

Variability of familial hemiplegic migraine with novel A1A2 Na⁺/K⁺-ATPase variants

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Abstract—A1A2 Na⁺/K⁺-ATPase mutations cause familial hemiplegic migraine type 2 (FHM2). The authors identified three putative A1A2 mutations (D718N, R763H, P979L) and three that await validation (P796R, E902K, X1021R). Ten to 20% of FHM cases may be FHM2. A1A2 mutations have a penetrance of about 87%. D718N causes frequent, long-lasting HM, and P979L may cause recurrent coma. D718N and P979L may predispose to seizures and mental retardation. A1A2 does not play a major role in sporadic HM; only one variant, R383H, occurred in 1 of 24 cases.

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Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine characterized by hemiparesis during the aura.¹ Half of the families carry mutations in the *CACNA1A* gene on chromosome 19p13 (FHM1) encoding the α_{1A} subunit of a neuronal voltage-gated P/Q-type calcium channel.² The phenotype of FHM1 is well established: In up to 50% of the families, permanent cerebellar signs such as gaze-evoked nystagmus or mild statokinetic ataxia are present; severe attacks with impaired consciousness may occur in up to 33% of the patients.³ More rarely, transient cerebral edema and hemispheric cerebral atrophy have been observed.³ Recently, the chromosome 1q23 *ATP1A2* gene encoding the A1A2 Na⁺/K⁺-ATPase subunit has been shown to be causative for FHM2.⁴ Until now, only four FHM2 mutations, each in a different family, have been described.^{4,5} The FHM2 phenotype of FHM2 is therefore not so well established. Features that have been observed in single families include epileptic seizures,^{4,8} severe attacks,^{6,7} and mental retardation.^{4,8}

Patients and methods. Patients provided written informed consent following guidelines of the local ethics committees. We included 26 unrelated families with FHM and 24 patients with sporadic HM, 8 of whom had a positive family history for migraine with aura or migraine without aura. Classification of migraine was established following the criteria of the International Head-

ache Society.¹ Clinical and linkage data on Family B have previously been reported.⁸

Linkage to the FHM2 locus and exclusion of the FHM1 locus were known for Family B⁸ and performed for Family A. The other 24 families consisting of two to three affected individuals were too small to draw conclusions from linkage; however, for 12 of the FHM families (including Families A, D, E, and F) and for all 24 sporadic cases (including G), the known *CACNA1A* mutations had been excluded.

Genomic DNA was isolated from peripheral blood lymphocytes. DNA samples were analyzed for mutations in the *ATP1A2* gene by direct sequencing of PCR products of exons 1 to 23 using intronic primer pairs (see the supplementary material on the *Neurology* Web site; go to www.neurology.org). All sequences showing base exchanges were verified by reverse sequencing of a new PCR product of the same DNA sample. Direct sequencing was further used to test each DNA sequence variant for co-segregation with the affected phenotype within families and to test for its frequency in unrelated healthy control subjects (n = 237 for the polymorphism and n = 300 for the other variants).

The clinical diagnosis of the individuals concerning migraine was performed prior to the genetic screening. Families A, C, D, E, F, and G (figure 1) were recontacted for a semistructured interview to obtain details for phenotype–genotype analysis (table). These patients were also seen personally by the authors, including Patients A1 and A6 who died during the course of the study (see figure 1). Only one unaffected individual was not available for re-examination; her clinical phenotype has therefore been marked with a question mark (see figure 1).

Results and discussion. Direct sequencing of the entire coding sequence of the *ATP1A2* gene in 26 unrelated cases from FHM families and 24 sporadic HM patients revealed eight different nucleotide changes leading to sequence alterations in A1A2 (figure 2, top). One of them (A2647C encoding I883L) was found in 12 (24%) of the unrelated migraineurs and 23% of 237 control subjects and therefore

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Table Clinical features of ATP1A2 variant carriers

