THE CLINICAL SPECTRUM OF FAMILIAL HEMIPLEGIC MIGRAINE ASSOCIATED WITH MUTATIONS IN A NEURONAL CALCIUM CHANNEL

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ABSTRACT

Background Familial hemiplegic migraine, an autosomal dominant disorder characterized by attacks of transient hemiparesis followed by a migraine headache, is divided into pure familial hemiplegic migraine (affecting 80 percent of families) and familial hemiplegic migraine with permanent cerebellar signs (affecting 20 percent of families). Mutations in *CACNA1A,* which encodes a neuronal calcium channel, are present in 50 percent of families with hemiplegic migraine, including all those with cerebellar signs. We studied the various clinical manifestations associated with mutations in *CACNA1A* in 28 families with hemiplegic migraine with and without cerebellar signs.

Methods *CACNA1A* was analyzed and nine mutations were detected in 15 of 16 probands of families affected by hemiplegic migraine and cerebellar signs, in 2 of 3 subjects with sporadic hemiplegic migraine and cerebellar signs, and in 4 of 12 probands of families affected by pure hemiplegic migraine. Genotyping of probands and relatives identified a total of 117 subjects with mutations whose clinical manifestations were assessed in detail.

Results Eighty-nine percent of the subjects with mutations had attacks of hemiplegic migraine. One third had severe attacks with coma, prolonged hemiplegia, or both, with full recovery. All nine mutations, including five newly identified ones, were missense mutations. Six mutations were associated with hemiplegic migraine and cerebellar signs, and 83 percent of the subjects with these six mutations had nystagmus, ataxia, or both. Three mutations were associated with pure hemiplegic migraine.

Conclusions Hemiplegic migraine in subjects with mutations in *CACNA1A* has a broad clinical spectrum. This clinical variability is partially associated with the various types of mutations. (N Engl J Med 2001;345: 17-24.)

MIGRAINE is a common condition, affecting about 12 percent of the population in Western countries.[1-4] In addition to environmental factors, genetic factors have been demonstrated to play a part in the pathogenesis of migraine.[5,6] In the two main types of migraine, migraine with aura and migraine without aura,[1] the familial aggregation cannot be explained by simple mendelian inheritance patterns. Familial hemiplegic migraine is the only variety of migraine in which a mendelian type of inheritance has been clearly established.[1,7-14] This rare variety of migraine with aura is characterized by its autosomal dominant pattern of inheritance and by the presence of some degree of hemiparesis during attacks.[1] In contrast to other types of migraine, familial hemiplegic migraine is characterized in some patients by severe coma with prolonged hemiplegia.[8,10,11] Some patients have permanent neurologic signs of disease, most often nystagmus and ataxia between attacks. These differences have led clinicians to distinguish families affected by pure hemiplegic migraine from families affected by hemiplegic migraine and cerebellar signs (in which at least one member has nystagmus or ataxia).[7-19] Some sporadic cases of hemiplegic migraine with cerebellar signs have also been reported.[19,20]

Familial hemiplegic migraine is genetically heterogeneous.[12,13,17,18] *CACNA1A,* the first gene that has been associated with the disorder, is located on chromosome 19 and encodes the α_{1A} subunit of voltage-gated P/Q-type calcium channels in neurons.[12,14] Thus far, familial hemiplegic migraine with cerebellar signs has been linked to mutations in *CACNA1A* in all families studied.[14,16,19-22] Pure hemiplegic migraine has been associated with mutations in *CACNA1A* in some families,[12,14,17,23] but in others a locus has been mapped on chromosome 1.[18,24] In still others the disorder is linked neither to *CACNA1A* nor to the locus on chromosome 1, suggesting the existence of at least a third locus.[18]

Eight mutations in *CACNA1A* have been identified in 18 families affected by hemiplegic migraine and two patients with sporadic hemiplegic migraine.[14,19-23] The absence of detailed clinical data on most of the families[19] has precluded the description of detailed correlations between genotype and phenotype.

We screened for mutations in *CACNA1A* in 28 families and 3 subjects with sporadic hemiplegic migraine. Our goal was to obtain a large, homogeneous group of subjects with mutations in *CACNA1A* that would allow a detailed clinical description of the disorder and of the correlations between genotype and phenotype.

