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CACNA1A gene mutations in familial hemiplegic migraine

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Abstract Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura. A few years ago, the gene linked to FHM was identified. CACNA1A encodes a voltage-activated, pore-forming α1A subunit of the P/Q-type calcium channel. At present, an increasing number of mutations have been identified in this gene in patients with FHM. Genotype-phenotype comparisons have become feasible only recently. The in vitro functional consequences on channel function of the first mutations have been deciphered. This is the moment to evaluate these recent discoveries and see how they can help us understand the pathophysiology of FHM and the common forms of migraine.

Key words FHM · Migraine · Calcium channel · CACNA1A gene

Introduction

Migraine research has intensified and gained direction with the discovery of the first familial hemiplegic migraine (FHM) gene, CACNA1A, a few years ago [1]. The finding that the pore-forming subunit of a P/Q-type calcium channel was mutated in FHM families gave new insight into the pathophysiology of this disease. It is now a challenge to test the functional consequences of the mutations and to unravel the exact pathways that are affected. Genetic evidence already indicates that the same locus plays a role in common forms of migraine (see below). A better understanding of the dysfunction of the FHM gene is important for the development of new anti-migraine drugs. This article describes migraine, the search for FHM genes, the characterisation of FHM mutations, and the current understanding of the functional consequences of these mutations.

Migraine with and without aura

Migraine is a common neurological paroxysmal disorder affecting up to 16% of the general population. Migraine is more frequent in women than in men. The disease is characterised by recurrent attacks of disabling, mostly unilateral headache, associated with other symptoms such as nausea, vomiting, photo- and phonophobia, and malaise (migraine without aura, MO). In about one-third of patients, the attacks are preceded or accompanied by transient focal neurological aura symptoms (migraine with aura, MA) that usu-

ally do not last longer than 60 minutes. The headache phase, which can vary from hours to days, is similar in MO and MA, although it may be less severe or of shorter duration in MA patients [2]. According to International Headache Society (IHS) criteria, a migraine patient has had at least 2 attacks of MA or 5 attacks of MO.

Family and twin studies have provided evidence that genetic as well as environmental factors are involved in the common forms of migraine [3–5]. Importantly, not the migraine attack itself, but the repeated recurrence of attacks is abnormal. Apparently, in migraine patients "the threshold" to get attacks is lowered or triggers (such as stress, exertion, lack of sleep) are particularly strong or frequent in patients.

The pathophysiology of migraine is still not well understood [6]. Abnormal activation of the trigeminovascular system seems to be important. This gives rise to abnormal transmission of nociceptive information to higher centers in the central nervous system (CNS) and results in excessive release of vasoactive peptides at nerve endings that surround pial vessels. Consequently, these vessels are dilated and cause the throbbing, pulsating headache. Aura symptoms are believed to be caused by a depolarising wave, known as cortical spreading depression. When this wave propagates across the brain cortex, it causes neuronal silencing, reduced ion homeostasis, and massive efflux of excitatory amino acids.

Familial hemiplegic migraine

FHM is a rare, autosomal dominant, subtype of migraine with aura characterised by transient hemiparesis or hemiplegia (one-sided weakness or paralysis of the body) during the attacks [2]. This ictal hemiparesis may last from minutes to hours or weeks. In addition, other aura symptoms may be present that are also observed in patients with MA. Occasionally, FHM attacks are accompanied by confusion or psychosis, alterations of consciousness, fever or an aseptic meningeal reaction. In about 20% of the families, FHM symptoms include permanent cerebellar ataxia. Importantly, the symptoms of the headache and aura phase of FHM are similar to those of "normal" migraine attacks, and both types may alternate within individuals and co-occur within families. These observations strongly suggest that FHM is part of the migraine spectrum. Thus, FHM can be used as a model to study the complex genetics and pathophysiology of the common types of migraine.