Family	General				Aura symptoms during attacks of HM				
	No.	Age, y	HM onset, y	Triggering factors of HM	H	S	V	A	Additional aura features
A	1	84	10	Physical exercise, angiography	+	+	+	+	Confusion, dysarthria, alien limb phenomenon, diplopia, impaired hearing
	2	47	8	Minor trauma	+	+	+	+	Dysarthria
	3	54	12	NS	+	+	+	-	Impaired hearing
	4	57	4	NS	+	+	+	+	Vertigo
	5	23	NA	NA	NA	NA	NA	NA	NA
	6	34	10	NS	+	+	+	+	Dysarthria, diplopia, impaired hearing, visual hallucinations
	7	16	3	Emotional stress	+	-	-	+	Confusion, fever, dysarthria
B	1*	59	<20	NS	+	NS	NS	NS	—
	2	50	23	Head trauma	+	+	+	-	Confusion, dysarthria
	3†	23	4	NS	+	NS	NS	NS	—
	4	33	6	NS	+	+	-	-	—
	5	30	3	Emotional stress	+	+	+	-	Coma, dysarthria
C	1	39	16	Sun exposure	+	-	-	-	Confusion, dysarthria
	2	15	5	NS	+	+	-	-	—
D	1	52	13	Head trauma, angiography, physical exercise	+	+	+	+	Confusion, fever, meningism, mild CSF pleocytosis, alien limb, apraxia, hyperacusis, visual hallucinations
	2	22	NA	NA	NA	NA	NA	NA	NA
	3	19	7	Physical exercise	+	-	+	+	—
E	1	31	0.7	Physical exercise, heat	+	-	?	+	Coma, confusion, fever
F	1	44	41	Stress, exercise, lack of sleep	+	+	+	+	—
G	1	45	11	Emotional and physical stress	+	+	+	+	—

* Alcohol abuse, incomplete interview.

† Memory disturbance, incomplete interview.

NS = not specified; NA = not applicable; HM = hemiplegic migraine; HA = headache; H = hemiplegia; S = sensory disturbances; V = visual disturbances; A = aphasia; (-) = not present; (+) = present; TR = temporal relationship of aura and headache; (=) = simultaneously; (>) = prior to; AU = aura; UL = unilateral; BL = bilateral; N/V/P/P = nausea/vomiting/photophobia/phonophobia; HA vs ND = relation to HA side to side of neurologic deficit; IL = ipsilateral; CL = contralateral; Var = variable; MA = migraine with aura; MO = migraine without aura; TT = tension-type HA.

represents a common polymorphism. Six additional sequence alterations were all found only in one FHM family: G2152A (D718N), C2936T (P979L), T3061C (X1021R followed by 27 residues prior to the stop codon, PHWKKN-QAWKDGELWRCCGDGDGEGWKKX), G2288A (R763H),

G2704A (E902K), and C2387G (P796R). A last variant, G1148A (R383H), was identified in one sporadic HM individual. There are several arguments in favor of the disease-causing character of these variants: 1) They affect highly conserved residues (see figure 2, bottom), 2) they

