Genetics of migraine and related syndromes

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CHAPTER 2

CACNA1A R1347Q: A FREQUENT RECURRENT MUTATION IN HEMIPLEGIC MIGRAINE

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ABSTRACT

Of the 18 missense mutations in the *CACNA1A* gene which are associated with familial hemiplegic migraine type 1 (FHM1), only mutations S218L, R583Q and T666M were identified in more than two independent families. Including the four novel families presented here, of which two represent *de novo* cases, the R1347Q mutation has now been identified in six families. A genotype-phenotype comparison of R1347Q mutation carriers revealed a wide clinical spectrum ranging from (trauma-triggered) hemiplegic migraine with and without ataxia, loss of consciousness and epilepsy. R1347Q is the third most frequent mutation in hemiplegic migraine patients and should therefore be screened with priority for confirmation of clinical diagnosis. This study clearly demonstrates that the availability of multiple families better reflects the full clinical spectrum associated with FHM1 mutations.

INTRODUCTION

Familial hemiplegic migraine (FHM) is a rare autosomal dominant inherited subtype of migraine with aura, mainly characterized by transient hemiparesis during the attacks.[1] The disease is genetically heterogeneous as mutations in three genes, *CACNA1A* (FHM1), *ATP1A2* (FHM2) and *SCN1A* (FHM3) are involved,[2-5] and FHM families exist who are not linked to these genes. Mutations in all three genes cause a broad spectrum of clinical symptoms (for review see Kors et al., 2004[6]). FHM1 mutations are associated with clinical symptoms of hemiplegic migraine, ataxia, attacks of confusion, alterations of consciousness, epilepsy, fever and even fatal brain edema.[7-11]

To date 18 FHM1 mutations in the *CACNA1A* gene have been reported, of which six are recurrent. The T666M mutation has been found in 21 unrelated families, R583Q was identified in seven families and S218L in three; mutations R1347Q, R1668W, I1710T and I1811L were reported twice.

The identification of recurrent mutations and comparison of detailed clinical information of mutation carriers make genotype-phenotype correlations feasible. Moreover, the detection of mutation-specific clinical symptoms could benefit diagnostic testing. Here, we present four unrelated families carrying the R1347Q *CACNA1A* mutation, establish R1347Q as the third most frequent recurring *CACNA1A* mutation and describe an expansion of the clinical spectrum associated with this mutation.

MATERIALS AND METHODS

Clinical diagnosis

Diagnoses were made by neurologists according to the International Classification of Headache Disorders-second edition (ICHD-II) criteria.[1] All subjects gave informed consent.

Mutation analysis

Genomic DNA was isolated from peripheral blood cells according to standard methods.[12] All 47 exons of the *CACNA1A* gene were analysed using direct sequencing analysis. Exons were amplified by PCR using exon-specific primer sets and genomic DNA of each proband. Details of intron-exon structure, primer sequences and conditions are available from the authors upon request. Double-strand sequencing was done by Cycle sequencing (Prism Big Dye Terminators Cycle Sequencing kit, Applied Biosystems, Foster City, CA) using the dideoxy termination method and an ABI3700 sequencer (Applied Biosystems, Foster City, CA).

Carrier detection

For detection of the R1347Q mutation (nt 4040 G>A, *CACNA1A* reference sequence: X99897), exon 25 was amplified by PCR using primers that were extended with an M13-tail (underlined): "exon25F" (5'TGTAAAACGACGGCCAGTCTACCCAACCTGACCTCTGC-3') and "exon25R" (5'-CAGGAAACAGCTATGACCCCATACACGATGGCTAGGATG-3'), resulting in a 329-bp product. Subsequently, PCR products were digested with restriction enzyme *Hinf*I using

standard protocols, and electrophoresed on a 3% agarose gel. The R1347Q mutation causes a loss of a *Hinf*I site, which results in an undigested product of 329 bps for the mutant allele and wild-type products of 124 and 205 bps.

