Expanding the Phenotypic Spectrum of the CACNA1A Gene T666M Mutation

A Description of 5 Families With Familial Hemiplegic Migraine

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Background: Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura. Missense mutations in the chromosome 19 CACNA1A calcium channel gene have been found in approximately half of the families. The T666M mutation, replacing a threonine by a methionine at residue number 666, is the most frequent mutation, reported in 14 independent FHM families; other mutations have so far been described in only 1 or 2 families each. The clinical features of T666M families have been reported, but the course is unknown.

Objective: To present a detailed description of the clinical features of new FHM families in which we identified the T666M mutation in our CACNA1A screening program.

Methods: As part of our ongoing genetic screening, mutation analysis of the CACNA1A gene was performed by single-strand conformational polymorphism analysis in 33 probands of families with FHM.

Results: We identified the T666M mutation in 5 unrelated FHM families. In 3 of the families, patients displayed cerebellar ataxia. In 1 family, some affected members with the mutation had attacks with confusion but without hemiparesis. In 1 family, patients had progressive cognitive dysfunction.

Conclusions: The T666M mutation is the most frequent CACNA1A mutation in FHM; it was found in 5 of 33 FHM families at our laboratory, and in 19 of 39 families with a known mutation reported in the literature (including the present study). Screening for the T666M mutation should therefore be the first step when screening families with FHM. There is a remarkable clinical heterogeneity among families with the T666M mutation.

Arch Neurol. 2003;60:684-688

Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura, characterized by hemiparesis during the aura. 

Approximately one third of FHM families also have progressive cerebellar ataxia. Most of the described FHM families, including all those with ataxia, have been assigned to chromosome 19p13. In 1996, Ophoff et al identified 4 different missense mutations in the CACNA1A gene on chromosome 19p13 in 5 unrelated FHM families. Since then, 14 different CACNA1A missense mutations have been reported in a total of 34 families with FHM. Eight mutations were associated with “pure” FHM, 5 with FHM plus cerebellar ataxia, and 1 (R1668W) was found in one family with FHM and cerebellar ataxia and one family with FHM without ataxia.

Of the 14 mutations, 8 were found once, 4 mutations (R192Q, S218L, R1667W, and I1811L) were found twice, the R583Q mutation was identified 4 times, and finally, the T666M mutation was reported in 14 unrelated families. Cerebellar ataxia was present in members of all 14 described FHM families with this mutation, where the amino acid threonine at residue number 666 of the coding sequence of the gene is replaced by a methionine. Recently, an overview of FHM mutations and their phenotype was published. However, only limited information on clinical features and course of the disorder was provided. We present herein a detailed description of the clinical features of 5 new FHM families in which we identified the T666M mutation in our CACNA1A screening program.

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As part of our ongoing genetic analysis of the CACNA1A gene, we studied 33 probands of families with FHM. Initially, only DNA samples of the proband of each family were analyzed. Polymerase chain reaction products of all 47 exons of the CACNA1A gene were screened for sequence aberrations by single-strand conformational polymorphism analysis. We identified the T666M mutation in 5 probands from 5 different families. This mutation is a substitution of C to T in exon 16, causing a threonine for methionine substitution at codon 666 of the coding sequence of the gene, which is located in the S5-S6 linker of the second domain of the protein. This linker is 1 of the 4 P segments that line the inner part of the ionic pore. After detection in the proband, the presence of the T666M mutation was investigated in all family members for whom DNA was available (Figure). Subsequently, haplotype analysis was performed, using 2 extragenic polymorphic microsatellite DNA markers from the Genethon linkage map, D19S221 and D19S226, that flank the CACNA1A gene and 2 intragenic polymorphisms, the D19S1150 satellite marker in intron 7 and the CAG repeat in exon 47 of the gene.

**METHODS**

**GENETIC ANALYSIS**

The pedigrees of families 1 through 5. Question mark indicates that information of the attacks was provided by other family members; plus sign, DNA was available. T666M+, family member carries the T666M mutation; and T666M−, family member does not carry the T666M mutation.

