

## Recurrence of the T666M Calcium Channel CACNA1A Gene Mutation in Familial Hemiplegic Migraine with Progressive Cerebellar Ataxia

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### Summary

Familial hemiplegic migraine (HM) is an autosomal dominant migraine with aura. In 20% of HM families, HM is associated with a mild permanent cerebellar ataxia (PCA). The CACNA1A gene encoding the  $\alpha$ 1A subunit of P/Q-type voltage-gated calcium channels is involved in 50% of unselected HM families and in all families with HM/PCA. Four CACNA1A missense mutations have been identified in HM: two in pure HM and two in HM/PCA. Different CACNA1A mutations have been identified in other autosomal dominant conditions: mutations leading to a truncated protein in episodic ataxia type 2 (EA2), small expansions of a CAG trinucleotide in spinocerebellar ataxia type 6 and also in three families with EA2 features, and, finally, a missense mutation in a single family suffering from episodic ataxia and severe progressive PCA. We screened 16 families and 3 nonfamilial case patients affected by HM/PCA for specific CACNA1A mutations and found nine families and one nonfamilial case with the same T666M mutation, one new mutation (D715E) in one family, and no CAG repeat expansion. Both T666M and D715E substitutions were absent in 12 probands belonging to pure HM families whose disease appears to be linked to CACNA1A. Finally, haplotyping with neighboring markers suggested that T666M arose through recurrent mutational events. These data could indicate that the PCA observed in 20% of HM families results from specific pathophysiologic mechanisms.

### Introduction

Familial hemiplegic migraine (HM; MIM 141500) is an autosomal dominant form of migraine with aura (Blau and Whitty 1955; Headache Classification Committee of the International Headache Society [IHS] 1988). Typical attacks include a unilateral motor deficit associated with paresthesias, speech disturbances, or visual signs. These aura symptoms last from 10 min to a few hours and are followed by a migrainous headache. In 20% of unselected families, some HM patients have permanent cerebellar symptoms, such as nystagmus and slowly progressive mild to moderate static ataxia. In some cases, cerebral magnetic resonance imaging reveals cerebellar atrophy. Cerebellar ataxia may be diagnosed prior to the first HM attack and progresses independently of the frequency and/or severity of these attacks. Autonomous gait remains generally possible, even after years of evolution (Ohta et al. 1967; Young et al. 1970; Zifkin et al. 1980; Fitzsimons and Wolfenden 1985; Joutel et al. 1993, 1994; Ophoff et al. 1994; Elliott et al. 1996; Terwindt et al. 1998). HM has been shown to be genetically heterogeneous (Joutel et al. 1994; Ophoff et al. 1994; Ducros et al. 1997).

The first responsible gene, located on chromosome 19p13.1 (Joutel et al. 1993), was identified in 1996 as being CACNA1A, which encodes the main pore-forming  $\alpha$ 1A subunit of P/Q-type voltage-gated calcium channels (Ophoff et al. 1996). These channels are expressed in a large variety of neurons, in which they play an important role in the control of membrane excitability, neurotransmitter release, and gene expression (Catterall et al. 1995). They are the predominant calcium channel in Purkinje cells (P-type currents) and are highly expressed in cerebellar granule cells (P- and Q-type currents) (Llinas et al. 1992; Stea et al. 1994; Westenbroek et al. 1995). CACNA1A has been determined to be involved (on the basis of linkage or mutation screening data) in

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**Table 1**  
No. of Sampled Individuals in the 16 HM/PCA Families

FAMILY	AFFECTED			HEALTHY	UNKNOWN STATUS	NO. OF SPOUSES	TOTAL	
	Total	Without PCA	With PCA					Not Examined
F1	20	1	16	3	13	3	7	43
F2	5	...	5	...	...	...	2	7
F3	3	...	3	...	7	...	1	11
F4	2	...	1	1	1	2	...	5
F5	17	6	11	...	10	...	6	33
F6	5	2	3	...	...	...	2	7
F7	2	...	2	...	...	...	1	3
F8	1	...	1	...	...	...	...	1
F9	1	...	1	...	...	...	...	1
F10	13	7	5	1	2	...	5	20
F11	6	...	6	...	...	4	5	15
F12	4	...	4	...	2	...	1	7
F13	2	1	1	...	...	...	1	3
F14	4	2	2	...	1	...	2	7
F15	1	...	1	...	...	...	...	1
F16	1	...	1	...	...	...	...	1
Total	87	19	63	5	36	9	33	165