Haplotype analysis

For haplotyping, genetic markers D19S221, D19S1150 and D19S226 were tested. For primer sequences and PCR conditions, see http://gdbwww.gdb.org/. After PCR, amplified products were separated using an ABI 3700 DNA sequencer. All genotypes were analyzed and independently double scored using Genescan and Genotyper 2.1 software (Applied Biosystems, Foster City, CA).

RESULTS

Pedigrees of the four hemiplegic migraine families are shown in Figure 1. We identified a heterozygous nt 4040 G>A substitution in exon 25 (*CACNA1A* reference sequence: X99897), in the probands of all four families. The mutation causes an Arginine to a Glutamine substitution at codon 1347 that is located in the S4 segment of the protein domain III (see Figure 2). The R1347Q mutation co-segregated in all eight family members with hemiplegic migraine from whom DNA was available. In addition, the mutation was found in one person who reported hemisensory aura symptoms, but no weakness. Unique haplotypes showed no

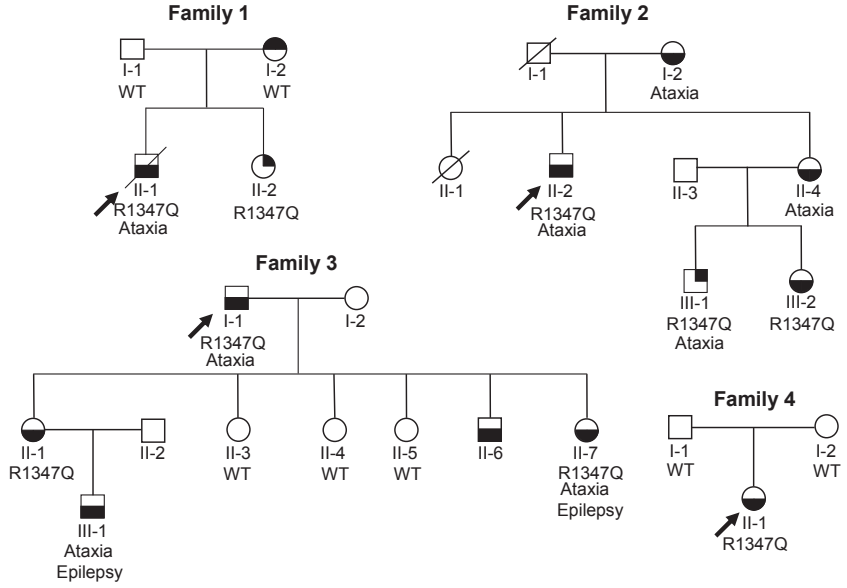

Figure 1. The pedigrees of the four R1347Q families.The arrow indicates the proband. Circles indicate females, squares indicate males. The following symbols indicate the clinical diagnosis: black lower half: hemiplegic migraine; black upper right quadrant: migraine with aura; black upper left quadrant: migraine without aura. The presence or absence of the R1347Q mutation is indicated by "R1347Q" and "WT" (wild-type), respectively.

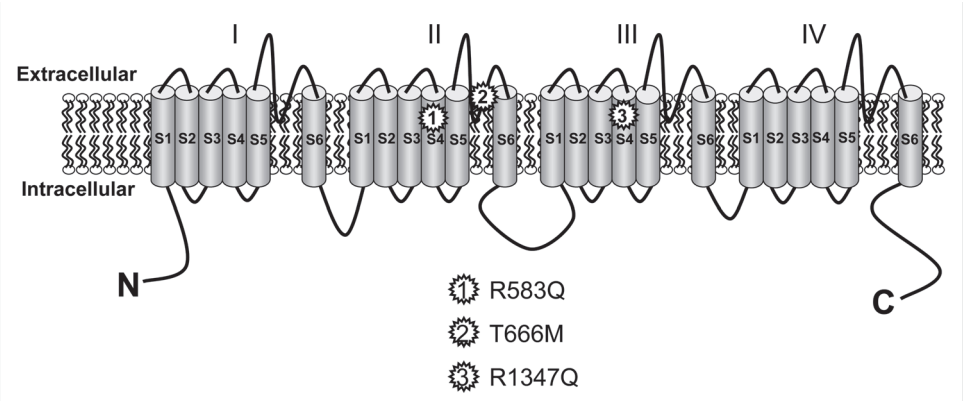

Figure 2. Schematic representation of the $Ca_v2.1\alpha1$ calcium channel subunit protein encoded by the *CACNA1A* gene. Each of the four protein domains (I to IV) has six transmembrane segments (S1-S6). The location of the three most frequent recurrent mutations is indicated.

