Systematic analysis of three FHM genes in 39 sporadic patients with hemiplegic migraine

B. de Vries, MSc*
T. Freilinger, MD*
K.R.J. Vanmolkot, MSc
J.B. Koenderink, PhD
A.H. Stam, MD
G.M. Terwindt, MD, PhD
E. Babini, PhD
E.H. van den Boogerd, BSc
J.J.M.W. van den Heuvel, BSc
R.R. Frants, PhD
J. Haan, MD, PhD
M. Pusch, PhD
A.M.J.M. van den Maagdenberg, PhD
M.D. Ferrari, MD, PhD
M. Dichgans, MD, PhD

Address correspondence and reprint requests to Dr. Michel D. Ferrari, Department of Neurology, Leiden University Medical Centre, PO Box 9600, 2300 RC Leiden, The Netherlands
m.d.ferrari@lumc.nl

ABSTRACT

Background: Familial (FHM) and sporadic (SHM) hemiplegic migraine are severe subtypes of migraine associated with transient hemiparesis. For FHM, three genes have been identified encoding subunits of a calcium channel (*CACNA1A*), a sodium–potassium pump (*ATP1A2*), and a sodium channel (*SCN1A*). Their role in SHM is unknown. Establishing a genetic basis for SHM may further the understanding of its pathophysiology and relationship with common types of migraine. It will also facilitate the often difficult differential diagnosis from other causes of transient hemiparesis.

Methods: We systematically scanned 39 well-characterized patients with SHM without associated neurologic features for mutations in the three FHM genes. Functional assays were performed for all new sequence variants.

Results: Sequence variants were identified in seven SHM patients: one *CACNA1A* mutation, five *ATP1A2* mutations, and one *SCN1A* polymorphism. All six mutations caused functional changes in cellular assays. One SHM patient later changed to FHM because another family member developed FHM attacks.

Conclusion: We show that FHM genes are involved in at least a proportion of SHM patients without associated neurologic symptoms. Screening of *ATP1A2* offers the highest likelihood of success. Because FHM gene mutations were also found in family members with "nonhemiplegic" typical migraine with and without aura, our findings reinforce the hypothesis that FHM, SHM, and "normal" migraine are part of a disease spectrum with shared pathogenetic mechanisms.
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GLOSSARY

ATPase = adenosine triphosphatase; **BAM** = basilar artery migraine; **Ctrl** = control; **cDNA** = complementary DNA; **Fam** = family; **FHM** = familial hemiplegic migraine; **HM** = hemiplegic migraine; **IHS** = International Headache Society; **MA** = migraine with aura; **MO** = migraine without aura; **SHM** = sporadic hemiplegic migraine; **WT** = wild type.

Hemiplegic migraine is a rare, often severe subtype of migraine with aura in which attacks are associated with hemiparesis.[1] Otherwise, the aura and headache symptoms are identical to those of common types of migraine.[2] Hemiplegic migraine may run in families (familial hemiplegic migraine [FHM]) or may be sporadic (SHM).[1] Clinically, FHM and SHM attacks are indistinguishable, and the majority of patients also have common attacks of migraine with or without aura, not associated with hemiparesis.[3]

Thus far, three genes for FHM have been identified. The *CACNA1A* gene (FHM1)[4] encoding the pore-forming subunit $Ca_v2.1$ of neuronal P/Q-type calcium channels, the

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*These authors contributed equally.

From the Departments of Human Genetics (B.d.V., K.R.J.V., E.H.v.d.B., R.R.F., A.M.J.M.v.d.M.) and Neurology (A.H.S., G.M.T., J.H., A.M.J.M.v.d.M., M.D.F.), Leiden University Medical Centre, Leiden, The Netherlands; Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-Universität, München, Germany (T.F., M.D.); Department of Pharmacology and Toxicology, Centre for Molecular Life Sciences, University Medical Centre St. Radboud, Nijmegen, The Netherlands (J.B.K., J.J.M.W.v.d.H.); Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Genoa, Italy (E.B., M.P.); and Department of Neurology, Rijnland Hospital, Leiderdorp, The Netherlands (J.H.).