The search for FHM genes

Our genetic research on migraine started with the search for an FHM gene using a genome-wide linkage analysis in two of our largest FHM families. Results by Joutel et al. [7] that showed linkage of FHM to chromosome 19p13 were confirmed in some of our Dutch families. Through positional cloning, the gene encoding the pore-forming α1A ($Ca_v2.1$) subunit of the brain-specific P/Q-type calcium channel (CACNA1A) was identified, and mutations were detected in this gene in five FHM families [1]. Mutations were identified in the same channel subunit in patients with episodic ataxia type 2 (EA-2) (truncating mutations) [6] and with spinocerebellar ataxia type 6 (SCA-6) (moderate expansions of a carboxyl terminal, polyglutamine CAG-repeat) [8]. However, recent reports have shown that missense mutations or moderate CAG-expansions can occur also in EA-2 patients [9, 10].

Only in 50% of the FHM families reported did the CACNA1A gene on chromosome 19p13 cause the disease [11, 12]. A second FHM locus has been mapped to chromosome 1q [13, 14]. Additional loci are expected, since a number of FHM families are linked neither to chromosome 19p nor 1q ([13] and RA Ophoff, personal communication). Clearly, these observations display the genetic heterogeneity of FHM. It is important to learn how functional defects in different genes converge into a common pathophysiological mechanism responsible for the neuronal instability observed in migraine patients.

FHM mutations in the CACNA1A gene

At least 13 FHM mutations in the CACNA1A gene have been reported (Fig. 1) [1, 15–19]. All of these mutations lead to a substitution of a single amino acid residue in the α1A subunit. The mutations involve highly conserved amino acid residues in various functional domains of the protein. For instance, mutations R192Q, R583Q and R1667W all involve arginine residues in S4 segments of the voltage sensors [20]. These mutations may affect gating properties of the channel. Other mutations, like T666M and V1457L, are located in P-loops close to key glutamate residues that form the binding site for divalent cations and that have a role in the ion selectivity and permeability of the channels [21]. Mutations V714A, I1811L and D715E are located in or near S6 segments that line part of the pore internal to the selectivity filter and control the channel's inactivation properties [22, 23]. Therefore, these mutations may well interfere with the inactivation function. Similarly, mutation K1335E is located in the S3-S4 linker of repeat 3, a region which controls the time course and voltage dependency of channel activation [24] in the α1B ($Ca_v2.2$) subunit of N-type channels. At present, it is unclear how mutations in S5 segments (e.g. Y1384C and V1695I) or nearby at the cytoplasmic face (L1682P and W1683R) might affect calcium channel function. The observation that mutations in

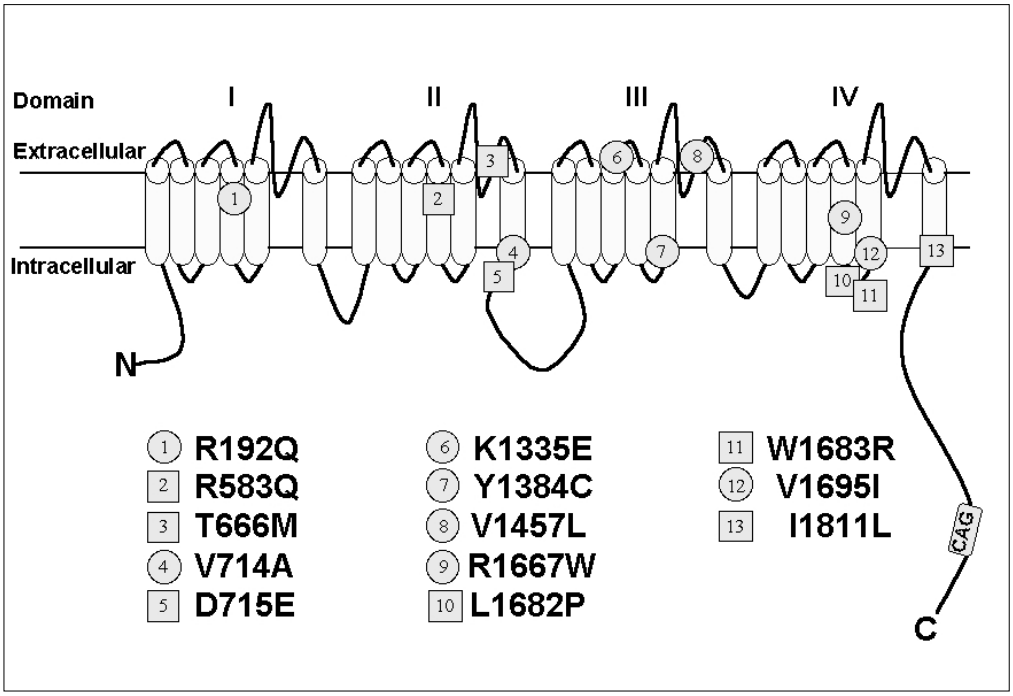

Fig. 1 FHM mutation in the α_{1A} Ca^{2+} channel subunit gene (CACNA1A). The positions of FHM mutations are illustrated for patients without (○) or with (□) cerebellar ataxia. Amino acid positions are given according to the authors that described the mutation first