Table Continued

Hemiplegic attacks		Characteristics of HA during attacks of HM						Other symptoms	
Duration, h	Frequency	TR	HA duration, h	HA side	HA vs ND	HA character	N/V/P/P	Other HA types	Interictal neurologic and other phenotypic features
"Hours"	1-2x/mo	AU = HA	6-8	UL	IL	Stabbing	++++	MO	—
NS	2 in total	NS	NS	UL	NS	Throbbing	++++	MA, MO, TT	—
"Hours"	1x/mo	HA > AU	Up to 24	UL	IL	Stabbing, burning	++++	MO	—
6-72	1x/mo to 1x/wk	AU > HA	6-72	UL	CL	Stabbing	++++	NS	Bilateral cerebellar infarcts on MRI, low IQ, nystagmus, obesity
NA	NA	NA	NA	NA	NA	NA	NA	MO	—
48-336	1x/mo	AU = HA	48-96	UL	IL	Stabbing, throbbing	++++	—	Obesity
48-240	2x/mo	AU > HA	48-72	UL	IL	NS	++++	—	Epileptic seizures, mental retardation
NS	NS	NS	NS	NS	NS	NS	NS	NS	Chronic alcohol abuse
0.5-48	1-4x/y	AU > HA	24	UL	IL	Pulsating	++++	MO, MA	Nystagmus
NS	NS	NS	NS	NS	NS	NS	NS	NS	Epileptic seizures, mild dysarthria
0.25	2x/y	AU > HA	3	UL	Var	Pulsating	----	—	—
0.5	3-4x/y	AU > HA	48	UL	IL	Pulsating	++++	MO, MA	Mental retardation, mild dysarthria, nystagmus
Up to 72	1-3x/y	AU > HA	4-72	UL	NS	Pulsating	----	—	Obesity
3	2 in total	AU > HA	3	BL	NS	Pressing	+-+-	MO	—
3-4	1x/2 mo	AU > HA	6-24	UL	CL	Pulsating	+(+)++	—	White matter lesions
NA	NA	NA	NA	NA	NA	NA	NA	—	—
0.5-0.7	3 in total	AU = HA	12-24	UL	CL	Stabbing, pressing	++(++)	—	—
Max 480	<1x/y	NS	NS	NS	NS	NS	NS	—	—
0.3-0.5	1 in total	AU = HA	12 h to "days"	BL	NS	Pressing, stabbing	+-++	MA	—
4-5	4x/y	NS	"Hours"	UL	NS	NS	++++	—	—

co-segregate with FHM when assuming two cases of incomplete penetrance (see figure 1), and 3) they are not present on 600 control chromosomes. D718N (Family A), which affects an Mg²⁺ interaction site, has been functionally examined in a1a2, a1a1, and the homologous ATPase a2a1. In all three cases, this amino acid change has been shown to result in a complete loss of function due to lack of catalytic activity,⁹ which is in agreement with the loss of function hypothesis proposed to cause FHM2.⁴ Similarly, various mutations of the R763 residue (Family D) in a2a1 are either not expressed in the cell membrane at a significant level or completely nonfunctional.⁹ R763H has also

been described in a large US family¹⁰ and may therefore represent a frequent mutation. At present, the disease causality for the other variants cannot be considered as certain; however, for P979L (Family B),⁸ the co-segregation is strongly supportive, and therefore, for the phenotype discussion, FHM2 may be considered as confirmed in Families A, B, and D.

Clinical data of the carriers of all variants are presented in the table. In Families A, B, and D, the average ages at onset for HM were 8, 9, and 10 years. Not yet described in FHM2, angiography and exertion were reported to trigger HM, each for individuals of two families