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ATP1A2 gene (FHM2)[5] encoding the $\alpha 2$ subunit of sodium–potassium pumps, and the *SCN1A* gene (FHM3)[6] encoding the $\alpha 1$ subunit of neuronal sodium channels.

Although clinically indistinguishable,[3] it is unknown whether and to what extent SHM and FHM are pathophysiologically related and whether and to what extent FHM genes are also involved in SHM. Previous studies identified mutations in the *CACNA1A* gene in SHM patients.[7-11] Most of these patients showed cerebellar signs, suggesting an involvement of the *CACNA1A* gene in SHM with associated cerebellar and other neurologic signs or symptoms, such as cerebral edema and coma after minor head trauma. In contrast, the role of the FHM genes in "pure" SHM without associated neurologic symptoms is less clear. One *CACNA1A* mutation (R583Q)[8] and one *ATP1A2* mutation (R383H)[12] were reported in such patients.

Investigating the involvement of FHM genes in sporadic patients with hemiplegic migraine is important because it may further the insight into the pathophysiology of SHM and the relationship with other types of migraine. Moreover, understanding and establishing the genetic basis of SHM may help clinicians in diagnostic and therapeutic decision making. Many patients are initially misdiagnosed and mistreated. We therefore set out to search systematically for mutations in the known FHM genes in a large set of 39 clinically well-characterized patients with "pure" SHM, who had no interictal neurologic symptoms.

METHODS

Patients. Sporadic hemiplegic migraine was diagnosed according to the criteria of the International Headache Society (IHS).[1] Patients with interictal neurologic symptoms, in particular ataxia, were excluded because these patients have a high a priori probability of carrying a *CACNA1A* mutation.[7-11] All available family members were directly interviewed, and their headache was diagnosed according to the IHS criteria. In addition to newly recruited SHM patients, we included 25 of the 27 patients from our previous study in which only the FHM1 *CACNA1A* gene was investigated.[8] Two patients from that study were excluded because of associated symptoms; one had ataxia and carried the T666M mutation, the other patient had childhood epilepsy and did not carry a *CACNA1A* mutation. Approval was obtained by local ethical committees in accordance with national legislation; all patients gave informed consent.

Mutation scanning. Genomic DNA was isolated from peripheral leukocytes using a standard salting out extraction method. The *CACNA1A*, *ATP1A2*, and *SCN1A* genes were screened for mutations by sequencing.[6,8,13] In brief, all exons and flanking intronic regions were amplified by PCR, using genomic DNA as a template. Direct sequencing was performed with Cycle Sequencing (Prism Big Dye Terminators Cycle Sequencing kit, Applied Biosystems, Foster City, CA) using the dideoxy termination method and an ABI3700 automated sequencer (Applied Biosystems). For each exonic variant identified, 150 healthy controls were screened, by restriction enzyme analysis or direct sequencing. Detailed information is available from the authors on request.

Functional analysis. Functional analysis of mutations in the $Ca_v2.1$-α_1 calcium channel subunit was not performed because the single *CACNA1A* mutation found in this study was thoroughly investigated before.[14] Functional analysis of *ATP1A2* variants was performed by survival assays. Human Na,K–adenosine triphosphatase (ATPase) α_2-subunit complementary DNA (cDNA) was subcloned into a modified pCDNA3.1 vector.[15] To distinguish endogenous Na,K-ATPase activity from that of transfected Na,K-ATPase, we used a cDNA construct encoding ouabain-resistant wild-type (*ATP1A2*-WT).[15,16] Mutations E120A, E492K, P786L, R834X, and R908Q were introduced in the ouabain-resistant wild-type α_2-subunit construct by site-directed mutagenesis (Quikchange, Stratagene, La Jolla, CA). HeLa cells (5×10^5) were transfected with plasmid DNA of either *ATP1A2*-WT or *ATP1A2*-mutant (*ATP1A2-E120A*, *ATP1A2-E492K*, *ATP1A2-P789L*, *ATP1A2-R834X*, *ATP1A2-R908Q*) using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA). Two days after transfection, two-thirds of the cells were harvested for immunoblotting, and the α_2-subunit protein was detected using the specific polyclonal antibody HERED.[15,16] The remaining one-third of the cells was seeded on 10-cm petri dishes, and subsequently 1 μM ouabain was added to the culture medium. After 5 days of ouabain challenge, colonies were stained with 1% methylene blue in 70% methanol, scanned, and analyzed with Image Pro Plus (MediaCybernetics, Silver Spring, MD). Each transfection was performed 7 to 15 times. In case of partial survival, statistical significance was tested using Student *t* test ($p < 0.05$).