all five families with HM and progressive cerebellar ataxia (HM/PCA) reported thus far (Joutel et al. 1993; 1994; Ophoff et al. 1996; Terwindt et al. 1998). In contrast, pure HM has been linked to at least three different genes: CACNA1A (Joutel et al. 1994; Ophoff et al. 1994; 1996); a second, unidentified gene mapped on chromosome 1q21-q23 (Ducros et al. 1997; Gardner et al. 1997); and at least a third gene, still to be localized (Ducros et al. 1997).

Only six HM families—three with HM/PCA and three with pure HM—have been screened thus far for CACNA1A mutations (Ophoff et al. 1996; Terwindt et al. 1998). In HM/PCA, two missense mutations have been identified: T666M in one family and I1811L in two unrelated families. In pure HM, two other missense mutations have been detected: R192Q and V714A, each in a single family. Because of the small number of families analyzed to date, genotype-phenotype correlations have not been possible.

Distinct types of CACNA1A mutations have been identified in other neurologic conditions in which cerebellar ataxia is the major symptom. Mutations leading to protein truncation have been identified in familial and sporadic episodic ataxia type 2 (EA2; MIM 108500), which is characterized by recurrent attacks of paroxysmal ataxia associated with interictal cerebellar symptoms similar to those observed in HM/PCA (Ophoff et al. 1996; Yue et al. 1998). Small expansions of a CAG repeat, located within the 3' end of the gene and predicted to code for a polyglutamine tract in three of the six known human splice variants, are causing spinocerebellar ataxia type 6 (SCA6; MIM 183086), a late-onset moderate to severe permanent and progressive ataxia

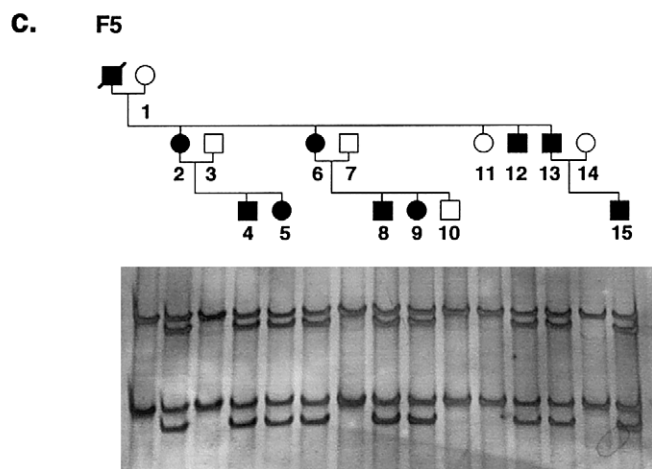
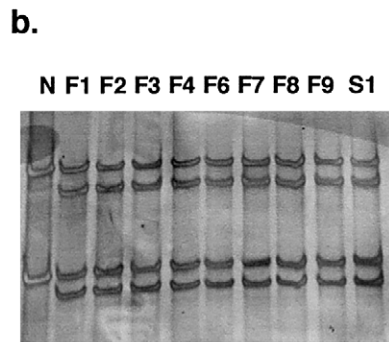
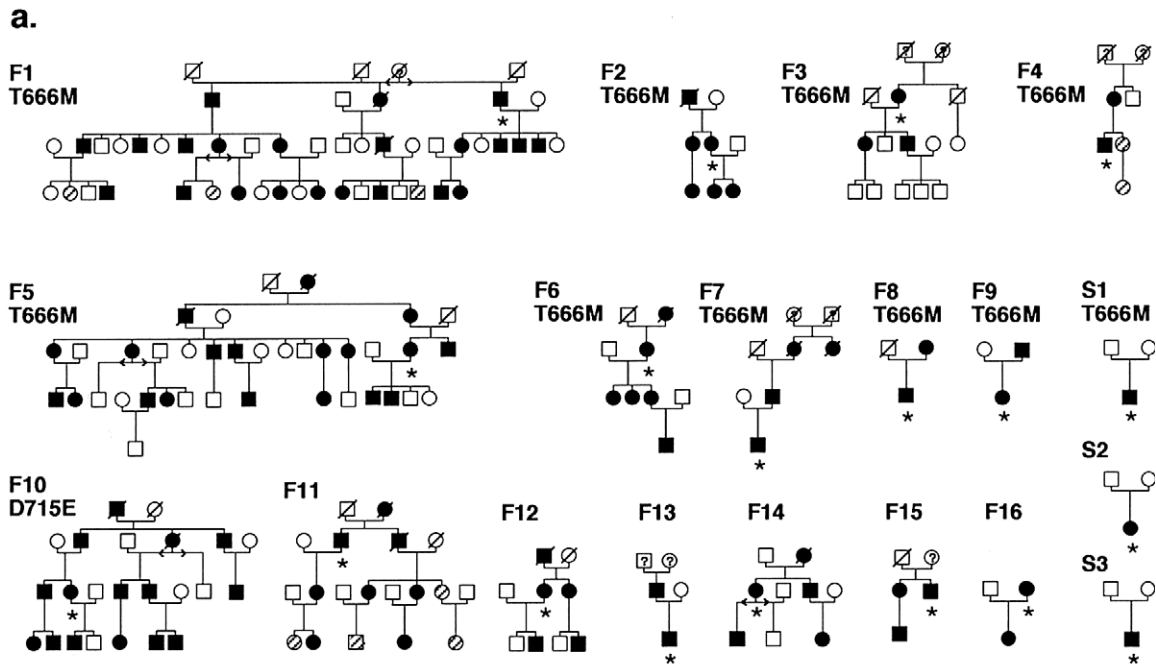
(Zhuchenko et al. 1997). Surprisingly, CAG expansions were also identified in three families with paroxysmal and permanent progressive ataxia (Jodice et al. 1997; Geschwind et al. 1997). Finally, a missense mutation was detected in a single family in which patients suffered from both paroxysmal episodes indistinguishable from those observed in EA2 and a rapidly progressive and very severe permanent ataxia (Yue et al. 1997).