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METHODS

Subjects and Controls

All subjects provided written informed consent, as required by appropriate committees on the protection of research subjects, and were interviewed and examined by one of us. Diagnostic criteria of the International Headache Society were used to define familial hemiplegic migraine and other varieties of migraine.[1] Twenty-eight unrelated families were studied: 16 families affected by hemiplegic migraine with cerebellar signs, defined by the presence of nystagmus, ataxia, or both in at least one member (Families 1 through 16), and 12 families affected by pure hemiplegic migraine, defined by the absence of nystagmus and ataxia in all of the examined members (Families 17 through 28).[19] In all of these families, previous data on linkage and haplotypes suggested linkage to chromosome 19, although the data were inconclusive in some small families.[19] Three subjects with sporadic hemiplegic migraine with cerebellar signs were also studied (Patients 1, 2, and 3). One hundred healthy spouses of subjects affected by hemiplegic migraine who had the same ethnic background were selected as controls.

Detection of Mutations and Genotyping

DNA was prepared according to standard procedures. Screening for mutations was performed in the proband from each of the 28 families and in subjects with sporadic cases with the use of single-strand conformation polymorphism (SSCP) analysis[25] as previously described (information on primers and conditions is available as Supplementary Appendix 1 with the full text of this article at http://www.nejm.org).[19] The DNA sequence of conformation variants was determined with the use of a dye-terminator cycle-sequencing kit (Perkin–Elmer, Foster City, Calif.) according to the supplier's instructions. Human messenger RNA (mRNA) sequence X99897 for the α_{1A} subunit of the P/Q-type calcium channel was used as the reference.[14] Additional DNA sequencing of *CACNA1A* — including sequencing in all four repeated domains of the junctions between the third and fourth membrane-spanning segments (S3 and S4); of S4, S5, and S6; and of the P loops — was performed in the probands from Families 16, 18, and 28 and in Patient 3, in whom SSCP analysis did not reveal any abnormal conformer. SSCP analysis was used to test each DNA-sequence variant for its cosegregation with the affected phenotype within families and to determine its frequency in 100 unrelated normal controls.

Statistical Analysis

Among subjects with mutations in *CACNA1A*, Fisher's exact test was used to compare the frequency of symptoms in those who were from families affected by hemiplegic migraine with cerebellar symptoms and in those who were from families affected by pure hemiplegic migraine.[26] An overall test for equality of proportions with the extension of Fisher's exact test (the Freeman–Halton method) was used to compare the frequency of symptoms in carriers of the three most frequent mutations associated with hemiplegic migraine with cerebellar symptoms.[27] All P values were two-tailed.

RESULTS

Genetic Studies

SSCP analysis showed abnormal conformers in 15 of the 16 probands from families affected by hemiplegic migraine and cerebellar signs, in 4 of the 12 probands from families affected by pure hemiplegic migraine, and in 2 of the 3 subjects with sporadic hemiplegic migraine and cerebellar signs. Sequencing of these conformers revealed nine distinct sequence variants of *CACNA1A*. All variants were missense mutations that segregated with the disease phenotype within the families of the probands (i.e., all carriers were heterozygous). None of the sequence variants were detected in the 200 chromosomes from the controls. All variants were located in important functional domains of the protein. Therefore, these nine variants were considered to be mutations associated with hemiplegic migraine. Five mutations were novel (R195K, K1336E, R1668W, W1684R, and V1696I), and four have already been described (R583Q, T666M,

TABLE 1. MUTATIONS IN *CACNA1A* ASSOCIATED WITH FAMILIAL HEMIPLEGIC MIGRAINE.

MUTATION	FAMILY OR PATIENT NO.	EXON	NUCLEOTIDE CHANGE	DOMAIN OF THE α_{1A} SUBUNIT	NO. OF SUBJECTS WITH MUTATION	NO. OF SUBJECTS AFFECTED BY HEMIPLEGIC MIGRAINE	NO. OF SUBJECTS AFFECTED BY CEREBELLAR SIGNS*
Hemiplegic migraine with cerebellar signs							
R583Q	Families 11, 12, and 14	13	CGA→CAA	Second S4	16	13	13/16
T666M	Families 1, 2, 3, 4, 5, 6, 7, 8, and 9 and Patient 1	16	ACG→ATG	Second P loop	55	54	43/50
D715E	Family 10	17	GAC→GAG	Second S6	14	9	9/12
Y1385C	Patient 2	26	TAC→TGC	Third S5	1	1	1/1
R1668W	Family 15	32	CGG→TGG	Fourth S4	1	1	1/1
W1684R	Family 13	32	TGG→CGG	Fourth S4–S5 junction	2	2	1/2
Pure hemiplegic migraine							
R195K	Family 19	4	AGG→AAG	First S4	5	5	0/5
K1336E	Family 17	25	AAA→GAA	Third S3–S4 junction	13	11	0/12
R1668W	Family 21	32	CGG→TGG	Fourth S4	7	6	0/3
V1696I	Family 25	33	GTC→ATC	Fourth S5	3	2	0/3

*For each mutation, the value shown is the number of subjects with cerebellar signs over the number of subjects who had the mutation and were examined.

