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ORIGINAL ARTICLE

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A G301R Na^+/K^+-ATPase mutation causes familial hemiplegic migraine type 2 with cerebellar signs

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Abstract Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with hemiparesis during the aura. In over 50% of cases the causative gene is *CACNA1A* (FHM1), which in some cases produces a phenotype with cerebellar signs, including ataxia and nystagmus. Recently, mutations in *ATP1A2* on chromosome 1q23 encoding a Na^+/K^+-ATPase subunit were identified in four families (FHM2). We now describe an FHM2 pedigree with a fifth *ATP1A2* mutation coding for a G301R substitution. The phenotype was particularly severe and included hemiplegic migraine, seizure, prolonged coma, elevated temperature, sensory deficit, and transient or permanent cerebellar signs, such as ataxia, nystagmus, and dysarthria. A mild crossed cerebellar diaschisis during an attack further supported the clinical evidence of a cerebellar deficit. This is the first report suggesting cerebellar involvement in FHM2. A possible role for *CACNA1A* in producing the phenotype in this family was excluded by linkage studies to the FHM1 locus. The study of this family suggests that the absence of cerebellar signs may not be a reliable indicator to clinically differentiate FHM2 from FHM1.

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Introduction

Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura. According to the International Headache Society, the aura is characterized by hemiparesis [1]. Variable degrees of impaired consciousness, such as drowsiness, confusion, and coma, have been described [2, 3, 4, 5, 6, 7]. The neurological deficits may be prolonged and outlast the associated migraine headache [8, 9, 10]. Permanent residual brain damage is rare [11] and death has occurred in only a few cases [12]. Typical migraine triggers such as food, odor, stress, exertion, head trauma, and cerebral angiography may provoke FHM attacks [13, 14, 15, 16, 17, 18]. The disease onset is in the 1st or 2nd decade of life and the attacks decrease in frequency with age [19].

Two variants can be discriminated genetically, FHM1 linked to chromosome 19p13, with mutations in the *CACNA1A* gene encoding the voltage-gated calcium channel subunit $Ca_v2.1$ [20], and FHM2 linked to chromosome 1q21–23, with mutations in the *ATP1A2* gene encoding a Na^+/K^+-ATPase subunit [21]. Cerebellar signs ranging from nystagmus to progressive ataxia occur in up to 50% of FHM1 families [16, 19, 22, 23]. More than half of the affected individuals develop cerebellar degeneration [19], especially of the vermis [24, 25]. Occasionally, focal seizures [6], in one case accompanied by mental retardation [26], have been described in FHM1. Transient cerebral edema during attacks [6, 26, 27] and hemispheric cerebral atrophy [6, 11, 26] have been reported rarely. In contrast, in FHM2 families, several cases with seizures have been reported [21, 28, 29, 30]. Confusion [17, 28], coma [31], aphasia [28], fever [17], and mental retardation [21, 29] have also been described. Generally, the penetrance of FHM2 is thought to be less than in FHM1 and cerebellar dysfunction and atrophy, frequent findings in FHM1, have not been detected in FHM2 [17, 28]. This

study describes an FHM2 family with a novel *ATP1A2* mutation that presents with signs of cerebellar involvement.

Materials and methods

Patients

The FHM family was of Italian origin and included 28 members who were clinically evaluated at least once by one of the authors. This included a neurological examination and semi-structured interview for patient history. Several of the 8 affected family individuals were repeatedly re-examined, including the proband, 732. The diagnosis of FHM was made according to the criteria of the International Headache Society [1], i.e., the affected individuals fulfilled the criteria for migraine and their aura included hemiparesis. Single-photon emission computerized tomography (SPECT) was performed with a Tomomatic 564 apparatus (Medimatic, Denmark) using Xenon-133 by inhalation.

All the procedures were in accordance with the Declaration of Helsinki and were approved by the local ethics committees of Ulm University and the Institute of Neurobiology and Molecular Medicine, Rome. Blood was taken from affected and unaffected family individuals after informed consent.

Linkage analysis

Linkage analysis of the FHM1 region on chromosome 19p13 and the FHM2 region on chromosome 1q23, including the migraine susceptibility locus on chromosome 1q31 [32], was performed using D19S221, D19S226, and D1S2707, D1S1679, D1S403, D1S518, D1S249, D1S2782, D1S180, respectively [33] (Genome Database http://genome.ucsc.edu). DNA typing was performed by PCR amplification using 20 ng of genomic DNA in a final volume of 10 μl containing a mixture of dNTPs (200 μM dATP, dTTP, and dCTP and 2.5 μM dGTP), 100 ng of each primer, 1.5 mM $MgCl_2$, and 0.25 U Taq polymerase. PCR was performed under standard conditions (denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, annealing at 55° for 30 s, 72° for 30 s, and final extension at 72° for 5 min). After addition of 4 μl of loading buffer, 4 μl of each sample was denatured and resolved on a 6% denaturing polyacrylamide gel. Fragments were either visualized by autoradiography if the PCR contained 2 μCi (α-thio) dGTP (^{35}S) or by automated detection of Cy5-marked primers using the ALF sequencer from Pharmacia Biotech. Two- and three-point LOD scores were calculated with the MLINK and LINKMAP programs of the LINKAGE package [34] (http://www.hgmp.mrc.ac.uk) using uniform allele frequencies, a prevalence of 0.1% reflecting worst case (higher phenocopy rate) and a penetrance comparable to FHM1 of 90%.