Since only three HM/PCA families have been tested so far, we analyzed a panel of 16 families and 3 non-familial patients affected by this condition to search for specific CACNA1A mutations, investigate the role of a CAG expansion in this peculiar phenotype, and compare chromosome 19 disease-linked haplotypes. Subsequently, the frequency of these HM/PCA mutations was evaluated in 12 unrelated pure HM patients belonging to families with linkage to chromosome 19p13.1.

## Subjects and Methods

### Familial Cases

From 1992 to 1997, >50 families fulfilling the IHS criteria for HM (1988) were referred to our laboratory. All subjects included in this study gave informed consent. In total, 165 family members, including 33 spouses, were interviewed directly, and most were examined by one of the authors (table 1). The 16 families (F1–F16) in which at least one HM patient had associated permanent cerebellar symptoms were selected for the present study. Pedigrees are shown in figure 1A. Families F1 and F10 were described as families A and J by Joutel et al. (1993, 1994). Both showed significant linkage to chromosome



**Figure 1** T666M mutation in nine families and one nonfamilial case of HM/PCA. *a*, Pedigrees of the 16 families and the 3 nonfamilial case patients. Individuals are represented as males (squares), females (circles), unaffected (unblackened symbol), affected (blackened symbol), and having an unknown status (hatched symbol). A question mark (?) indicates subjects for whom no history was available. Probands are indicated by an asterisk (\*). All living subjects represented on this figure were sampled, except in families F8, F9, F15, and F16, in which only the proband was sampled, and the nonfamilial case S1, from whose parents blood had not been drawn. *b*, T666M abnormal conformers. SSCP was performed with DNA from the 16 family probands and the 3 nonfamilial case patients. SSCP and DNA sequencing revealed the same C→T substitution at codon 666, substituting a threonine for a methionine (T666M) in nine families (F1–F9) and in sporadic case S1. The banding pattern of a normal control (N) is also shown. *c*, Cosegregation of the T666M mutation with HM/PCA was demonstrated by SSCP in each multiplex family, as shown here for part of family F5. The numbering in this part of family F5 is given according to the gel lanes. All affected members are heterozygous for the wild type and the mutant sequence, and all unaffected subjects (10 and 11) and unrelated spouses (3, 7, and 14) are homozygous for the normal sequence.