D715E, and Y1385C) (Table 1 and Fig. 1 and 2).[19,20] T666M, the most frequent mutation, was present in Families 1 through 9 and in Patient 1, all of whom were affected by hemiplegic migraine with cerebellar signs. R583Q was detected in three families affected by hemiplegic migraine and cerebellar signs (Families 11, 12, and 14). R1668W was detected in a proband affected by hemiplegic migraine and cerebellar signs (from Family 15) and also in a small family affected by pure hemiplegic migraine (Family 21). All other mutations were each found in a single family. Y1385C was identified in Patient 2 and was shown to be a spontaneous mutation.[20]

No sequence variant of *CACNA1A* associated with the disorder was identified in nine probands or in Patient 3, most likely because of the genetic heterogeneity of hemiplegic migraine.

Clinical Spectrum in 117 Subjects with Mutations in *CACNA1A*

Genotyping of 169 relatives of the 19 probands with identified mutations in *CACNA1A* detected 96 additional subjects with mutations (Fig. 2). A total of 117 subjects with mutations in *CACNA1A* (58 women and 59 men) were therefore studied, including the 2 subjects with sporadic hemiplegic migraine and cerebellar signs (Patients 1 and 2), 87 members of families affected by hemiplegic migraine and cerebellar signs, and 28 members of families affected by pure hemiplegic migraine. The mean (±SD) age of the 117 people we studied was 37.8±19.1 years (range, 6 to 86).

Phenotype of the 104 Subjects with Mutations and Attacks of Hemiplegic Migraine

Attacks of hemiplegic migraine that fulfilled the criteria of the International Headache Society were reported by 104 of the 117 subjects with mutations (53 women and 51 men, 89 percent), whose mean age was 38±19 years (range, 6 to 86) (Tables 1 and 2). Most attacks had the typical pattern of migraine with aura, with neurologic symptoms lasting a mean of 60 minutes, followed by headache lasting from 30 minutes to 5 days. However, some prominent features were remarkable. The hemiparesis during the aura, whatever its severity, was never isolated; it was always associated with sensory, language, or visual disturbances (Table 2). Among these disturbances, sensory signs (paresthesias and numbness) were the most frequent (93 per-