relation between the families, indicating that all mutations occurred independently (data not shown). The mutation was not found in 200 control chromosomes by restriction enzyme analysis with *Hinf*I.

Family 1

The proband (II-1) was a 54-year-old male who since the age of three had hemiplegic migraine attacks, with a frequency of one every four to six weeks. Severe hemiplegic migraine attacks could last up to a week. During these attacks he could only be aroused briefly by vigorous stimulation and had an elevated temperature. From the age of three he gradually developed gait ataxia and dysarthria over the years. After the FHM attacks his gait ataxia and dysarthria worsened for days to weeks, but subsequently returned to the pre-ictal level. Interictal examination at the age of 51 showed saccadic pursuit eye movements, mild dysarthria, abnormal tandem gait, dysdiadochokinesis, intention tremor on finger-to-nose testing and overshoot on finger chase testing. Cerebral MRI showed evidence of cerebellar atrophy. The patient died at age 54 of a colon carcinoma. No autopsy was performed. His mother, now aged 82, has suffered from migraine without aura since puberty and recently started to have attacks of visual aura always followed by a non-migrainous headache (ICHD-II 1.2.2). The sister of the proband has migraine with visual aura since the age of 34. Mother and sister never experienced migraine attacks associated with hemiplegia.

Family 2

The proband (II-2) is a now 54-year-old male who has suffered from attacks of hemiplegic migraine since the age of three. Some attacks were associated with drowsiness and fever. Cerebral MRI scans were unremarkable. Interictal neurological examination at age 47 showed mild cognitive deterioration, saccadic ocular pursuit, gaze-evoked nystagmus, mild limb ataxia and more marked gait ataxia. Since the age of 47 he has been treated with acetazolamide which has dramatically reduced the number of attacks. His mother (I-2)

suffers from hemiplegic migraine and has a marked interictal gait ataxia. His eldest sister (II-1), who suffered from schizophrenia, died at the age of 19 in her sleep after a presumed epileptic attack. His youngest sister (II-4) suffers from typical attacks of hemiplegic migraine associated with mild confusion and disorientation, somnolence, general malaise and deafness. Interictal neurological examination at age 38 showed saccadic ocular pursuit, mild dysarthria, mild symmetrical limb ataxia and marked gait ataxia. Her son (III-1) now aged 18 has had a total of about 10 attacks of migraine with hemisensory symptoms since the age of five, but no weakness was reported. Some episodes were provoked by head trauma. Between episodes he has a gaze-evoked nystagmus to the left, mild dysarthria and mild gait ataxia. Her daughter (III-2), now aged 17 had episodes of blurred vision and paraesthesia in both upper and lower limbs, followed by weakness, and accompanied by headache, nausea and vomiting. Attacks are often brought on by flickering light. She had episodes of loss of consciousness, which were considered to be of epileptic origin and were successfully treated with valproate.