For functional analysis of the *SCN1A* variant, we used the closely related *SCN5A* cDNA, because of the known stability problems of recombinant bacteria with *SCN1A* cDNA.[6,17] R1914G, which corresponds to *SCN1A* R1928G, was introduced by site-directed mutagenesis into full-length human *SCN5A* cDNA subcloned in pCDNA3.1 (QuikChange XL Kit, Stratagene). *SCN5A-R1914G* and *SCN5A*-WT cDNAs were transfected into human tsA201 cells and were each coexpressed with accessory human sodium channel subunit β_1.[6] Macroscopic sodium currents were recorded using the whole-cell configuration of the patch clamp technique.[6] Steady state activation, steady state inactivation, time constants of inactivation (e.g., time constants τ_{fh} and τ_{sh}), and recovery from inactivation (e.g., τ_{fast} and τ_{slow} time constants) were measured using protocols, as described before.[6]

RESULTS

Patients. Thirty-nine patients with "pure" SHM were included; 37 originated from Western Europe (mostly The Netherlands or Ger-

Table 1 Clinical and genetic characteristics of SHM patients

Patient	Mutation in HM gene	Age at onset, y	Frequency of attacks, per y	Total duration of attack	Duration of paresis	BAM symptoms	Unconsciousness during attacks	Attacks triggered by minor head trauma	MO or MA in first-degree family members
1	No	27	3	1–2 d	Minutes	No	No	No	MO
2	No	20	4	4 h–2 d	0.5–2 h	No	No	No	MA
3	No	12	?	Days	Hours	No	No	No	MA
4	No	18	24	15–60 min	15 min	No	No	No	MO
5	No	20	0–3	8 h	1 h	No	No	No	No
6	*ATP1A2- R834X*	10	1	4 d	2 d	No	No	Yes	MO
7	No	22	12	12 h	0.5–2 h	No	No	No	MO and MA
8	No	9	12–30	1–3 d	1 h–1 d	No	No	No	MA
9	No	25	24	Hours–3 d	0.5–1 h	Yes	No	No	MO
10	No	12	4–36	1–2 d	5–10 min	No	Yes	No	No
11	No	19	2 in lifetime	20 min–1 h	20 min	No	No	No	MA
12	*ATP1A2-E120A*	13	12–250	1 d	1 h	No	No	No	No
13	No	25	48	10–24 h	?	?	No	No	MO and MA
14	No	13	2–6	Few hours	0.5 h	No	No	No	MO and MA
15	No	29	2–100	Minutes–hours	Minutes–hours	Yes	Yes	Yes	No
16	No	4	24	1 d	Few hours	Yes	No	No	MA
17	No	10	Once	1 d	1 h	No	No	No	MO and MA
18	No	20	0–2	1 wk–1 mo	1 wk–1 mo	?	No	No	MO
19	*ATP1A2-P786L*	5	0.5	3 wk	6 d	No	No	No	No
20	No	16	12	1 d	1 h	No	No	No	MO and MA
21	No	13	Once	Few days	Few days	No	No	No	MO and MA
22	No	13	2–52	1 d	0.5–1 h	No	No	No	No
23	*ATP1A2-E492K*	19	2	?	?	No	No	No	MA
24	No	32	2–12	Days	Hours–days	No	No	No	No
25	*CACNA1A-R583Q*	13	2	1 d	0.5 h	No	No	No	MA
26	No	34	8	0.5?	Hours?	No	No	No	No
27	No	37	36–48	3–48 h?	Hours–48 h	No	No	No	MO or MA?
28	*SCN1A-R1928G*	38	2 in lifetime	Few hours	1.5 h	No	Yes	No	MO and MA
29	No	42	2 in lifetime	3–4 hours	0.5 h	No	No	No	Migraine unspecified
30	No	11	30 in lifetime	Hours	1 h	No	No	No	MA and migraine unspecified
31	No	17	24	Up to 4 h	3–4 h	No	No	No	MA in grandmother
32	No	22	up to 50	Hours	Hours	No	No	No	No
33	No	15	4 to 10	1 h	15–30 min	No	No	No	Migraine unspecified
34	*ATP1A2-R908Q*	5	2 to 5	2 h	30–90 min	Yes	No	No	No
35	No	27	5 in lifetime	Up to 7 h	Up to 7 h	No	No	No	MO and MA
36	No	16	4	1.5–3 h	1–3 h	No	No	No	No
37	No	4	4	Hours	24–72 h	No	No	No	No
38	No	8	2–12	Hours	15–60 min	No	No	No	No
39	No	35	5	Few hours	15 min–3 h	No	Yes	No	MA