19p13.1. Except for family F11, which originated from Denmark, all families were from France. In four families (F8, F9, F15, and F16), only the proband was sampled, but at least one first-degree affected relative was interviewed to confirm the hereditary nature of HM. All together, 87 subjects within the 16 families had hemiplegic migraine attacks fulfilling the IHS diagnosis criteria (1988) and were classified as affected. Among those HM patients, 63 had permanent cerebellar symptoms such as nystagmus and/or mild to moderate statokinetic cerebellar ataxia, and 19 had normal neurologic findings on examination; the last five patients could not be examined. Thirty-six at-risk individuals >15 years old were free of any type of migraine, had normal neurologic findings on examination, and were classified as healthy. Finally, nine at-risk subjects had nonhemiplegic migraine, isolated cerebellar symptoms, or both. Those clinical features may be part of the phenotypic spectrum of HM/PCA; however, in the absence of HM attacks, these subjects were classified as having an unknown status. Blood samples were collected from all of the 165 consenting family members.

Probands belonging to 12 pure HM families (P1–P12) linked to chromosome 19p13.1 were also selected. These families were identified on the basis of the IHS criteria for HM and classified as affected by the pure form of the condition on the basis of the absence of cerebellar symptoms in any of the 96 at-risk family members who were examined (table 2). Three of these families have been reported by Joutel et al. (family P1 as family B in 1993, families P2 and P3 as families E and F in 1994). Linkage analysis was conducted prior to the present study. In brief, seven microsatellite markers spanning the CACNA1A genetic interval on chromosome 19p and including two intragenic markers (D19S1150 and the CAG repeat) have been tested (Gyapay et al. 1994; Ophoff et al. 1996). Within the five first families

(P1–P5), positive LOD scores were obtained with several markers, including a maximum LOD score ( $Z_{\max}$ ) >1.5 with at least one marker. In the seven last families, weakly positive LOD scores were obtained because of the small size of the pedigrees and the lack of informativeness of several markers. However, haplotyping using both intragenic markers has shown that all affected subjects in families P6–P12 were carrying the D19S1150-CAG disease-linked haplotype.

#### Nonfamilial Cases

Three sporadic case patients with HM and permanent cerebellar symptoms were also analyzed (S1–S3). All three patients fulfilled the IHS diagnosis criteria for HM but did not have any affected relative. Blood was collected from those three nonfamilial case patients and from both parents of subjects S2 and S3.

#### Mutation Analysis

DNA was prepared according to standard procedures. Mutation screening using SSCP analysis (Orita et al. 1989) was performed in the proband of each family and in the three nonfamilial case patients. Sixty-four pairs of primers were used to amplify the 47 published exons and their corresponding 92 exon/intron boundaries. We used 58 primers published by Ophoff et al. (1996) and 70 newly designed primers to limit the size of amplified fragments to ~200 bp and thus to optimize the sensitivity of SSCP analysis (sequences of the new primers and details of the PCR conditions are available on request). After electrophoresis of heat-denatured PCR products on 10% acrylamide gels at 20°C on a Mighty Small II apparatus (Pharmacia-Biogen), the conformers were revealed by means of the Silver Stain Plus kit (Bio-Rad), according to the supplier's protocol. Genomic DNA samples from one unrelated normal individual were used

**Table 2**

**Clinical and Genetic Features of the Families of the 12 Pure HM Probands**

Family	Affected	Healthy	Unknown Status	Spouses	Total	Linkage Analysis and Haplotyping with Chromosome 19p Markers
P1	10	3	3	6	22	$Z_{\max} = 2.49$ at $\theta = .01$ from D19S226
P2	5	4	1	3	13	$Z_{\max} = 1.68$ at $\theta = .00$ from D19S906
P3	5	2	...	2	9	$Z_{\max} = 1.72$ at $\theta = .00$ from D19S394
P4	6	1	2	3	12	$Z_{\max} = 1.72$ at $\theta = .00$ from D19S199
P5	6	4	2	3	15	$Z_{\max} = 2.41$ at $\theta = .00$ from D19S221
P6	6	2	1	2	11	No affected obligate recombinant <sup>a</sup>
P7	5	3	1	2	11	No affected obligate recombinant <sup>a</sup>
P8	3	1	...	1	5	No affected obligate recombinant <sup>a</sup>
P9	3	...	...	...	3	No affected obligate recombinant <sup>a</sup>
P10	3	...	...	1	4	No affected obligate recombinant <sup>a</sup>
P11	6	...	...	1	7	No affected obligate recombinant <sup>a</sup>
P12	4	4	...	2	10	No affected obligate recombinant <sup>a</sup>
Total	62	24	10	26	122	