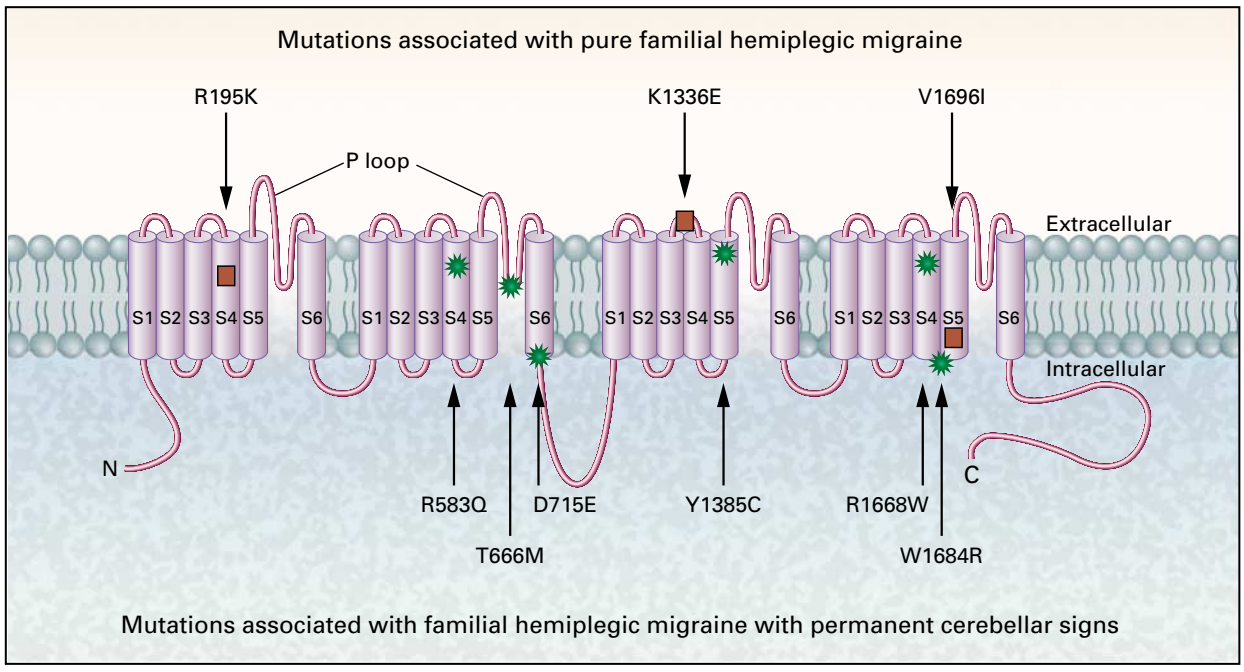

Figure 1. Mutations in *CACNA1A* Causing Hemiplegic Migraine.

The structure of the α_{1A} pore-forming subunit of P/Q-type voltage-gated calcium channels is shown. This subunit, which is located in the neuronal membrane, contains four repeated domains. Each domain includes six membrane-spanning segments (S1 to S6) and a so-called P loop between S5 and S6. The four S4 segments form the voltage sensor of the channel, the four S5 and S6 segments form the inner part of the pore, and the four P loops line the inside of this pore. The positions of the nine missense mutations studied here are indicated. Mutations causing pure hemiplegic migraine are shown in red, and mutations causing hemiplegic migraine with permanent cerebellar signs are shown in green. The positions of the mutations are given according to the human messenger RNA sequence X99897.[14]