SHM = sporadic hemiplegic migraine; HM = hemiplegic migraine; BAM = basilar artery migraine; MO = migraine without aura; MA = migraine with aura.

many) and 2 came from the United States (table 1). As expected,[3,18] some of the patients exhibited basilar-type migraine symptoms during the attacks, but they were all free of interictal signs or symptoms. Age at onset of hemiplegic attacks ranged from 4 to 42 years. The number of attacks varied from 2 per lifetime to more than 200 per year. Likewise, duration of hemiparesis was variable, from several minutes to 1 week. Four patients reported loss of consciousness during

Table 2 DNA variants identified in pure SHM patients

Gene	Amino acid change	Nucleotide change	Abnormality in functional test
CACNA1A	R583Q	nt2021G>A	Electrophysiologicconsequence
ATP1A2	E120A	nt 463A>C	Partial cell survival
ATP1A2	E492K	nt 1578G>A	Partial cell survival
ATP1A2	P786L	nt 2604C>T	No cell survival
ATP1A2	R834X	nt 2461C>T	No cell survival
ATP1A2	R908Q	nt 2827G>A	No cell survival
SCN1A	R1928G	nt 5782C>G	No electrophysiologic consequence

SHM = sporadic hemiplegic migraine.

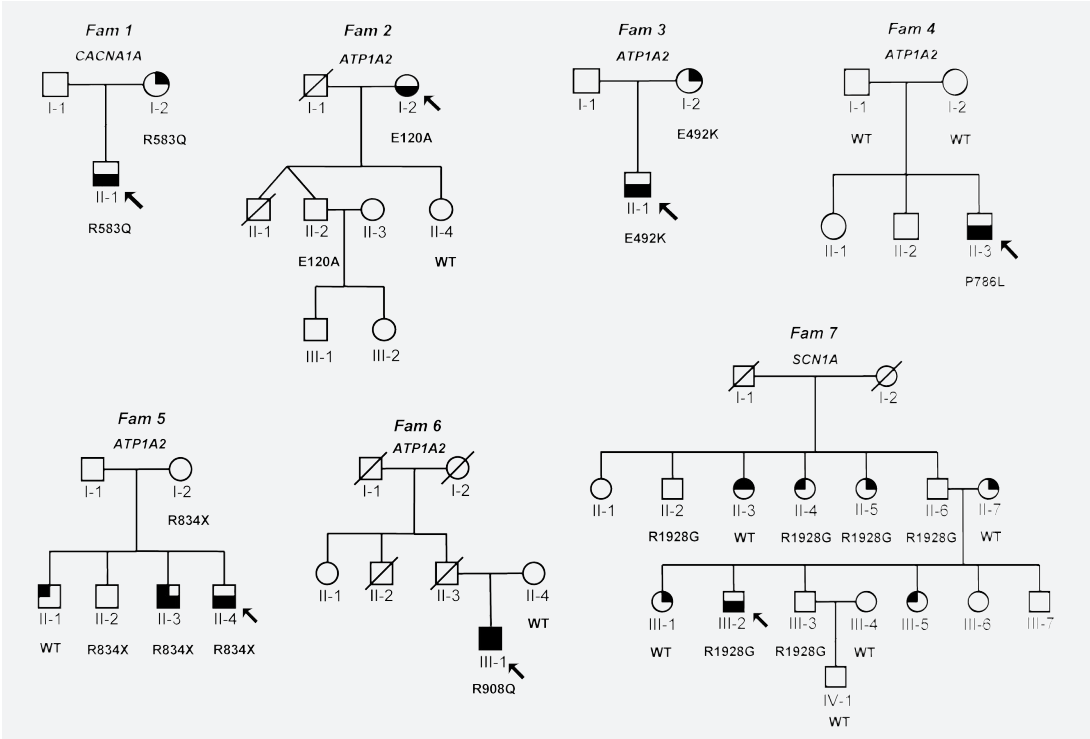
attacks; 2 patients reported triggering of attacks by minor head trauma. In 1 patient, the initial diagnosis was later changed to FHM, when a family member developed hemiplegic migraine attacks. Notwithstanding, this patient was kept in the SHM group because he fulfilled the inclusion criteria of SHM at the time of clinical presentation. In approximately 70% of the families of our 39 SHM patients, attacks of common nonhemiplegic migraine with aura (one-third), without aura (one-third), or both (one-third) were present in one or more first-degree relatives.