<sup>a</sup> With intragenic markers D19S1150 and CAG.

as controls in each SSCP analysis. In the case of abnormal SSCPs, the DNA sequence was determined by using a dye-terminator cycle-sequencing kit (Perkin-Elmer) according to the supplier's instructions. For each DNA sequence abnormality, cosegregation with the disorder was analyzed by SSCP. For each of the three largest HM/PCA families (F1, F5, and F10), a subset of 15 or 16 members was analyzed. In the other, smaller pedigrees, cosegregation with the disease was analyzed with all available family members. In addition, 100 unrelated normal individuals and the 12 pure-HM probands were tested, with SSCP analysis, for the absence of each mutation cosegregating with the disease; PCR products from those controls were run on the same gels as the PCR product of a known mutation carrier.

### Genetic Analysis

To identify the haplotypes cosegregating with HM/PCA, all subjects were genotyped with four polymorphic markers: D19S221 and D19S226, which are two CA repeats flanking the 6-cM interval containing CACNA1A (Gyapay et al. 1994; Genome Database), and two intragenic markers, D19S1150, which is a CA repeat located in intron 7 of CACNA1A, and the CAG repeat contained in exon 47. D19S1150 and the CAG repeat are separated by a maximum of 350 kb (Ophoff et al. 1996; Zhuchenko et al. 1997). PCR was performed with published primers and conditions (Gyapay et al. 1996; Ophoff et al. 1996; Zhuchenko et al. 1997). The frequencies of the D19S1150 alleles, determined by genotyping 55 unrelated healthy individuals, including the 33 spouses sampled in this study, were as follows: 164 bp: 0.069; 162 bp: 0.009; 160 bp: 0.294; 158 bp: 0.157; 156 bp: 0.078; 154 bp: 0.009; 152 bp: 0.363; 150 bp: 0.009; and 148 bp: 0.009. The frequencies of the CAG repeat alleles in the same control population were as follows: CAG<sub>17</sub>: 0.009; CAG<sub>14</sub>: 0.009; CAG<sub>13</sub>: 0.309; CAG<sub>12</sub>: 0.173; CAG<sub>11</sub>: 0.427; CAG<sub>7</sub>: 0.054; and CAG<sub>4</sub>: 0.018. For D19S221 and D19S226, published frequencies from CEPH families were used. No D19S1150 allele was in linkage disequilibrium with a CAG allele. The expected frequencies of the dinucleotide- and trinucleotide-repeat alleles and of the haplotypes of the studied families were calculated from the frequencies observed in control subjects. Expected and observed allele and haplotype frequencies in the affected population were compared by means of the  $\chi^2$  test (with Yates's correction when necessary).

## Results

### *T666M as a Predominant Mutation in HM/PCA*

Mutations were identified in 10 of the 16 families and in one of the three patients with nonfamilial HM. Only

two different missense mutations were detected throughout the 47 exons and flanking intron sequences (table 3). The same C→T substitution in exon 16, causing a threonine-for-methionine substitution at codon 666 (T666M), was found in nine families (F1–F9) (fig. 1). Cosegregation with the disease was demonstrated in all of these nine families by means of SSCP. This mutation was shown to be absent in 200 normal chromosomes. We also detected the T666M substitution in a nonfamilial case (S1). In the absence of DNA samples from both parents, false paternity or incomplete penetrance could not be ruled out.

A new missense mutation located in exon 17, a C→A substitution at codon 715, resulting in an aspartic acid-to-glutamic acid substitution (D715E), was identified in family F10 (figs. 2 and 3). Cosegregation with HM/PCA was demonstrated by means of SSCP analysis. This mutation was not detected in 200 control chromosomes.