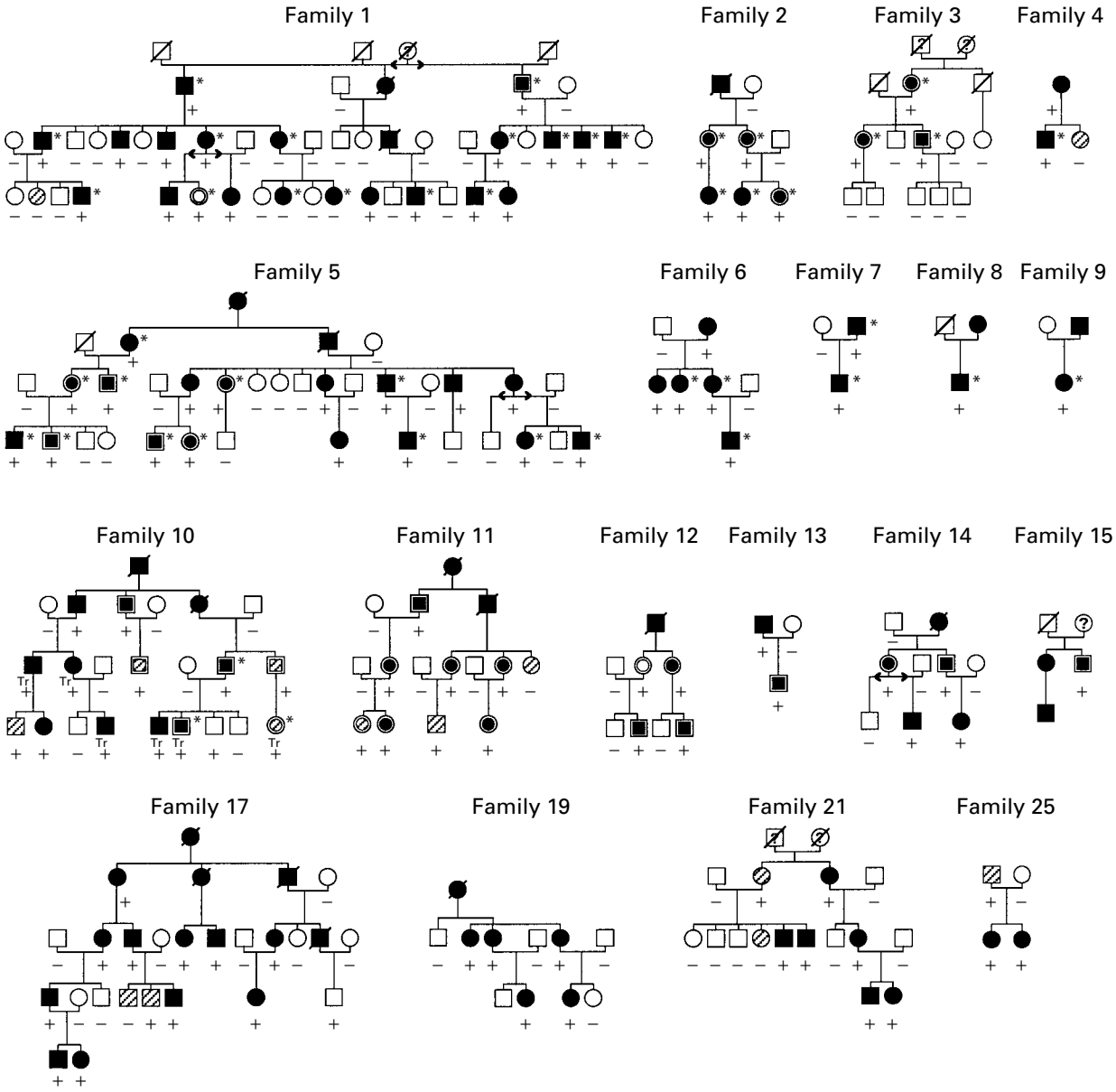

Figure 2. Pedigrees.

Pedigrees are shown for the 19 families with an identified mutation in *CACNA1A*. Squares denote men, circles women, slashes subjects who have died, open symbols healthy subjects, solid symbols subjects affected by hemiplegic migraine, hatched symbols subjects affected by migraine without aura or with nonhemiplegic aura, symbols with a white border subjects with cerebellar ataxia, asterisks subjects with nystagmus, Tr subjects with tremor, plus signs subjects with mutations in *CACNA1A,* minus signs subjects with normal genotypes, and question marks subjects for whom no medical history was available.

cent of subjects), followed by language disturbances such as dysarthria and various degrees of dysphasia (83 percent) and visual symptoms such as scintillating scotoma, phosphenes, hemianopia, and blurred vision (74 percent). A mild degree of confusion or somnolence was occasionally reported (21 percent). Thirty-five percent of the subjects had attacks with bilateral signs, occurring either simultaneously or in succession, and dysphasia occurred irrespective of the side of hemiparesis.

There were 66 atypical attacks in 44 of 104 subjects (42 percent). These attacks were characterized either by a prolonged aura lasting up to five days (7 attacks in 6 patients) or by signs of diffuse encephalopathy (59

TABLE 2. CLINICAL SPECTRUM OF FAMILIAL HEMIPLEGIC MIGRAINE ASSOCIATED WITH MUTATIONS IN *CACNA1A*.*

CLINICAL FEATURE	ALL FAMILIES AND PATIENTS (117 SUBJECTS WITH MUTATIONS)	FAMILIES AFFECTED BY PURE HEMIPLEGIC MIGRAINE (28 SUBJECTS WITH MUTATIONS)†	FAMILIES AND PATIENTS AFFECTED BY HEMIPLEGIC MIGRAINE WITH CEREBELLAR SIGNS (89 SUBJECTS WITH MUTATIONS)†
Age — yr			
Mean ±SD	37.8±19.1	38±19	37.7±19
Range	6–86	10–86	6–85
Hemiplegic migraine — no./total no. (%)	104/117 (89)	24/28 (86)	80/89 (90)
Age at onset — yr			
Mean ±SD	11.7±8.1	11.7±5.4	11.7±9
Range	1–51	6–28	1–51
Aura with sensory disturbance — no./total no. (%)	90/97 (93)	19/21 (90)	71/76 (93)
Aura with language disturbance — no./total no. (%)	73/88 (83)	14/21 (67)	59/68 (87)
Aura with visual disturbance — no./total no. (%)	66/89 (74)	17/21 (81)	49/68 (72)
Atypical attacks — no./total no. (%)	44/104 (42)	5/24 (21)‡	39/80 (49)‡
Severe attacks with coma or confusion	38/104 (37)	5/24 (21)	33/80 (41)
Severe first attack	21/104 (20)	0/24‡	21/80 (26)‡
Subjects with mutations with permanent cerebellar signs — no./total no. (%)	68/105 (65)	0/23	68/82 (83)
Nystagmus	47/105 (45)	0/20	47/82 (57)
Ataxia	36/105 (34)	0/20	36/82 (44)
Dysarthria	9/105 (9)	0/20	9/82 (11)

*The denominators (total numbers) indicate the number of family members for whom data were available.