Genetic and functional findings. Sequencing of all exons and flanking intronic sequences in the 39 index cases revealed seven different sequence variants which were present in seven probands (table 2 and figure 1): one in *CACNA1A*, five in *ATP1A2*, and one the *SCN1A* gene. None of the sequence variants was present in 150 healthy controls (data not shown).

CACNA1A. The single variant in the *CACNA1A* gene (R583Q) has been reported as part of our earlier study.[8] R583Q was present in the index case and his unaffected mother, who had migraine with aura but no hemiplegic attacks indicating incomplete penetrance. R583Q has previously been identified in families with FHM and shown to affect $Ca_v2.1$ Ca^{2+} channel gating in functional studies.[14] Thus, R583Q can be considered causative in our case.

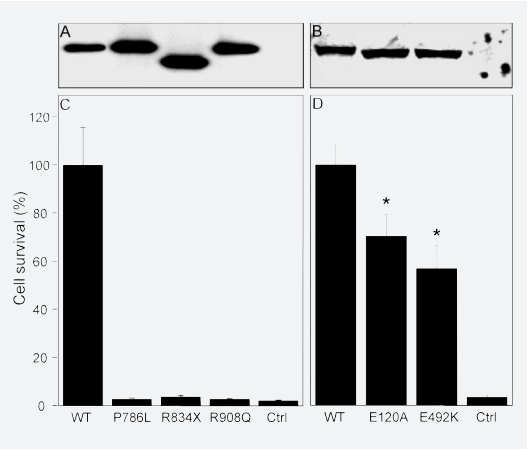
ATP1A2. The five DNA variants in *ATP1A2* included four missense variants (E120A, E492K, P786L, R908Q) and one nonsense mutation (R834X). P786, R908, and R834 are completely

Figure 1 Pedigrees of sporadic hemiplegic migraine cases with a mutation in one of the familial hemiplegic migraine genes (*CACNA1A*, *ATP1A2*, and *SCN1A*)



The following symbols are used to indicate the diagnosis: filled lower half = familial hemiplegic migraine; right upper quadrant = migraine with aura; left upper quadrant = migraine without aura. Circle = female; square = male. Arrows indicate probands. Individuals homozygous for the wild-type allele are indicated by WT; individuals heterozygous for a DNA variant are indicated by the respective variant. Fam = family.