**RESULTS**

**DESCRIPTION OF THE T666M CARRIERS**

Pedigrees of the 5 T666M families, in which all mutation carriers are indicated, are shown in the Figure. Clinical features of families 1 and 4 have been reported previously at a time when the causative mutation in these families was still unknown. A summary of clinical features of all families is presented in the Table. No similar haplotypes were found in the families, so it can be concluded that the families are unrelated and each mutation occurred independently.

**FAMILY 1 (BRITISH ORIGIN)**

In family 1, the proband, a 32-year-old man (II:3), had attacks of hemiplegic migraine since the age of 17 years. In the past 2 years, he experienced 3 to 12 attacks per month. He is the only affected sibling of 5 siblings. His 60-year-old mother (I:2) had a 15-year history of hemiplegic migraine, occurring approximately twice a month. Both patients showed interictal jerky ocular pursuit, gaze-evoked horizontal nystagmus, impaired vestibuloocular reflex suppression, and mild limb and gait ataxia. Magnetic resonance imaging (MRI) of the brain of the proband revealed mild cerebellar atrophy. Acetazolamide, 250 mg twice a day, reduced the attack frequency in both patients during a period of months, but the attacks returned in the proband despite continued use of the drug.

**FAMILY 2 (BRITISH ORIGIN)**

In family 2, the proband (IV:20) had had several attacks of migraine with right-sided hemiplegia that lasted for 30 minutes since the age of 12 years. At the age of 15 years, he had a different type of attack, starting with blurred vision and followed after 5 minutes by headache and weakness of the left arm. He became drowsy and was admitted to the hospital, where he further deteriorated into coma. Subcutaneous sumatriptan succinate (probably 6 mg but not documented) was given, but the patient continued to deteriorate. Computed tomography of the brain was normal at that time. A cerebral MRI 4 days later showed evidence of right middle cerebral artery territory infarction (MRI not available). The patient died 3 days later while having tonic-clonic seizures. No postmortem examination was performed. Several other family members (II:5, II:9, III:12, II:11, and III:16) had migraine attacks with hemiplegia and confusion and severe attacks with coma lasting up to a week, associated with high fever and cerebrospinal fluid pleocytosis.

Cerebral MRI results of patient II:11 showed multiple high T2-signal foci scattered throughout both hemispheres, which in this age group would be in keeping with minor ischemic change. Cerebral MRI results of patient III:16 showed a certain amount of cerebral cortical atrophy. In addition, some tiny bright objects in both ventricles were present, which were small and not further specified. Cerebral MRI showed no abnormalities for patient IV:17.
Three family members (II:5, II:9, III:12) showed cognitive impairment with a rather stereotypical course: they initially had normal early development, but after onset of the (severe) attacks, there was a progressive cognitive decline. It is unknown whether they got worse in a stepwise fashion after each attack or whether it was a slowly progressive deterioration. Their cognitive decline resulted in inability to live independently in adulthood. All affected members had cerebellar ataxia. Family members IV:17, IV:18, IV:19, and IV:21 have frequent attacks of migraine without aura; family member II:5 had 2 attacks of migraine with visual aura in his teens. None of them carried the T666M mutation.

**FAMILY 3 (AMERICAN ORIGIN)**

This family consists of 8 affected members in 3 generations. Clinical data were limited and not detailed. Starting at the age of 17 years, the proband (II:9) had migraine headaches preceded by blurred vision, dark spots, and incoherent speech, often accompanied by aggressive behavior, approximately once a year. In 70% of these attacks, he had hemiparesis for a half hour. He felt sleepy during the attacks and once became unconscious. Other family members (II:7, II:8, III:11, III:13, III:14, III:17, and IV:18) had migraine with hemiparesis. All of them but patient III:11 were carriers of the T666M mutation. It is not documented whether these family members had signs of cerebellar ataxia.

