearly are pathogenic in the sense that they are the cause of the renal and ocular problems, it is less obvious why they would cause the migraine and hemiplegia phenotypes that are very common and sometimes hard to diagnose.

A fourth monogenic syndrome that is associated with migraine is MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like syndrome) which is caused by a mitochondrial DNA (mtDNA) 3243 A>G tRNALeu point mutation.[73] Fifth, many carriers of mutations in the *POLG* gene, which encodes the nuclear polymerase-γ that is essential for the maintenance of mitochondrial DNA, suffer from migraine as well.[74-76] Lastly, Alternating Hemiplegia of Childhood (AHC) can be considered a monogenic model that has relevance to migraine. AHC is a rare syndrome characterised by recurrent

hemiplegic attacks, movement disorders, seizures, and developmental delay starting before the age of 18 months.[1] Due to the considerable clinical overlap in severely affected hemiplegic migraine patients and AHC patients, it is sometimes impossible to establish the correct diagnosis.[77] The clinical overlap prompted investigating the FHM genes *CACNA1A* and *ATP1A2* in AHC patients. A *CACNA1A* mutation was found in monozygous twins with overlapping clinical features of both disorders[78] and an *ATP1A2* mutation was identified in a Greek atypical AHC family.[79,80] No *CACNA1A* or *ATP1A2* mutations were found in any of the patients meeting all diagnostic criteria.[81,82] Due to the overlapping clinical and genetic features, advances in genetic research of AHC may also provide important information about the genetics of monogenic and complex forms of migraine.

Genetic Studies in Common Forms of Migraine

Identifying genes involved in complex disorders has proven difficult, especially before the era of genome-wide association studies (GWAS) (see below). Two main hypotheses are used to explain the genetic origin of complex diseases.[83] One hypothesis proposes that common disease is caused by common variants (CD-CV), which means that relatively frequent genetic variants cause disease, each with a small effect size cause disease but none of them is sufficient. In contrast, the common disease - rare variant (CD-RV) hypothesis assumes that multiple, relatively rare variants with a larger effect size may explain susceptibility to disease. To date, most findings in complex diseases test the first hypothesis only although a few rare variants with a moderate effect have been detected, such as Factor V Leiden in deep venous thrombosis.[84]

Linkage and candidate gene association studies in migraine

Until a few years ago, the main approach used in genetic studies of common migraine was family-based linkage analysis, which led to the identification of many chromosomal susceptibility regions, but did not result in the discovery of migraine genes. A second popular approach consisted of candidate gene association studies that search for significant differences in allele frequencies between migraine cases and controls in genes that had emerged from other knowledge of migraine pathophysiology. These association studies tested only one or at best a few DNA polymorphisms in such a gene. Candidate gene-based association studies in theory are a powerful tool, if carefully designed to overcome methodological issues regarding sample size, selection of cases and controls, selection of variants, correction for multiple testing, and replication of findings in independent populations. Unfortunately, the great majority of candidate gene association studies performed in migraine suffered from one or more methodological weakness and led to the conclusion that most results must be false positives.[85] Among the selected candidate genes that were most often tested are genes in the dopaminergic and serotonergic systems, hormone

receptors, and inflammatory pathways (for review see De Vries et al, 2009[27]). The best replicated finding is the association with the C677T polymorphism in the 5',10'-methylenetetrahydrofolate reductase (*MTHFR*) gene that increases the risk of migraine in carriers of the T-allele,[86-91] although other large and well-designed studies could not find such association.[92,93] Two meta-analyses showed an association of this polymorphism with migraine with aura, but not with migraine without aura.[94,95] *MTHFR* codes for an enzyme with an important role in homocysteine and folate metabolism.[96] Carriers of the T-allele have increased homocysteine concentrations, which is a well-known risk factor for cardiovascular disease. It is hypothesized that high homocysteine levels may induce vascular endothelial dysfunction and thereby increase migraine risk.

Genome-wide association studies in migraines

Over the last few years, genome-wide association studies (GWAS) have become the most used approach to identify genes that confer susceptibility to complex disorders. In a GWAS, hundreds of thousands of SNPs that are distributed over the genome are tested in a hypothesis-free manner for association with a disease trait. For each SNP, the allele frequencies are compared between cases and controls. Significant differences in allele frequency either pinpoint the SNP itself as a genetic susceptibility factor or provide statistical evidence that a causal gene variant is in close vicinity, i.e. is the causal variant is in linkage disequilibrium. Several major migraine GWA studies have been performed investigating so-called end-diagnoses migraine with aura[97] or migraine without aura[98] in two well-defined clinic-based studies, migraine in a population-based cohort[99], and, most recently, a systematic migraine meta-analysis.[97] Over a dozen migraine susceptibility gene variants have been identified that point to genes that cluster into five main different pathways related to (i) glutamatergic neurotransmission; (ii) synapse development and plasticity; (iii) pain sensing; (iv) metalloproteinases; and (v) vasculature & metabolism. The pathophysiological and clinical implications of these variants, however, still have to be determined.