Figure 2 Ouabain survival assay of novel *ATP1A2* DNA variants identified in sporadic hemiplegic migraine patients



(A and B) Western blot analysis of HeLa cells transfected with wild-type (WT) or mutant *ATP1A2* complementary DNA (cDNA). (C and D) Ouabain sensitivity of cells transfected with either wild-type or mutant *ATP1A2* cDNA. Bars represent cell survival after 5 days of ouabain treatment (error bars = SEM). * Partial survival is significantly lower than for WT ($p < 0.05$). Mutants P786L, R834X, and R908Q gave no survival. Ctrl = control.

conserved across multiple homologs and orthologs, whereas E120 and E492 are less well conserved (figure E-1 on the *Neurology*® Web site at www.neurology.org). The P786L mutation was not present in the proband's parents. False paternity was excluded in this case. Thus, P786L represents a de novo mutation. E120A, E492K, and R834X were all present in one or more relatives who had no hemiplegic attacks, thus suggesting incomplete penetrance. R908Q was not present in the proband's mother, but DNA from additional family members was not available (figure 1).

Functional consequences of all five *ATP1A2* variants were investigated using survival assays in HeLa cells as previously reported for FHM2 mutations.[5,16] The survival assays test for the ability of mutant protein to compensate for the loss of endogenous Na,K pump function, as achieved by ouabain treatment (figure 2, C and D). Because of an altered ouabain binding site, the transfected wild-type (*ATP1A2*-WT) and mutant (*ATP1A2-E120A*, *ATP1A2-E492K*, *ATP1A2-P786L*, *ATP1A2-R834X*, *ATP1A2-R908Q*) Na,K-ATPase α_2 subunits are ouabain insensitive. Western blot analysis showed that the constructs were expressed at comparable levels (figure 2, A and B). In the survival assay, cells expressing the wild-type construct survived ouabain treatment. In contrast, *ATP1A2* mutants gave no (*ATP1A2-P786L*, *ATP1A2-R834X*, *ATP1A2-R908Q*) or partial (*ATP1A2-E120A* [$p = 0.03$], *ATP1A2-E492K* [$p = 0.002$]) cell survival, indicating a clear functional consequence for all mutants (figure 2, C and D).

SCN1A. DNA variant R1928G in the *SCN1A* gene was present in the index case and five additional family members, two of whom had non-hemiplegic migraine. R1914G (which is equivalent to *SCN1A-R1928G*) was introduced to highly homologous human *SCN5A* (*SCN5A-R1914G*) and functionally tested for its biophysical properties by patch clamp experiments in transiently transfected human tsA201 cells.[6] Cells expressing the *SCN5A-R1914G* showed no significant difference in current density, steady state activation, steady state inactivation, and recovery from inactivation when compared with wild type (table E-1). These results indicate that the variant may be a DNA variant without a biologically significant effect on $Na_v1.1$ channel functioning.

DISCUSSION We screened 39 patients with "pure" SHM without ataxia or other additional neurologic features for mutations in the three known FHM genes. In 7 patients, we found a sequence variant (table 2). None was found in 300 control chromosomes. Six of these showed obvious functional changes and can be considered causal mutations. These results indicate that genes for FHM are involved in at least a proportion of patients with "pure" SHM. Our findings have important pathogenetic, clinical, and diagnostic implications.

With our findings, a sensible approach to genetic testing in SHM has become available to confirm the often difficult and clinically important diagnosis of SHM.[19] Because SHM patients with de novo mutations may represent the founder of a new family with highly disabling FHM, genetic confirmation of the diagnosis may have consequences for genetic counseling. When genetic testing is considered in a patient with "pure" SHM, the *ATP1A2* gene should be screened first. We found an *ATP1A2* sequence variant in five of the seven SHM cases with a confirmed sequence variant corresponding to 13% of the overall SHM sample. This is a strikingly higher prevalence compared with a previous study of the *ATP1A2* gene that included patients with SHM but provided no specific clinical details.[12]

Our findings are in line with earlier smaller studies showing that the yield of *CACNA1A* mutations in SHM patients is low in the absence of ataxia.[20,21] In contrast, *CACNA1A* mutations were found in 50% of SHM patients with associated cerebellar signs.[7,9] The present study is the

first to evaluate the role of the recently identified FHM3 gene[6] in SHM. The likelihood of finding *SCN1A* mutations in "pure" SHM, however, seems very low.