†The frequency of symptoms was compared between subjects who were from families affected by hemiplegic migraine with cerebellar signs and subjects who were from families with pure hemiplegic migraine with the use of Fisher's exact test.

‡In addition to the presence or absence of cerebellar signs, the only significant differences between the two groups were in the frequency of atypical attacks ($P=0.02$) and the frequency of severe first attacks ($P=0.003$).

attacks in 38 patients). Most of the 38 subjects with diffuse encephalopathy were admitted to intensive care units with a suspicion of meningoencephalitis because of confusion or coma, fever (temperature, up to 40°C), severe hemiplegia, and in some patients, seizures (Table 3). These signs lasted up to six weeks, but the subjects recovered fully. Such severe attacks occurred mostly in younger subjects (mean age, 21±16 years; range, 2 to 83), were the first symptoms of the disease in 21 subjects, were triggered by mild head trauma in 10 subjects, and were triggered by cerebral or coronary angiography in 2 subjects.

Attacks of hemiplegic migraine started at a young age in the majority of subjects, with a mean age of onset of 11.7±8.1 years (range, 1 to 51). The natural history was highly variable. The frequency of attacks ranged from one per day to less than five in a subject's lifetime (mean, two or three per year), and long attack-free intervals (range, 2 to 37 years) were reported by 25 subjects. Emotional stress was the most frequent triggering factor, and minor head trauma was the second most frequent. Nine percent of the subjects affected by hemiplegic migraine also reported attacks of migraine with a nonhemiplegic aura, and 23 percent had had attacks of migraine without aura.

Finally, permanent cerebellar signs were found in 62 of the 104 subjects with mutations who had attacks of hemiplegic migraine, all of whom were members of families with cerebellar signs. No subject had permanent motor, sensory, language, or visual symptoms.

TABLE 3. FREQUENCY OF CLINICAL SIGNS AND SYMPTOMS DURING SEVERE ATTACKS WITH IMPAIRMENT OF CONSCIOUSNESS.

SIGNS AND SYMPTOMS	SUBJECTS (N=38)	ATTACKS (N=59)
	number (percent)	
Somnolence	14 (37)	20 (34)
Mild coma	14 (37)	18 (31)
Severe coma	6 (16)	10 (17)
Confusion	22 (58)	27 (46)
Severe agitation	5 (13)	5 (8)
Complex visual and auditory delusions	4 (11)	5 (8)
Prolonged hemiplegia (>6 hr)	30 (79)	53 (90)
Prolonged aphasia (>6 hr)	23 (61)	25 (42)
Meningismus	6 (16)	9 (15)
Fever	18 (47)	32 (54)
Epileptic seizures	3 (8)*	10 (17)

*A subject in Family 13 had partial or generalized tonic–clonic seizures in seven of his eight severe attacks, Patient 2 had bouts of tonic eye deviation during one attack and diffuse hypertonia during another attack, and the proband of Family 15 had clonic movements of the weak side during his only severe attack.

Phenotype of the 13 Subjects with Mutations but No Attacks of Hemiplegic Migraine

Thirteen subjects with mutations in *CACNA1A* had no attacks of hemiplegic migraine. They belonged to six unrelated families and carried six different mutations (Table 1). Their mean age was 36.5±16 years

(range, 15 to 65). Two had no symptoms. Five were affected by migraine with nonhemiplegic aura and one by migraine without aura as defined by the criteria of the International Headache Society. One had recurrent headaches with loss of consciousness. Three subjects had single transient episodes of unknown clinical significance that were characterized by dysarthria, unilateral paresthesia, and confusion with fever, respectively. In addition, six of these subjects had permanent neurologic signs (nystagmus and ataxia).

Characteristics of Permanent Neurologic Signs

Neurologic examination was performed in 105 of the 117 subjects with mutations. Permanent neurologic signs were observed in 68 subjects, 62 of whom also had attacks of hemiplegic migraine; they included Patients 1 and 2 and 66 members of families affected by hemiplegic migraine with cerebellar signs (Tables 1 and 2). The most frequent sign was nystagmus, which was observed in 47 subjects. Gait ataxia was the second most frequent sign (in 36 subjects) and was associated with limb ataxia in 27 subjects, nystagmus in 19, and dysarthria in 9 (Fig. 2). The mean age of subjects with permanent neurologic signs was 40±18 years (range, 11 to 78). Six subjects with ataxia never had any attacks of hemiplegic migraine; two of these subjects, who were 18 and 61 years of age, had isolated ataxia without any history of migraine. The severity of ataxia increased with age and caused gait impairment in all nine affected subjects who were over 50 years of age, but none required a wheelchair. The frequency of severe attacks was similar in subjects with and without ataxia who shared a given mutation (1.51 vs. 1.50 per year). Finally, early-onset postural tremor was present in six subjects from Family 10 (Fig. 2). This tremor affected both hands but not the head or the voice.