**FAMILY 4 (GERMAN ORIGIN)**

The proband of this family is a 36-year-old man who had yearly attacks of migraine with hemiparesis that lasted 10 minutes since the age of 10 years. At the age of 16 years, following a cerebral angiogram performed to exclude an arteriovenous malformation, he became comatose with high fever for several days. At that time, external ventricular drains were inserted for undocumented reasons. On another occasion, he was found unconscious on the street. On admission to the hospital, he was drowsy and aphasic and had a right-sided hemiparesis and unequal pupils. Except for a slight leukocytosis, laboratory results were normal. His temperature was normal. The results of computed tomography of the brain with contrast were unremarkable, except for a left frontoparietal trepanation. Two hours after admission, his temperature rose to 40°C. Lumbar puncture showed a normal cell count. The following day the temperature normalized, pupils became equal, and the aphasia and hemiparesis improved. After 3 days, the patient only had a minimal aphasia, which resolved completely. Cerebral MRI without gadolinium was performed but showed no pathological signs, especially not in the cerebellum. The results of the last neurologic examination, 1 year ago, were normal. The patient reported no further attacks while taking propanolol as a prophylactic drug (40 mg, 3 doses daily).

His father (II:5) has migraine attacks that occurred 6 to 8 times a year, accompanied by visual symptoms, abnormal speech, and confusion. Twice a year he has an attack with unconsciousness, lasting approximately 15 minutes. In 1980, he had an attack with hemiparesis, high temperature (39.4°C), and right hemispheric edema on computed tomography with normal cerebrospinal fluid levels. All symptoms improved within a few days. In the subsequent 6 years, he was admitted 5 times to the hospital. The father died of cardiac failure; no descriptions of cerebellar signs were found in his medical history. Another family member (II:4) experienced attacks of headache with hemiparesis, occasionally accompanied by unconsciousness for a half hour.

**FAMILY 5 (CZECH ORIGIN)**

The proband (II:5) had a history of frequent attacks of migraine with sensory aura that started at the age of 19 years. Attacks with hemiparesis that lasted for several hours occurred 3 times a year. During these attacks, he had a high temperature, was confused, and slept for several days. Neurologic examination showed no abnormalities except for nystagmus. His son (III:15) had attacks of confusion, lethargy, drowsiness, and altered behavior without headache. These attacks were provoked by minor head injury or physical exercise. Only recently, the attacks were accompanied by hemiparesis for 30 minutes. His daughter (III:14) had episodes of coma followed by headache 3 times a year but without hemiparesis. The brother of the proband (II:3) had attacks of headache twice a year, with hemiparesis and unconsciousness for 1 hour. Other family members (III:12, II:10, II:18, and I:2) had hemiplegic migraine, occasionally accompanied by altered consciousness, which could be triggered by physical activity. Patient III:19 was a mutation carrier but at the age of 24 years did not show any symptoms.

We describe 5 FHM families in which we have identified the T666M mutation in exon 16 of the CACNA1A
gene on chromosome 19p13. The T666M mutation is by far the most frequent mutation, being found in 19 of 39 probands with a CACNA1A mutation overall. In our cohort of hemiplegic migraine patients, with and without ataxia, the T666M mutation was found in 5 of 33 familial cases. Therefore, we suggest that the T666M mutation is sought first when a CACNA1A gene–related disorder is suspected. If no T666M mutation is detected, the remaining exons can be screened, or linkage analysis to the CACNA1A locus can be performed in the family.

Although all families with the T666M mutation described so far had chronic cerebellar ataxia, members of family 4 were interictally normal. This would be the first T666M family with pure hemiplegic migraine. It adds to previous findings that a strict genotype-phenotype relation in CACNA1A disorders does not exist.17

In family 5, 2 patients (III:14 and III:15) did not have hemiparesis during the attacks, so strictly they do not meet the International Headache Society criteria for hemiplegic migraine.3 Patients III:14 and III:15 had episodes of confusion, especially after a period of physical exercise, resembling acute confusional migraine.18 Episodes of acute confusion or psychosis have been described before but always in addition to hemiplegic migraine attacks.19,20 Only recently, at the age of 16 years, patient III:15 started to have attacks with hemiparesis. Acute confusional migraine might have been the first symptoms of the CACNA1A mutation in this patient.