Reduction of clinical heterogeneity for genetic studies of common migraine

Apart from genetic heterogeneity, the gene hunt in common migraine is also complicated by extensive clinical heterogeneity regarding for instance presenting symptoms or age-of-onset in addition to the lack of reliable biomarkers to establish a migraine diagnosis. Clearly, diagnostic criteria of the IHS that are useful to diagnose attacks in the clinic are less suited for genetic research, because multiple combinations of symptoms lead to the same end-diagnosis, not necessarily through the same pathophysiological mechanism. Two types of strategies can be applied to reduce clinical heterogeneity among participants of genetic studies in migraine. The first approach takes advantage of the well-known comorbidity of migraine with various disorders.[100] Although the observation can be

spurious due to selection bias or reflect a unidirectional causal relationship, that is migraine causes (or is caused by) the co-morbid disorder, it may also be that shared genetic and/or environmental factors underlie both migraine *and* the co-morbid disorder.[101] Migraine patients are at increased risk of epilepsy, ischemic stroke, myocardial infarction, major depressive disorder, anxiety disorders, bipolar disorder, asthma, and chronic pain disorders[102,103] and genetic heterogeneity might be reduced by selecting those migraine patients that also suffer from the co-morbid disorder. The second type of approach uses other classification than the ICHD-2 criteria[1] as a trait in genetic analysis. Two examples of this latter strategy are trait component analysis[104,105] (TCA; using individual symptoms instead of ICHD-2 diagnosis as traits in the analysis)) and latent class analysis[106-108] (LCA, using different classes of patients based on clustering of symptoms), which led to the discovery of various chromosomal regions potentially harboring migraine susceptibility genes. Until now, no susceptibility genes have been found using TCA or LCA.

An attractive alternative could be the use of a (set of) migraine biomarker(s) as a trait in genetic studies to search for novel migraine susceptibility genes. Past research on migraine biomarkers was hypothesis-driven and tested a small number of metabolites in small study populations[66,109-117] and failed to identify a clinically useful migraine biomarker. Current high-throughput proton nuclear magnetic resonance (^{1}H NMR) spectroscopy enables generating a profile of tens to hundreds low-molecular-weight metabolites from a blood sample in a single measurement.[118,119] Interestingly, GWAS using metabolite concentrations have led to the identification of genetic variants with much larger effect sizes than commonly encountered in GWAS using 'just' clinical diagnoses.[120] Combining the existing knowledge on genetics of metabolites with yet unidentified migraine biomarkers could be an attractive approach to identify novel migraine susceptibility genes.

Studying the genetics of cluster headache

Cluster headache is a rare, disabling, primary headache disorder.[1] Clinical characteristics and treatment options partly overlap with migraine and involvement of the trigeminovascular system may be a key feature of both disorders.[11,121,122] Cluster headache is much rarer than migraine and has a lifetime prevalence of only 0.12%.[123] In contrast to migraine, there is a striking male preponderance with a male-to-female ratio of 3:1.[123,124] Cluster headache is characterised by attacks of unilateral severe (supra)orbital and/or temporal pain accompanied by restlessness and/or ipsilateral autonomic symptoms, that is eyelid oedema, conjunctival injection, miosis, ptosis, lacrimation, nasal congestion, rhinorrhoea, forehead and facial sweating. Attacks have duration from 15 to 180 minutes and occur up to eight times a day.[1] Approximately 90% of patients have the episodic form of the disease. Patients with episodic cluster headache experience bouts of frequent attacks lasting weeks to months, followed by remission lasting several months to years. The

remaining 10% has chronic cluster headache, characterised by short (less than one month) or absent periods of remission.

Cluster headache is considered a complex genetic disorder, although an autosomal dominant pattern of transmission has been suggested in some cases.[124] The relative risk for family members of cluster headache patients is estimated to be 5-18 for first-degree relatives and 1-3 for second-degree relatives. Due to the low prevalence of the disorder, no large cohorts of patients are available for large-scale genetic studies such as GWAS. Therefore, genetic research in cluster headache has been limited mainly to candidate gene studies. Multiple small-scale studies have been performed, but many of them lack a replication sample, thereby precluding a final conclusion regarding the association of the reported genes with cluster headache. To date, only one genetic factor (i.e. a missense variant in the hypocretin type 2 receptor gene *HCRTR2*) was found to be associated with cluster headache in two of the three small conducted studies and in a meta-analysis of these studies.[125-128]