Correlations between Genotype and Phenotype

Mutations identified in families affected by hemiplegic migraine with cerebellar signs and in subjects with sporadic hemiplegic migraine with permanent cerebellar signs were recurrent and, with the exception of R1668W, were distinct from those identified in families affected by pure hemiplegic migraine. Six mutations (R583Q, T666M, D715E, Y1385C, R1668W, and W1684R) were found in families affected by hemiplegic migraine with cerebellar signs and in subjects with sporadic cases of hemiplegic migraine with cerebellar signs; 83 percent of the subjects examined who had these mutations had nystagmus, ataxia, or both. In contrast, the results of neurologic examination were normal for all the subjects examined who had R195K, K1336E, or V1696I mutations. The person who had K1336E but was not examined reported having no disability when interviewed by telephone. R1668W was found in the proband of Family 15, who was affected by hemiplegic migraine with ataxia, and also in Family 21, a small family with three subjects who were affected by pure hemiplegic migraine.

In our analyses of the 117 subjects with mutations, we first compared the frequency of symptoms in the 89 subjects from families affected by hemiplegic migraine with cerebellar signs (we included the 2 patients with sporadic hemiplegic migraine) with the frequency in the 28 subjects from families affected by pure hemiplegic migraine (Table 2). The frequency of atypical attacks and the frequency of severe first attacks were higher in subjects with mutations who were from families affected by hemiplegic migraine with cerebellar signs than in subjects with mutations who were from families affected by pure hemiplegic migraine (49 percent vs. 21 percent for atypical attacks, $P=0.02$; 26 percent vs. 0 percent for severe first attacks, $P=0.003$).

In a second step, we searched for correlations between genotype and phenotype by comparing the frequency of symptoms among the 85 subjects with the three most frequent mutations linked to hemiplegic migraine with cerebellar signs, R583Q, T666M, and D715E (Table 4). The phenotype in those with T666M was characterized by the highest frequency of hemiplegic migraine (98 percent), severe attacks with coma (50 percent), and nystagmus (86 percent). The phenotype in those with R583Q was characterized by the highest frequency of ataxia (81 percent) in the absence of any nystagmus. The phenotype in those with D715E was characterized by the lowest frequency of attacks of hemiplegic migraine (64 percent).

DISCUSSION

We characterized the clinical features of familial hemiplegic migraine in 117 subjects with nine distinct mutations in *CACNA1A*. Familial hemiplegic migraine is an unusual inherited variety of migraine with aura characterized by an autosomal dominant pattern of transmission and the presence of unilateral motor deficits during the aura. In addition to these well-known features, there are other interesting clinical characteristics of familial hemiplegic migraine. The hemiparesis during the aura is always associated with sensory, language, or visual symptoms. However, one fourth of the patients affected by familial hemiplegic migraine do not have visual symptoms, whereas in more common varieties of migraine with aura, visual symptoms are almost universal (occurring in close to 99 percent of patients).[28] Bilateral motor signs are observed in one third of patients affected by hemiplegic migraine but very rarely in patients affected by the other varieties of migraine with aura.[28] Severe attacks with impairment of consciousness occur in one third of patients with hemiplegic migraine, with full recovery. Such attacks may lead to major difficulties in diagnosis, as in two subjects from our series in whom recurrent coma with hemiplegia that lasted for weeks and seizures were the dominant pattern of presentation. Finally, permanent cerebellar ataxia, nystagmus, or both

TABLE 4. COMPARISON OF THE FREQUENCY OF SYMPTOMS IN SUBJECTS WITH THE THREE MOST COMMON MUTATIONS ASSOCIATED WITH HEMIPLEGIC MIGRAINE WITH CEREBELLAR SIGNS.*