the S4-S5 linker of potassium channel Shaker B alter the stability of the inactivated state and channel conductance may hint at a similar function of this region in P/Q-type calcium channels [25].

The fact that only missense mutations are associated with FHM suggests a molecular mechanism common to other channelopathies. Although no definite proof has been obtained, both alleles are likely to be expressed with the mutant allele resulting in gain-of-function variants of the α1A calcium channel pore-forming subunit. Similar findings have been described for mutations in the α subunit of the skeletal muscle sodium channel associated with hyperkalaemic periodic paralysis (reviewed in [26]).

Clinical variation with FHM mutations

A clinical comparison of FHM families linked and unlinked to chromosome 19p did not show significant differences for age at onset or frequency and duration of attacks [27]. However, unconsciousness during attacks and provocation of attacks by mild head trauma were reported more in the 19p-linked families. In about half of the FHM families linked to chromosome 19, chronic cerebellar ataxia is part of the clinical phenotype. This is interesting, because cerebellar ataxia is also present in patients with episodic ataxia type 2 (EA2) [1, 28]. This observation together with recent studies suggest a considerable overlap between the clinical phenotype of FHM and EA-2 [29].

With the identification of an increasing number of mutations in CACNA1A, genotype-phenotype correlation studies have become feasible (Table 1). Terwindt et al. [30] compared the phenotypical consequences of the I1811L and V714A mutations. Interestingly, cerebellar ataxia was observed only with the I1811L mutation. No other significant differences were identified, except that loss of consciousness during attacks was reported more often in patients with the V714A mutation. Patients with the T666M mutation have reported both increased loss of consciousness and cerebellar ataxia [17, 31]. In the pedigree published by Elliott et al. [31], all patients had abnormal eye movements which is consistent with vestibulocerebellar dysfunction and probably an early manifestation of the cerebellar atrophy.

Permanent cerebellar ataxia was also reported in FHM patients with the mutations D715E, R583Q, R1668W and W1683R. In contrast, ataxia was never observed with the mutations R192Q, V714A, K1335E, V1457L and V1695I. Although the number of patients with these mutations is small, it seems that the position of the mutation or the specific amino acid change controls the development of ataxia in an unknown manner. The mutations causing ataxia are neither clustered nor located in homologous domains of α1A (Fig. 1). Comparison of the functional consequences of mutations with the clinical phenotype might explain, for instance, the ataxic phenotype observed with some mutations.

Table 1 FHM mutations in the CACNA1A gene and clinical phenotypic variation

Mutation	Exon	Localisation	Ataxia[a]	Observations	Reference
R192Q	4	IS4	–	–	Ophoff et al. [1]
R583Q	13	IIS4	+	Second frequent mutation; No common haplotype in four families	Battistini et al. [15]
				Altered consciousness[b]	Ducros et al. [18]
				Acetazolamide-responsive[b]	
T666M	16	IIS5-S6 linker	+	Most frequent mutation; No common haplotype in over 10 families	Ophoff et al. [1]
				Sporadic case observed	Ducros et al. [17]
				Interictal nystagmus[b]	Friend et al. [9]
V714A	17	IIS6	–	–	Ophoff et al. [1]
D715E	17	Loop II-III	+	Essential tremor	Ducros et al. [17]
K1335E	25	IIIS3-S4 linker	–	–	Ducros et al. [18][c]
Y1384C	26	IIIS5	+	Sporadic case	Ducros et al. [18][c]
				Partial seizures, meningitis, fever, altered consciousness	Vahedi et al. [42][c]
				Moderate mental deficit, nystagmus	
V1457L	27	IIIS5-S6 linker	–	–	Carrera et al. [16]
R1667W	32	IVS4	–	–	Ducros et al. [18][c]
L1682P	32	IVS4-S5 linker	+	Interictal nystagmus[b]	Gardner et al. [19][c]
W1683R	32	IVS4-S5 linker	+	–	Ducros et al. [18][c]
V1695I	33	IVS5	–	–	Ophoff et al. [1]
I1811L	36	IVS6	+	–	Ophoff et al. [1]