Study populations

Leiden University Migraine Neuro-Analysis (LUMINA) population

The Leiden University Migraine Neuro-Analysis LUMINA program was initiated in March 2008 and inclusion is still ongoing. The aim of the program is to enroll a large number of self-reported migraine patients for genetic and epidemiological studies through the project's website (www.lumc.nl/hoofdpijn). Self-reported migraine patients from the Dutch population were invited to complete a validated screening questionnaire. Screen-positives were subsequently asked to complete a newly developed extended web-based questionnaire aiming to diagnose migraine based on the ICHD-2 criteria. Many previously developed questionnaires are good at assessing headache characteristics, but fail to make reliable aura diagnoses. Discriminating between migraine with and without aura is of particular interest in genetic studies and such migraine type information can be reliably obtained using questionnaire-based aura diagnoses with our LUMINA questionnaire. Five years after the start of the project, approximately 5,000 migraine patients have been collected and have contributed to the identification of susceptibility genes for common forms of migraine in various GWAS.[97,98,129]

Leiden University Cluster headache Analysis (LUCA) population

The Leiden University Cluster headache Analysis (LUCA) program is the cluster headache counterpart of the LUMINA study and started in April 2010. Genetic studies in cluster

headache are greatly hampered by the combination of low prevalence and putative complex genetic background of the disorder. The LUCA study aimed to collect a large group of self-reported cluster headache patients for genetic and epidemiological studies. Self-reported cluster headache patients were invited to complete a screening questionnaire. Screen-positive participants were asked to complete the newly developed web-based extended questionnaire to enable diagnosing cluster headache for genetic and epidemiological studies. Three years after the start of the project, 917 patients cluster headache patients were enrolled in the program, of which 560 provided a DNA sample, resulting in the largest single sample set of cluster headache patients to date.

Erasmus Rucphen Family (ERF) Study

The ERF population originates from a genetically isolated region in the southwest of the Netherlands.[130] The study population consists of the 3,465 living descendants of 22 founder couples that had at least six children baptized in the community church between 1850 and 1900. The pedigree of this large family is characterized by multiple consanguinity and increased inbreeding. The occurrence of disease in isolated populations is influenced by a less heterogeneous genetic and environmental background than the occurrence of disease in the general outbred population. Identification of susceptibility genes in isolated populations is therefore considered easier because genetic drift (that is rare variants tend to disappear or become overrepresented), facilitates investigating rare variants underlying common disease, while isolated populations are equally well suited to detect common variants associated with disease, as these variants in general have similar allele frequencies in genetic isolates and the general outbred population.[131]

Scope and outline of the thesis

The studies presented in this thesis aim to advance genetic knowledge of primary headache disorders with a focus on migraine and cluster headache.

In **Chapter 2** the identification is reported of two novel mutations in the *SCN1A* gene in Spanish families with familial hemiplegic migraine. The identification of such mutations is important as the overwhelming majority of mutations in this gene cause epilepsy and it is still unclear why certain *SCN1A* mutations cause FHM instead. **Chapter 3** describes a mutation in the *SLC2A1* gene in a patient that links hemiplegic migraine with alternating hemiplegia of childhood, which may be of use to understand how one mutation causes features of both monogenic disorders and may be of relevance in dissecting mechanisms of migraine pathophysiology. Also attempts were made to investigate the possible role of the *SLC2A1* and *ATP1A3* genes in familial and sporadic hemiplegic migraine.

Chapters 4 and **5** are dedicated to the validation of extended web-based questionnaires that were developed to enrol self-reported headache patients for large-scale genetic studies. **Chapter 4** focuses on the LUMINA program that aims to recruit migraine patients and addresses the difficulties in obtaining reliable aura diagnoses using questionnaire data. In **Chapter 5** a similar approach was applied to recruit patients with cluster headache for the LUCA (Leiden University Cluster headache Analysis) program. One aim was to select which questions are best predictors for cluster headache diagnosis in the Dutch cluster headache population, generating a shorter questionnaire suitable for case-finding in large population-based studies.

Chapter 6 contains the results of a candidate gene study and subsequent meta-analysis investigating the role of a missense variant in the *HCRTR2* gene in cluster headache susceptibility. That variant had been associated with cluster headache in several small-scale association studies and *HCRTR2* is considered the only replicated cluster headache susceptibility gene. Using patients recruited in the LUCA program this association was re-investigated, now including the LUCA population as the largest single sample set of patients with cluster headache to date.

Chapter 7 contains the results of a genetic epidemiology investigation using the genetically isolated population of the Erasmus Rucphen Family study that aimed to investigate whether atherosclerosis is the cause of the previously observed increased rate of cardiovascular disease in migraine patients. The study also addresses, to certain extent, the question how useful it can be to select migraine patients that have a comorbid disease, in this case cardiovascular disease. In **Chapter 8** the same ERF cohort was used for metabolic profiling in serum using nuclear magnetic resonance (NMR) spectroscopy in a first attempt to identify molecular biomarkers for migraine. This study aims to identify a set of metabolites that may predict disease status.

Finally, **Chapter 9** provides a general discussion of the main findings presented in this thesis in relation to current literature, their implications and possibilities for future research.

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