CHARACTERISTIC	SUBJECTS WITH R583Q (N=16)	SUBJECTS WITH T666M (N=55)	SUBJECTS WITH D715E (N=14)	P VALUE†
Age — yr				
Mean ±SD	39.2±18.5	37.4±19.4	38.5±19.3	
Range	11–78	6–85	12–76	
Hemiplegic migraine — no./total no. (%)	13/16 (81)	54/55 (98)	9/14 (64)	<0.001
Age at onset — yr				
Mean ±SD	10.1±1.5	12.7±10.1	10.2±3.5	
Range	7–12	1–51	4–14	
Aura with sensory disturbance — no./total no. (%)	12/13 (92)	47/52 (90)	9/9 (100)	
Aura with language disturbance — no./total no. (%)	3/4 (75)	44/52 (85)	8/9 (89)	
Aura with visual disturbance — no./total no. (%)	2/4 (50)	36/52 (69)	8/9 (89)	
Atypical attacks — no./total no. (%)	3/13 (23)	30/54 (56)	3/9 (33)	
Severe attacks with coma or confusion	2/13 (15)	27/54 (50)	2/9 (22)	0.04
Severe first attack	1/13 (8)	18/54 (33)	0/9	0.03
Permanent cerebellar signs — no./total no. (%)	13/16 (81)	43/50 (86)	9/12 (75)	
Nystagmus	0/16	43/50 (86)	3/12 (25)	<0.001
Ataxia	13/16 (81)	15/50 (30)	5/12 (42)	0.001
Dysarthria	2/16 (12)	6/50 (12)	1/12 (8)	

*The denominators (total numbers) reflect the numbers of family members for whom data were available or who were examined.

†P values are for the comparison of the three phenotypes. An overall test for equality of proportions with the use of the extension of Fisher's exact test (the Freeman–Halton method) was used to compare the frequency of symptoms in subjects with R583Q, T666M, and D715E.

are found in about 80 percent of subjects with mutations who are from families with hemiplegic migraine and cerebellar signs.

The five novel mutations in *CACNA1A* that we identified are all missense mutations, as are the eight previously known mutations. All these mutations are located in important functional domains of the calcium channel: the voltage sensor, the pore, and the pore-lining loops.[14,19-23] Other autosomal dominant neurologic disorders have been associated with distinct types of mutations in *CACNA1A*. Episodic ataxia type 2, which is characterized by attacks of paroxysmal ataxia, is mostly associated with truncations of the gene,[14,29,30] and spinocerebellar ataxia type 6, a late-onset progressive ataxia, is associated with CAG-repeat expansions.[31,32]

Our data strongly suggest that pure familial hemiplegic migraine and familial hemiplegic migraine with cerebellar signs, which were previously considered to be two clinical subtypes, are associated with distinct mutations in *CACNA1A*.[14,19-23] The presence of R1668W in two families, one without and the other with cerebellar signs, could be the result of a clinical misclassification due to the low number of examined subjects or a low penetrance of cerebellar signs in subjects with R1668W. If confirmed in larger samples, these correlations between genotype and phenotype may provide clues about the mechanisms leading to cerebellar dysfunction in people with mutations in *CACNA1A*.

When we examined familial hemiplegic migraine with cerebellar signs, we found that subjects with T666M had the highest penetrance of hemiplegic migraine (98 percent), severe attacks with coma (50 percent), and nystagmus (86 percent). Subjects with R583Q had the highest penetrance of gait ataxia (81 percent) in the absence of any nystagmus. Subjects with D715E had the lowest penetrance of attacks of hemiplegic migraine (64 percent); tremor was found in some subjects with D715E. The association of hemiplegic migraine with nystagmus and tremor was previously described in another family, without a molecular analysis.[33] Tremor may be part of the clinical spectrum induced by mutations in *CACNA1A;* however, the current data are insufficient to prove this hypothesis.

In conclusion, hemiplegic migraine caused by mutations in *CACNA1A,* a gene that encodes a neuronal calcium channel, has a broad clinical spectrum, including paroxysmal attacks and permanent signs, both of which have a highly variable severity. The variability of the mutations in *CACNA1A* partly accounts for this variability in disease severity. In vitro electrophysiological studies have compared P/Q-type calcium currents in cells expressing wild-type and mutant *CACNA1A*. Seven mutations linked to familial hemiplegic migraine, including R583Q, T666M, and D715E, have been shown to modify both the density and the gating properties of functional channels.[34-36] When in vitro models were used, no clear difference

was found between mutations associated with hemiplegic migraine with and without cerebellar signs. However, these studies probably do not reproduce the complexity of the situation in vivo. In addition, the clinical variability among subjects with the same mutation suggests that other genetic or environmental factors also influence the expression of these phenotypes. Finally, screening for missense mutations in *CACNA1A* may be useful in the diagnosis of disease in patients with recurrent coma with hemiplegia or in patients with sporadic hemiplegic migraine.

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