[a] As prominent symptom

[b] Phenotypic variation observed with some of the carriers of the mutation

[c] Publication in abstract form with only partial clinical information; amino acid positions are given according to the authors that described the mutation first

Functional consequences of mutations in CACNA1A

So far, the functional consequences of only a few mutations in the α1A protein have been studied. Abnormalities in calcium channel function can be investigated by measuring disturbances in electrophysiological parameters using patch clamp techniques, either on the whole-cell level, or by single channel measurements. Mutations have been introduced in rabbit and human α1A cDNAs which were subsequently transfected into *Xenopus* oocytes and human embryonic kidney 293 cells. Different mutations in α1A caused an identical clinical phenotype, suggesting that these mutations have a similar effect on calcium channel functioning: a loss-of-function or gain-of-function. However, results from two research groups have shown complicated patterns for the individual mutants (Table 2) [32–34].

Certain parameters, such as the voltage at which the mutant channels open, did change in the same direction for most of the mutations tested. Mutated Ca^{2+} channels are opened already at weak depolarisations. However, other parameters, like channel inactivation or the recovery from inactivation, showed different effects for the various mutations. In addition, the expression level of functionally active channels varied considerably among the mutations.

Mutation R192Q resulted in a 2-fold increase in current that was mainly due to an increased expression of mutated channels on the plasma membrane and, to a lesser extent, to an increased open probability of the channels at all voltages. It is expected that more calcium ions enter through such a mutated channel.

In contrast, the T666M mutation showed both a strongly decreased expression of functionally active channels, as well as a reduced single-channel current. This explains the observed reduced whole-cell current density. As a result, calcium influx is probably lower with this mutation than in the wildtype situation.

More difficult to understand are the functional consequences of the V714A and I1811L mutations. The V714A mutation and, to a lesser extent, the I1811L mutation allow

Table 2 Functional consequences of FHM mutations

Mutation	Activation voltage	Steady-state inactivation kinetics	Inactivation time constant	Channel inactivation during pulse trains	Recovery from inactivation time constant	Peak current density	Channel density on plasma membrane*	Most likely consequence of mutation on calcium influx
R192Q	↔	↔	↔	↔	↔	↑	↑	Gain of function
R583Q	Negative shift	Negative shift	↔	↑	↑	↓	ND	Loss of function
T666M	Negative shift	↔	↑	↑	↑	↓	↓	Loss of function
V714A	Negative shift	↔	↑	↓	↓	↓	↓	Gain or loss of function
D715E	Negative shift	Negative shift	↑	↑	↔	↓	ND	Loss of function
V1457L	Negative shift	↔	↓	↔	↑	↓	ND	Gain or loss of function
I1811L	Negative shift	↔	↔	↓	↓	↓	↓	Gain or loss of function

ND, not determined

The changes are in respect to wildtype calcium channels. In all cases, ↔ : not different from wildtype; ↓ : less than wildtype; ↑ : more than wildtype. *All data are from Kraus et al. [32, 33], except for channel density on the membrane [34]

channel opening at more negative potentials combined with an increased open probability. This implies that more calcium ions enter the cell through a single channel. However, when these data are combined with the reduced expression of these mutated channels (~40% and ~20% of wildtype expression, respectively), it remains unclear whether this increased single-channel influx is counterbalanced at the whole cell level by reduced channel expression.

The question arises if any of the altered biophysical properties account for the episodic character of migraine. Indeed, for mutations R583Q, T666M, V714A, D715E, and I1811L an increased or decreased Ca^{2+} current decay was observed during rapid trains of depolarisations. This was changed to altered channel inactivation during individual test pulses and/or changes in the recovery from inactivated channel states between pulses. It is therefore likely that pronounced alterations in neuronal Ca^{2+} influx occur in FHM neurons especially during episodes of high neuronal activity. This mechanism could link excessive neuronal stimulation (e.g. induced by migraine triggers) to the neuronal instability observed in functional studies.