Family 3

Family 3 is a Jewish family that was originally reported in 1997.[13] The proband (I-1) is a 70-year-old male who has had attacks of hemiplegic migraine since the age of 26. He currently has an attack once every three to four months. None of the attacks has ever been accompanied by seizures. Interictal neurological examination reveals dysmetria on finger-to-nose testing, dysdiadochokinesis and an abnormal tandem gait. One of his children, II-6, now aged 29, has had three attacks of hemiplegia with dysarthria lasting between five and fifteen minutes not accompanied by headache. His interictal neurological examination is normal. Individual II-7 is a now 27-year-old woman who has suffered from attacks of hemiplegic migraine once every three months since the age of six. Attacks are sometimes associated with focal seizures always on the same side as the hemiplegia. She never experiences seizures independently from hemiplegic attacks. She has progressive ataxia**.** Individual II-1, aged 36 years, has had two events of acute hemiplegia at the ages of five and six years. She has a normal neurological examination. Her 17-year-old son (III-1) has had attacks of hemiplegic migraine since the age of three years now occurring once every three to six months. In addition, he has progressive ataxia. He also suffers from focal (tonic or clonic) seizures, independent of the hemiplegic migraine attacks, which can be secondary generalized. Three other daughters of I-1 do not suffer from any type of migraine.

Family 4

The proband (II-1) of this family is a 13-year-old girl. Her first attack of hemiplegic migraine occurred when she was nineteen months old. Later she had on average one to two attacks a year; the last one occurring two years ago. The attacks are always precipitated by trivial head trauma and include severe headache accompanied by nausea and vomiting, hemiplegia, hemianesthesia, psychotic alterations and stupor. Attacks always completely resolved within three days. She has a normal psychomotor and mental development. An MRI made 6 years ago showed no cerebellar atrophy. Neither of her parents nor any other family member has migraine or ataxia.

DISCUSSION

We identified the *CACNA1A* R1347Q mutation in four unrelated hemiplegic migraine families. This makes R1347Q the third frequently occurring FHM1 mutation. The mutation changes a positively charged Arginine to a neutral Glutamine and is located in the S4 segment of the third protein domain. The positively charged amino acids of the S4 segment act as the 'voltage sensor', which detect changes in the membrane electrical field. Mutations that neutralize highly conserved Arginine residues (i.e., R192Q, R195K, R583Q R1347Q, and R1677W) affect channel functioning by altering the voltage dependence[14] and are associated with disease.

The R1347Q mutation was previously identified in four hemiplegic migraine patients: two in a Portuguese family[15] and two in a German family.[16] All patients also had progressive cerebellar ataxia. With the present study, the total number of families with the R1347Q mutation has increased to six and the total number of mutation carriers to 13. Three additional hemiplegic migraine patients - two with ataxia - likely have the mutation, but genetic confirmation was not possible.

The newly identified R1347Q families reveal a broader clinical spectrum than previously reported. Attacks start at a young age in the majority of patients (range 19 months- 26 years; median four years) and the frequency of attacks is highly variable, from two attacks in a lifetime to one attack per month. Most mutation carriers had attacks of hemiplegic migraine: six with and three without interictal cerebellar signs. Patient III-1 in family 2 had no weakness during attacks, but hemisensory symptoms. In two patients attacks were triggered by head trauma and in two other patients attacks were associated with altered consciousness and fever. The young proband from family 4 had psychotic alterations and stupor. The proband of family 1 suffered from remarkably long hemiplegic attacks, which could last up to a week. After the attacks, there was often a temporary worsening of gait ataxia and dysarthria. The hemiplegic attacks of the proband of family 2 responded to acetazolamide, which has been reported before.[17]

Epilepsy now also appears to be part of the phenotypic spectrum of R1347Q, being present in two hemiplegic migraine patients of family 3. Patient II-7 had focal seizures ipsilateral to the hemiplegia during hemiplegic migraine attacks. Patient III-1 suffered from focal seizures that occurred independently from hemiplegic migraine attacks. *CACNA1A* mutations have been associated with epilepsy; in most cases occurring during hemiplegic attacks.[7-11;18,19] In only one FHM1 family did epileptic seizures occur independently from hemiplegic migraine attacks.[10]

In conclusion, although hemiplegic migraine and progressive ataxia are the predominant clinical features in these families, the R1347Q mutation, like other FHM1 *CACNA1A* mutations, shows considerable phenotypic variation. Identification of recurrent mutations has implications for genetic screening, as they should be screened with priority, that is before screening the entire *CACNA1A* gene.

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