Most *ATP1A2* mutations in this study were also found in asymptomatic relatives and in relatives with nonhemiplegic migraine. They thus showed reduced penetrance, as has also been noticed for *ATP1A2* mutations associated with FHM2.[22-24] This might explain why mutations in the *ATP1A2* gene are relatively common among sporadic patients. In contrast, all *SCN1A* mutations previously identified in FHM families showed complete penetrance.[6,17] This might relate to the low yield of mutations in this gene in our sample of sporadic cases.

Although we found FHM gene mutations in 18% of our patients with pure SHM, we did not find mutations in the majority of our patients. It is likely that when additional genes for FHM are discovered, greater proportions of patients with SHM will prove to have mutations in FHM genes. Until then, a diagnosis of SHM remains based on the exclusion of other causes of recurrent hemiparesis, careful physical examination, detailed personal and family history, and regular follow-up. In one patient (with *ATP1A2* mutation R834X; Family 5: figure 1), we had to change the initial diagnosis of SHM to FHM when an additional family member developed hemiplegic migraine attacks several years after our initial investigation. A diagnosis of pure SHM is likely when transient hemiparesis occurs in the course of a typical attack of migraine with aura, when there are no interictal abnormalities, and when "normal" attacks of migraine with or without aura are present in first-degree relatives.[25] Approximately 70% of pure SHM cases and 60% of the mutation carriers had first-degree relatives with common types of migraine.

Our findings provide genetic evidence that FHM genes are also involved in SHM and thus extend and reinforce the growing clinical, epidemiologic, genetic, and pathophysiologic evidence that FHM and SHM share neurobiological mechanisms.[26] Moreover, because the majority of hemiplegic migraine patients also have "normal" attacks of migraine without hemiparesis, both diseases can be considered extremes of the pathogenetic migraine spectrum with shared common pathways with "normal" migraine with and without aura.[26-29]

We identified five sequence variants in *ATP1A2* (figure 1; Families 2 through 6). All conferred reduced survival in cellular assays (figure 2) and therefore are likely to be causative mutations. P786L occurred de novo and could thus be the founder of a new FHM family. R908Q was found in a patient whose mother did not carry the mutation. Because DNA from the father was not available, it could not be established whether the mutation had occurred de novo. E120A and E492K showed partially reduced survival (figure 2D). Both mutations were identified in other family members who were unaffected or had nonhemiplegic migraine with aura. The single *CACNA1A* mutation (R583Q) we found was previously shown to affect $Ca_v2.1$ Ca^{2+} currents in cellular models by changing channel gating.[14] The mutation was inherited from the mother, who has attacks of nonhemiplegic migraine with aura. The R1928G DNA variant that was identified in the *SCN1A* gene did not reveal significant effects on channel properties as investigated by electrophysiologic recordings. Also, this variant poorly segregates with the migraine phenotype. It was present in five nonhemiplegic family members; only one of them has migraine with aura, and another has migraine without aura. R1928G may therefore be a rare sequence variant without functional consequences.

Screening of FHM genes in sporadic patients with hemiplegic migraine may help to establish the diagnosis, enable counseling, and prevent unnecessary diagnostic and therapeutic trial with potentially harmful drugs. Scanning of the FHM2 *ATP1A2* gene seems to offer the highest likelihood of success. Because FHM mutations were also found in SHM and common types of migraine with or without aura, our findings reinforce the growing evidence that FHM, SHM, basilar-type migraine, and "normal" migraine are part of a disease spectrum with at least some shared pathogenetic pathways. Unraveling these pathways may help to identify novel migraine prophylactic drugs.

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REFERENCES

1. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders: 2nd edition. Cephalalgia 2004;24:1–160.
2. Ferrari MD. Migraine. Lancet 1998;351:1043–1451.