It is difficult to predict the amount of calcium ions that enter a specific neuronal cell expressing mutated P/Q-type calcium channels. Many additional parameters, such as (1) the relative expression of the mutant and wildtype channel proteins in a given neuronal cell, (2) the contribution of various splice forms of the CACNA1A calcium channel subunit, and (3) the contribution of (alternatively spliced) auxillary β and α2δ subunits, which have not been studied yet.

The strange observations that some mutated channels can switch modes over time and seem to function as wildtype channels for short time periods are not understood but might be of importance [34]. At present, we do not know if compensatory mechanisms operate when these mutated calcium channels are expressed in neuronal nerve terminals or soma together with other channels like the L-type and N-type calcium channels.

Implications for migraine pathophysiology

One of the major questions is how mutated calcium channels cause FHM. We also would like to know how altered calcium channel functioning affects pathophysiology of the common forms of migraine. From the previously described electrophysiological studies, one may suggest that disturbances in calcium channels result in either an increase or a decrease of calcium ion flux into neuronal cells. In ways not understood, the abnormalities in calcium channel functioning could result in neuronal instability which renders patients susceptible to migraine attacks that are triggered by neural stimuli, such as stress or sensory afferentiation. The observation that the functional disturbances were more prominent at high frequency stimulation explains the episodic nature of the associated disease. In FHM patients with V714A or I1811L mutations, sustained neuronal firing due to an incorrect integration of (otherwise normal) neural stimuli may result in a relative decreased inactivation of calcium channels. Ultimately, this will result in an increased influx of calcium ions and perhaps abnormal down-stream neuronal signalling pathways. Abnormal Ca^{2+} influx can explain the observed cell death which results in cerebellar atrophy.

Since P/Q-type calcium channels control neurotransmitter release from nerve terminals (including serotoninergic

caudal raphe neurons [35]), it is important to prove that altered Ca^{2+} influx from FHM mutations translates into changes in neurotransmitter release. This hypothesis is strongly supported by recent findings in *tottering* mice. These mice have a missense mutation changing a proline into a leucine at position 601 of the P/Q-type calcium channel, and are considered models for absence epilepsy [36]. We have shown that spontaneous acetylcholine release in peripheral synapses of the neuromuscular junction is increased in *tottering* mice [37]. However, at high frequency stimulation, there is a decrease in transmitter release in homozyous mice. Field-potential studies in thalamic nuclei and hippocampal areas show that neurotransmitter release is also altered in the CNS of *tottering* mice [38, 39]. In thalamic nuclei, transmitter release at specifical glutamatergic synapses is reduced [38]. Interestingly, reduced calcium influx through P/Q-type channels, and a coinciding decrease in neurotransmitter release from these channels, do not prevent compensation of neurotransmitter release via N-type channels in *tottering* mice [39]. Future studies will have to test whether only neurons that are unable to rely on compensatory mechanisms are compromised in this mouse model.

Although tentative at present, the observations by Ferrari et al. [40, 41] that serotonin and glutamate levels in migraine patients are altered, especially during attacks, fit into an integrated hypothesis on migraine pathophysiology that now includes abnormalities in neurotransmitter release due to P/Q-type calcium channel mutations.

Future directions

Although much insight has been gained using in vitro cell systems, it clearly is a major challenge to study the effects of FHM mutations in their natural environment, i.e. in neuronal cells. Only then can we evaluate their contribution to the pathogenesis of FHM and perhaps to the common forms of migraine. In years to come, transgenic knock-in mice carrying FHM mutations in the endogenous *CACNA1A* gene will be useful in this respect.

Genetic approaches that include parametric and non-parametric mapping, such as sib-pair analysis that investigates the increased sharing of alleles between affected sibs with MA and/or MO, will be extremely important for identifying new migraine genes. The use of genetic isolates may limit the genetic heterogeneity and thus help overcome the difficulties that we face today when mapping complex diseases. In the future, the discovery and characterisation of new migraine genes and associated pathways will help put the pieces of the complex puzzle together. A better understanding of migraine pathophysiology is crucial to the development of novel drugs that will hopefully result in a better (prophylactic) treatment for the common types of migraine.

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