No disease-associated mutation was detected in six families (F11–F16). In addition, four polymorphisms were observed in HM/PCA patients (table 4).

To establish that both T666M and D715E mutations were strongly associated with the HM/PCA phenotype, the 12 probands belonging to pure HM families linked to chromosome 19p13.1 were screened by SSCP for the abnormal conformer generated by each mutation. For exon 16 (T666M), PCR products of the 12 pure HM probands, 1 unrelated healthy control, and 1 T666M mutation carrier were run on the same gel. The same control procedure was used for exon 17 (D715E). No abnormal conformer was observed in exons 16 and 17 of the 12 probands, demonstrating the absence of mutations T666M and D715E.

### *Evidence against a Major Founder Effect in T666M Patients*

All HM/PCA families and nonfamilial case patients were genotyped with the two intragenic markers, D19S1150 and the CAG repeat, as well as the markers flanking the 6-cM interval containing CACNA1A, D19S221, and D19S226. Alleles and haplotypes cosegregating with the disease were established in 12 multiplex families (table 1). In the last four families (F8, F9, F15, and F16) in which only the proband has been analyzed, both alleles were taken into account. There was no significant difference in the frequencies of the various marker alleles in HM/PCA patients and normal controls, except for one CAG allele. Indeed, this allele, CAG<sub>7</sub>, was observed on HM/PCA chromosomes with a higher frequency than on control chromosomes (observed frequency = 21.05%, expected frequency = 5.45%,  $\chi^2 = 6.20$ ,  $P < .02$ ). However, the frequency of this CAG<sub>7</sub> allele on chromosomes carrying T666M was not significantly different from its frequency on other HM/

**Table 3****Haplotypes and Mutations in 16 Families (F1 to F16) and 3 Nonfamilial Case Patients (S1 to S3) Affected with HM/PCA**

FAMILY OR NONFAMILIAL SUBJECT	HAPLOTYPE				CACNA1A MUTATION			
	D19S221	D19S1150	CAG	D19S226	Exon	Nucleotide	Codon	Domain
F1	199	160	13	251	16	aCg→aTg	T666M	IIS5-S6 linker
F2	209 <sup>a</sup>	164 <sup>a</sup>	11 <sup>a</sup>	243 <sup>a</sup>	16	aCg→aTg	T666M	IIS5-S6 linker
F3	209 <sup>a</sup>	164 <sup>a</sup>	11 <sup>a</sup>	243 <sup>a</sup>	16	aCg→aTg	T666M	IIS5-S6 linker
F4	199	158	13	235	16	aCg→aTg	T666M	IIS5-S6 linker
F5	205	160	7	245	16	aCg→aTg	T666M	IIS5-S6 linker
F6	207 <sup>b</sup>	156 <sup>b</sup>	7 <sup>b</sup>	243 <sup>b</sup>	16	aCg→aTg	T666M	IIS5-S6 linker
F7	207 <sup>b</sup>	156 <sup>b</sup>	7 <sup>b</sup>	243 <sup>b</sup> /247	16	aCg→aTg	T666M	IIS5-S6 linker
F8	209/207	160/150	13/13	247/241	16	aCg→aTg	T666M	IIS5-S6 linker
F9	205/191	160/152	12/11	245/235	16	aCg→aTg	T666M	IIS5-S6 linker
S1	...	158/156	12/11	245/253	16	aCg→aTg	T666M	IIS5-S6 linker
F10	207	152	11	235	17	gaC→gaG	D715E	IIS6
F11	197	156	13	253	...	...	...	...
F12	207	152	12	245	...	...	...	...
F13	205	164	7	235	...	...	...	...
F14	211	160	12	235	...	...	...	...
F15	207/201	160/152	13/13	247/243	...	...	...	...
F16	...	164/158	12/12	251/235	...	...	...	...
S2	203/197	156/150	13/13	241/235	...	...	...	...
S3	209/205	164/160	13/11	247/235	...	...	...	...

<sup>a</sup> Haplotype shared in families F2 and F3 (frequency in the 16 families: 12.5%; frequency expected: 0.028%;  $P < 10^{-10}$ ).

<sup>b</sup> Haplotype shared in families F6 and F7 (frequency in the 16 families: 12.5%; frequency expected: 0.011%;  $P < 10^{-10}$ ).