3. Thomsen LL, Ostergaard E, Olesen J, Russell MB. Evidence for a separate type of migraine with aura: sporadic hemiplegic migraine. Neurology 2003;60:595–601.
4. Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell 1996;87:543–552.
5. De Fusco M, Marconi R, Silverstri L, et al. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 2003;33:192–196.
6. Dichgans M, Freilinger T, Eckstein G, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet 2005;336:371–377.
7. Ducros A, Denier C, Joutel A, et al. The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. N Engl J Med 2001;345:17–24.
8. Terwindt G, Kors E, Haan J, et al. Mutation analysis of the CACNA1A calcium channel subunit gene in 27 patients with sporadic hemiplegic migraine. Arch Neurol 2002;59:1016–1018.
9. Ducros A, Denier C, Joutel A, et al. Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. Am J Hum Genet 1999;64:89–98.
10. Vahedi K, Dernier C, Ducros A, et al. CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. Neurology 2000;55:1040–1042.
11. Curtain RP, Smith RL, Ovcaric M, Griffiths LR. Minor head trauma-induced sporadic hemiplegic coma. Pediatr Neurol 2006;34:329–332.
12. Jurkat-Rott K, Freilinger T, Dreier JP, et al. Variability of familial hemiplegic migraine with novel A1A2 Na^+/K^+-ATPase variants. Neurology 2004;62:1857–1861.
13. Vanmolkot KRJ, Kors EE, Hottenga JJ, et al. Novel mutations in the Na+, K+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. Ann Neurol 2003;54:360–366.
14. Kraus RL, Sinnegger MJ, Koschak A, et al. Three new familial hemiplegic migraine mutants affect P/Q-type Ca2+ channel kinetics. J Biol Chem 2000;13:9239–9243.
15. Koenderink JB, Zifarelli G, Qiu LY, et al. Na,K-ATPase mutations in familial hemiplegic migraine lead to functional inactivation. Biochim Biophys Acta 2005;1669:61–68.
16. Vanmokot KRJ, Kors EE, Turk U, et al. Two de novo mutations in the Na,K-ATPase gene ATP1A2 associated with pure familial hemiplegic migraine. Eur J Hum Genet 2006;14:555–560.
17. Vanmolkot KRJ, Babini E, De Vries B, et al. The novel L1649Q mutation in the SCN1A epilepsy gene is associated with familial hemiplegic migraine: genetic and functional studies. Hum Mutat 2007;28:522.
18. Haan J, Terwindt GM, Ophoff RA, Frants RR, Ferrari MD. Is familial hemiplegic migraine the hereditary form of basilar artery migraine? Cephalalgia 1995;15:477–481.
19. Black DF. Sporadic hemiplegic migraine. Curr Pain Headache Rep 2004;8:233–238.
20. Thomsen LL, Olesen J. Sporadic hemiplegic migraine. Cephalalgia 2004;24:1016–1023.
21. Carrera P, Piatti M, Stenirri S, et al. Genetic heterogeneity in Italian families with familial hemiplegic migraine. Neurology 1999;13:26–33.
22. Ducros A, Joutel A, Vahedi K, et al. Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence for further heterogeneity. Ann Neurol 1997;42:885–890.
23. Gardner K, Barmada M, Ptacek L, et al. A new locus for hemiplegic migraine maps to chromosome 1q31. Neurology 1997;49:1231–1238.
24. Riant F, De Fusco M, Aridon P, et al. ATP1A2 mutations in 11 families with familial hemiplegic migraine. Hum Mutat 2005;26:281.
25. Thomsen LL, Ostergaard E, Romer SF, et al. Sporadic hemiplegic migraine is an aetiologically heterogeneous disorder. Cephalalgia 2003;23:921–928.
26. Ferrari MD, Goadsby PJ. Migraine as a cerebral ionopathy with abnormal central sensory processing. In: Gilman S, Pedley T, eds. Neurobiology of disease. New York: Elsevier, 2006:333–348.
27. May A, Ophoff RA, Terwindt GM, et al. Familial hemiplegic migraine locus on 19p13 is involved in the common forms of migraine with and without aura. Hum Genet 1995;96:604–608.
28. Terwindt GM, Ophoff RA, van Eijk R, et al. Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. Dutch Migraine Genetics Research Group. Neurology 2001;56:1028–1032.
29. Moskowitz MA, Bolay H, Dalkara T. Deciphering migraine mechanisms: clues from familial hemiplegic migraine genotypes. Ann Neurol 2004;55:276–280.