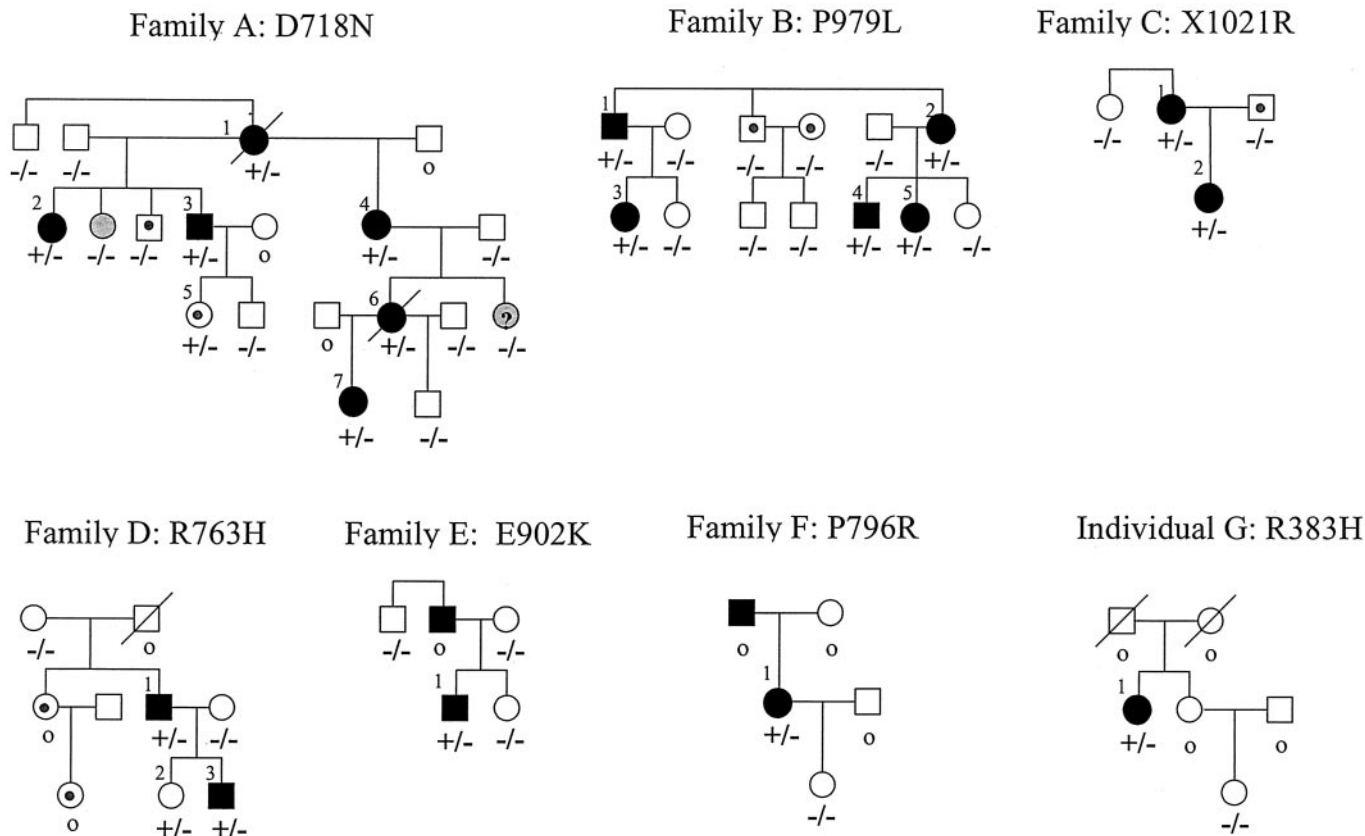


Figure 1. Pedigrees of the familial hemiplegic migraine (FHM) and sporadic HM cases in which *ATP1A2* mutations were identified. Squares = males; circles = females; shaded symbols = individuals with different types of migraine (black = FHM; gray = migraine with aura [MA]; dot = migraine without aura; white = unaffected). Mutation carriers are numbered for easy reference. The genotype is indicated by (+) for mutation carriers and by (–) for wild type. An (o) indicates that DNA was unavailable for testing. Note the two cases of incomplete penetrance, A5 and D2, both women ages 22 to 23. The individual with (?) in Family A was not available for the structured interview, and therefore the MA phenotype is not directly confirmed by the authors.

with mutations. Rare aura symptoms such as alien limb phenomenon, diplopia, apraxia, dysarthria, visual hallucinations, and vertigo were repeatedly found in FHM2 mutation carriers (see the table) and may indicate an especially widespread propagation of the cortical depression that produces these symptoms.

D718N seems to cause a higher frequency and longer duration of HM attacks than the other two mutations. For P979L, severe attacks may be accompanied by recurrent coma lasting 1 to 12 days, as in Individual B5. These two mutations may be associated with seizure susceptibility: One individual, A7 (D718N), had two generalized tonic-clonic seizures at ages 4 and 7 years; the other, B3 (P979L), had generalized tonic-clonic seizures without fever between ages 8 months and 2 years. Additionally, these two mutations may lead to mental retardation: A7 (D718N) showed delayed language development and cognitive impairment requiring education at a specialized institution, B5 (P979L) was determined to be mentally retarded at age 4 years,⁸ and A4 (D718N) exhibited an under-average performance on formal neuropsychological testing.