PCA chromosomes ( $\chi^2 = 0.195$ , not significant), which did not favor a founder effect.

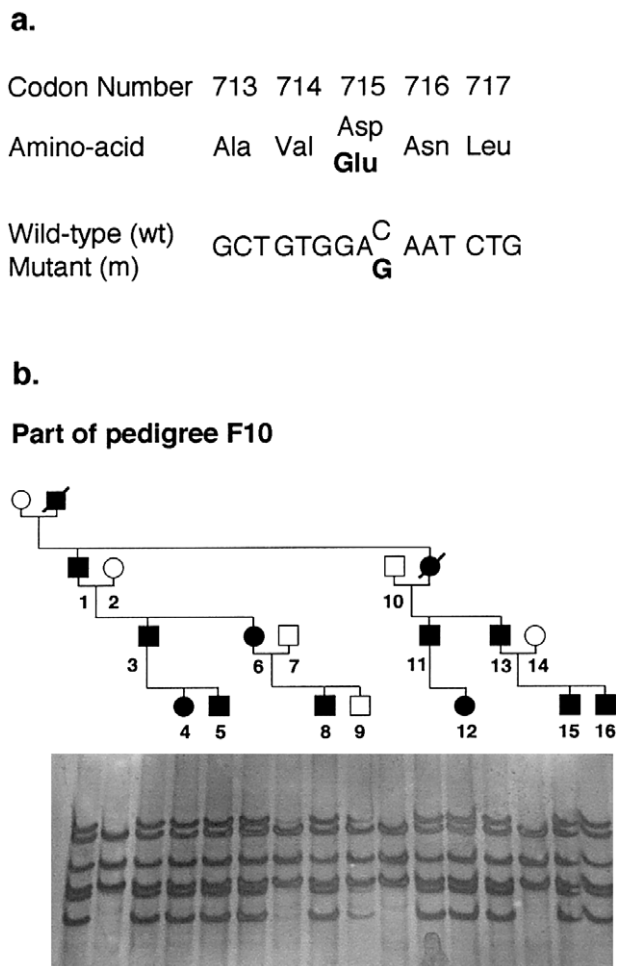
Within the nine families (F1–F9) with the T666M mutation, affected haplotypes were established in seven families (F1–F7): five different haplotypes were clearly identified, strongly suggesting the absence of a major founder effect. A common haplotype was found in families F2 and F3, but tracing of family trees to a common ancestor was impossible because the parents of the transmitter in family F2 were unknown. A second common haplotype was shared in families F6 and F7. Again, tracing of family trees to a common ancestor was impossible because of the lack of information about ancestors in family F7. In addition, the five distinct haplotypes observed in kindreds F1–F7 were different from the various combinations of alleles observed in familial cases F8 and F9 and in the nonfamilial case S1, suggesting the existence of eight different genetic backgrounds for the same T666M mutation. The haplotype cosegregating with the D715E mutation in family F10 was not observed in any other HM/PCA patient.

Finally, haplotypes segregating with the disease were determined in four families without any identified CACNA1A mutation (F11–F14). They were different from one another, and comparison with the combination of alleles observed in familial cases F15 and F16 and sporadic cases S2 and S3 suggested the existence of a

total of eight different haplotypes in patients without identified mutations.

#### *Absence of CAG Repeat Expansion*

The CAG repeat length was determined in all 16 families and 3 nonfamilial case patients affected with HM/PCA. No intergenerational CAG repeat expansion was observed. In the 90 affected subjects, the CAG stretch ranged from 4 to 17 repeat units (mean = 10.98), which is not different from the range observed in the 55 normal unrelated controls (range = 4–17 repeats, mean = 11.5). The CAG allele cosegregating with HM/PCA was determined in 15 families and 1 homozygous nonfamilial case and ranged from 7 to 13 repeat units (table 1). The CAG allele inherited from the healthy parent was determined in 77 patients who had been examined. These alleles in trans ranged from CAG<sub>4</sub> to CAG<sub>13</sub> in the 15 patients with normal neurologic findings and from CAG<sub>4</sub> to CAG<sub>17</sub> in the 63 patients with permanent cerebellar symptoms. Allele CAG<sub>4</sub> was observed more frequently in patients with normal neurologic findings on examination ( $\chi^2 = 5.066$ ,  $P < .05$ ), in only four patients (three without and one with PCA). No difference was detected in the frequencies of the other alleles between patients with and without cerebellar symptoms.