In a recent analysis of 28 families with FHM, 6 persons had had 10 attacks with severe coma. The index case of family 4 had severe attacks with pyrexia and coma that lasted up to a week. In family 2, 6 members had, in addition to hemiplegic attacks, sporadic severe attacks with coma and meningeal signs. Patients with a T666M mutation were characterized by the highest frequency of severe attacks with coma or confusion (50% of mutation carriers).11 Other mutations in the CACNA1A gene have been described to be associated with coma during FHM attacks as well.7,21-23 Fitzsimons and Wolfenden14 described a family in which members had recurrent attacks with hemiplegic migraine and severe coma. Recently, an S218L mutation in the CACNA1A gene was identified in this family.10

In the family with the S218L mutation, several affected members were considered to be intellectually slow compared with their unaffected siblings; one patient was mentally retarded before the onset of the attacks. Cognitive impairment is a symptom not often associated with FHM. Remarkably, in family 2, 3 members who carried the mutation showed progressive cognitive decline after normal early development. In another FHM family, without proven CACNA1A mutation, 3 of 4 patients showed permanent mild cognitive impairment following the onset of the FHM attacks.23 Lastly, Vahedi et al22 described a patient with hemiplegic migraine with no other affected family members with developmental delay and severe mental retardation who had a de novo Y1385C mutation in the CACNA1A gene. Cognitive dysfunction is also observed in episodic ataxia type 2 (EA-2), another disorder caused by CACNA1A mutations.4 Cognitive symptoms in EA-2 range from marked learning difficulties at school to major mental retardation requiring a specialized institution.26 It is unclear whether cognitive impairment can be assigned to damage during attacks of FHM or EA-2 or to a process of progressive neuronal damage. In family 2, it was suggested that the cognitive decline was stepwise, worsening after each attack.

Acetazolamide was given to patients II:3 and I:2 of family 2, and the patients seemed to respond well for a period. Acetazolamide is a well-known treatment for patients with EA-2.27 Also, FHM and other aura subtypes have been reported to respond to acetazolamide.7,28 In addition, a patient with migraine with aura and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a hereditary arteriopathy leading to recurrent cerebral infarcts and dementia) showed a marked reduction in migraine attacks after he began taking acetazolamide, 250 mg/d.29 The mechanism of action of acetazolamide in migraine aura, however, is unknown.

Although the prognosis of FHM seems generally favorable, the families presented herein show that FHM is a complex syndrome, which in some patients can cause permanent deficits or even death. The age at onset in affected persons in this study is generally younger than 30 years, but the disease progress is variable. At one end of the spectrum is the proband of family 2, who had an infarct in the territory of the middle cerebral artery during one of his migraine attacks and died at the age of 14 years. It is, however, not known whether this infarct occurred as a direct result of the mutation or as a complication of the attack or treatment. In contrast, patient I:2 of family 1 is currently 60 years old and shows no signs of FHM, apart from mild cerebellar ataxia. Predicting the outcome for individual patients on genotypic data of the CACNA1A gene is therefore impossible.

The clinical variety in carriers of the T666M mutation in the CACNA1A gene confirms that single-gene disorders with “simple” mendelian inheritance, such as FHM, can behave as a complex trait. In all likelihood, environmental factors and/or modifier genes act at some point on the comprehensive function of P/Q type calcium channels in the brain.

Accepted for publication November 12, 2002.

Author contributions: Study concept and design (Drs Kors, Haan, Terwindt, Frants, and Ferrari); acquisition of data (Drs Kors, Giffin, Pazdera, Schnittger, Lennox, and Van den Maagdenberg and Mr Vermeulen); analysis and interpretation of data (Drs Kors, Giffin, Terwindt, Van den Maagdenberg, and Ferrari and Mr Vermeulen); drafting of the manuscript (Drs Kors, Pazdera, Schnittger, and Van den Maagdenberg and Mr Vermeulen); critical revision of the manuscript for important intellectual content (Drs Haan, Giffin, Schnittger, Lennox, Terwindt, Van den Maagdenberg, Frants, and Ferrari); obtained funding (Drs Frants and Ferrari); administrative, technical, and material support (Drs Kors, Schnittger, Lennox, and Van den Maagdenberg and Mr Vermeulen); study supervision (Drs Haan, Terwindt, Van den Maagdenberg, Frants, and Ferrari).

This study was supported by the Netherlands Organisation for Scientific Research (The Hague) (grant 903-52-291) and Migraine Trust (London, England).
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