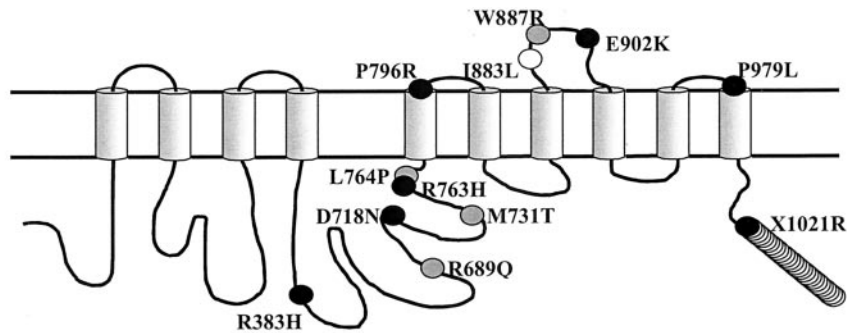
Should the other three variants, X1021R, E902K, and P796R, not be confirmed as causative mutations, they may exert a modulatory effect on the phenotype. This may be of interest when considering E902K, because the only carrier experienced a coma lasting 20 days with reversible unilat-

eral meningeal enhancement and cortical edema in the MRI similar to *CACNA1A* mutation carriers.³

When considering all of our FHM families—the 26 included in this study plus an additional 4 families with known *CACNA1A* mutations—the portion of *ATP1A2* mutations in our families is 3/30 = 10%. Depending on whether the other three variants we found are confirmed, it may be up to 20%. In contrast, *ATP1A2* variants in our sporadic HM cases was much lower (only 1/24 = 4%), suggesting that FHM2 and sporadic HM may have a different molecular basis. These values give only a crude estimation for the Central European population.

Previous authors have emphasized an incomplete penetrance of FHM2.⁷ In our *ATP1A2* mutation carriers, we found a penetrance of 13/15 = 87% for the presence of HM. If the other variants were confirmed as mutations, this value would not differ (18/20 = 90%), so that the penetrance in FHM2 is comparable with that reported for FHM1 of 89%.³

Our study has shown that FHM2 may have a similar clinical variability as FHM1. The FHM2 mutations are located in the intracellular ATP catalytic domain and the extracellular loops involved in β -subunit binding and ion conduction (see figure 2, top).⁹ Judging by their frequency, *ATP1A2* mutations may be a second major cause of FHM aside from *CACNA1A*.



	R383H	D718N	R763H/L764P	P796R	I883L	W887R	E902K	P979L	STOP
P50993 A1A2_HUMAN	TQNRMTV	GVNDSPA	EEGRLLIFD	ANIELPL	LLGIRLDWDDR	YGQEWTY	RMYPPLKV	TYY-	
P13637 A1A3_HUMAN	TQNRMTV	GVNDSPA	EEGRLLIFD	ANIELPL	LVGIRLNWDDR	YGQQWTY	RMYPPLKP	TYY-	
P05023 A1A1_HUMAN	TQNRMTV	GVNDSPA	EEGRLLIFD	ANIELPL	LLGLRVDWDDR	YGQQWTY	RMYPPLKP	TYY-	
Q13733 A1A4_HUMAN	TQNRMTV	GVNDSPA	EEGRLLIFD	LGIELPL	LLGIRLHWEDK	YGQQWTY	RMYPPLKI	TYY-	
P20648 ATHA_HUMAN	TQNRMTV	GVNDSPA	EEGRLLIFD	VSVELPL	CVGLRAQWEDH	YGQEWTF	NFMPIRF	TYY-	
P13607 ATNA_DROME	TQNRMTV	GVNDSPA	EEGRLLIFD	CDIELPL	LFGIRKMWDSK	YGQEWTY	RMYPPLKL	TYY-	
	*****	*****	* *****	****	* * *	*** **	*	****	

Figure 2. (Top) Structural model of the Na⁺/K⁺-ATPase. Known mutations (gray), novel variants (black), and the polymorphism (white) are marked. (Bottom) Multiple alignments of different members of the Na⁺/K⁺-ATPase family in several regions of the protein. Conserved residues are shaded black (if corresponding to a variant identified in our study), gray (if corresponding to a mutation previously described), or white (if corresponding to a polymorphism).

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