**Figure 2** Mutation D715E in exon 17 of the CACNA1A gene in family F10. *a*, SSCP and DNA sequence analysis of individual F10-4 revealed a C→G substitution at codon 715, resulting in a substitution of an aspartic acid for a glutamic acid. *b*, The cosegregation of this mutation with HM/PCA was demonstrated in family F10 by the SSCP analysis of 16 of the 20 family members. The numbering in this part of family F10 is given according to the gel lanes. All affected members are heterozygous (wt/m). All unaffected spouses are homozygous for the wild-type sequence. Individual 9, a 24-year-old man free of any migraine attack and with normal neurologic findings on examination, is heterozygous (wt/m). In the 90 HM/PCA patients in this study, the mean age of onset was 11.4 years old, but in 7% of those patients, attacks started after 24 years of age. Individual 9 may represent an instance of nonpenetrance or a case of delayed onset.

**Discussion**

We screened the CACNA1A gene in 16 families and 3 nonfamilial subjects affected by HM/PCA. Nine of the 16 families and 1 nonfamilial subject had the same T666M mutation. This substitution was shown to be absent in probands belonging to 12 unrelated pure HM families linked to chromosome 19p13.1, indicating a

strong correlation between the T666M genotype and the HM/PCA phenotype. Haplotyping, using four markers including two intragenic ones (D19S1150 and the CAG repeat), suggested that the predominance of T666M may be due to recurrent mutations rather than to a founder effect. Of interest, one of the three HM/PCA families reported thus far also had the T666M mutation (Ophoff et al. 1996).

Two other mutations have been identified in HM/PCA: I1811L (Ophoff et al. 1996) and D715E (present study). The I1811L mutation was detected in two HM/PCA families, including one initially diagnosed as having pure HM (Terwindt et al. 1998). Both families originated from different countries (The Netherlands and the United States) and displayed different disease haplotypes. Thus, two of the three missense mutations identified thus far in HM/PCA are recurrent on different genetic backgrounds, favoring the hypothesis of a restriction of the number of CACNA1A substitutions susceptible to producing this phenotype.

The CACNA1A gene codes for the main pore-forming  $\alpha$ 1A subunit, which, in association with auxiliary  $\beta$  and  $\alpha$ 2/ $\delta$  regulatory subunits, forms P/Q-type voltage-dependent calcium channels (Catterall 1995; Gurnett and Campbell 1996).  $\alpha$ 1A subunits control the voltage dependence, ionic selectivity, and major gating kinetics of P/Q-type channels. They are formed by four repeated domains (I–IV), which contain six integral transmembrane segments (S1–S6). The positively charged S4 helix represents the voltage sensor. The four S5–S6 linkers (also called “P-loop” [for pore-loop]) line the inner part of the ionic pore. How do these T666M, V715E, and I1811L substitutions alter P/Q-type calcium currents and result in the permanent cerebellar dysfunction characteristic of the HM/PCA phenotype?

The three HM/PCA mutations are neither clustered in a specific part of the  $\alpha$ 1A subunit nor located in homologous segments within the four repeated domains: The T666M mutation is located in the S5–S6 linker of domain II, the D715E mutation alters the residue located just after the intracytoplasmic end of IIS6, and the I1811L substitution is located at the intracytoplasmic end of the IVS6 helix. The HM/PCA phenotype could be due to a shared functional effect of these substitutions located in different channel parts. Recently, using expression of mutant  $\alpha$ 1A subunits in *Xenopus laevis* oocytes, Kraus et al. (1998) demonstrated that both T666M and I1811L mutations change the channel’s inactivation kinetics. However, the T666M mutation was shown to slow recovery from inactivation and the I1811L mutation to accelerate recovery from inactivation. Consequently, a similar clinical phenotype may be due to mutations showing distinct functional consequences, at least in *Xenopus* oocytes.

One would have expected mutations causing pure HM





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## Electronic-Database Information

URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for familial hemiplegic migraine, episodic ataxia type 2, and spinocerebellar ataxia type 6)

Genome Database, <http://www.gdb.org> (for markers)

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