Mutation screening

Mutation screening was performed by PCR amplification and direct sequencing (primers reported in Table 1). PCR products were loaded on a 2% agarose gel, stained with ethidium bromide, and the band cut out under UV light. Bands were purified using the Amersham Pharmacia kit, and cycle-sequenced with 1 pmol primer using the dye terminator kit (Applied Biosystems). Sequencing was performed on 6% denaturing polyacrylamide gels in an ABI 377 HT automated sequencer. All sequences with base exchanges were verified by reverse sequencing of a new PCR product of the same DNA sample.

After having identified the G901A base exchange coding for G301R, DNA from 100 Italian and 79 non-Italian Central European random subjects was screened for the putative mutation in exon 8 of the *ATP1A2* gene. DNA was amplified with the primers CGGACACCCATAGCAATGG and CGATGAGGAAGATGACTGCC, which produced a 136-bp fragment. PCR products (15 μl) were incubated for 16 h at 37°C with the restriction enzyme *Mnl*I. The digestion produced three DNA fragments of 93, 25, and 18 bp for

Table 1 *ATP1A2* primer sequences

ATP1A2 exons	Forward and reverse primers	Length (bp)	Tm (°C)
1	GGAGAGGGGGAGAAGGACC CTTCATCCTTCCTCAGCAGC	231	55
2+3	CTGGCTGAGTGGTGGGAATG TGGATTACAGTTTTAAGGCTCCTGC	503	55
4	AAGGGATGGGCATGGTGAC CCCTTTCCAGCCTAAGATGCA	414	55
5+6	GATGGCACTGCCTGCTCAT TGTTGACATTTGGACCTGGGT	689	55
7	TTCCTCGTGTCATGAAGATTGAG TTGCCAGGTAAGTACTACCAGAGC	314	55
8	TCCCTGGGAGCCACAAGG AGCAACTGTGCCTCTATCACTCTGAG	394	55
9+10+11	GCCACGGTCTAGGGTAAGGTT TGATTCCCCTTGGCTTTGTC	901	55
12+13	CTGCTCTATGCCGCGCTAC CTGCAGCTCCTTGAACTCTGG	624	55
14	CACATGCCCTTATTTCGGTTG AATGCTATCCAAATACTCCCAGG	365	55
15+16	GGGCTGGTACAGGTGCCA ACAGGGAACAGAGGTGCCG	575	55
17+18	CACTTTAGCTTCCCTTGAACACAA TGTCCTGGAATTTTGCTTGGG	746	55
19+20	TGTGCCCCTTCTGCTTCC ACAGCTCTGGTCCAGGGCT	619	55
21+22	TCTTTCCCTTGCACCCCAG GGAGTCTGCGTGTGGTCCTG	508	55
23	CCCTTCACCAGGCTTCTCC CCATGCCTGGTTCTTCTTCC	171	55

the wild type DNA and four fragments of 69, 26, 25, and 18 bp for the mutant alleles. Digested fragments were separated on 3% agarose gels (Sigma) and stained with ethidium bromide.

Results

Patients: case reports and clinical examination

The FHM family has 8 clearly affected individuals in three generations (Fig. 1). In addition, there is one obligate carrier, 737. She is a 57-year-old woman who has repeatedly denied any form of headache and hemiparesis. However, her daughter, 850, has recurrent hemiplegic aura with confusional state and aphasia, which is occasionally followed by a mild migraine headache and is always followed by a terminal sleeping phase. Two individuals, 863 and 864, for whom the diagnosis of FHM could not be made at the time of the first visit when they were in their teens, refused further contact and could not be classified.

The proband, 732, is a 28-year-old woman suffering from migraine with visual aura since the age of 8–9 years. At 12 years, she experienced a tonic-clonic seizure followed by elevated temperature (39.2°C), a coma lasting 5 days, and, subsequently, a right-side hemiparesis and a global aphasia that resolved completely in 25 days. Laboratory findings were normal and cerebrospinal fluid showed a normal protein level and cell count. During the coma, the computed tomographic (CT) scan showed a diffuse swelling of the brain and electroencephalography (EEG) displayed a diffuse slow delta and theta activity that turned normal in 2 weeks. At 13 years of age, she presented with a similar attack, beginning with a seizure followed by coma and subsequent left-side hemiparesis, hemihypoaesthesia, and severe right frontal migraine with nausea and vomiting. Five months later she was admitted to the emergency room because of a new tonic-clonic seizure followed by psychomotor agitation with incoherent speech. After the admission she lost consciousness, became comatose, and developed an elevated temperature of 38.4°C. Her temperature normalized within a few hours while the coma state persisted for a week. Upon awakening from the coma, she presented with severe frontal headache associated with left hemiparesis and cerebellar deficit, including horizontal nystagmus, dysarthria, dysmetria, gait ataxia, and intentional tremor. A SPECT image (Fig. 2) showed a reduction of blood perfusion on the right frontal and on the left cerebellar hemisphere (crossed cerebellar diaschisis). During the subsequent 8 years, she had at least four episodes with the same features, the last of which occurred 4 years ago. Since the second attack, she has been taking 100 mg phenobarbital and 800 mg carbamazepine daily. Additionally, every 2–3 months, she has a migraine attack preceded by visual aura and/or transient hemiparesis and hemihypoaesthesia. Aphasia may also be present if the right side is involved. During these uncomplicated attacks, standard analgesic drugs relieve the pain. The interictal neurological examination performed on several occasions failed to show any abnormality.

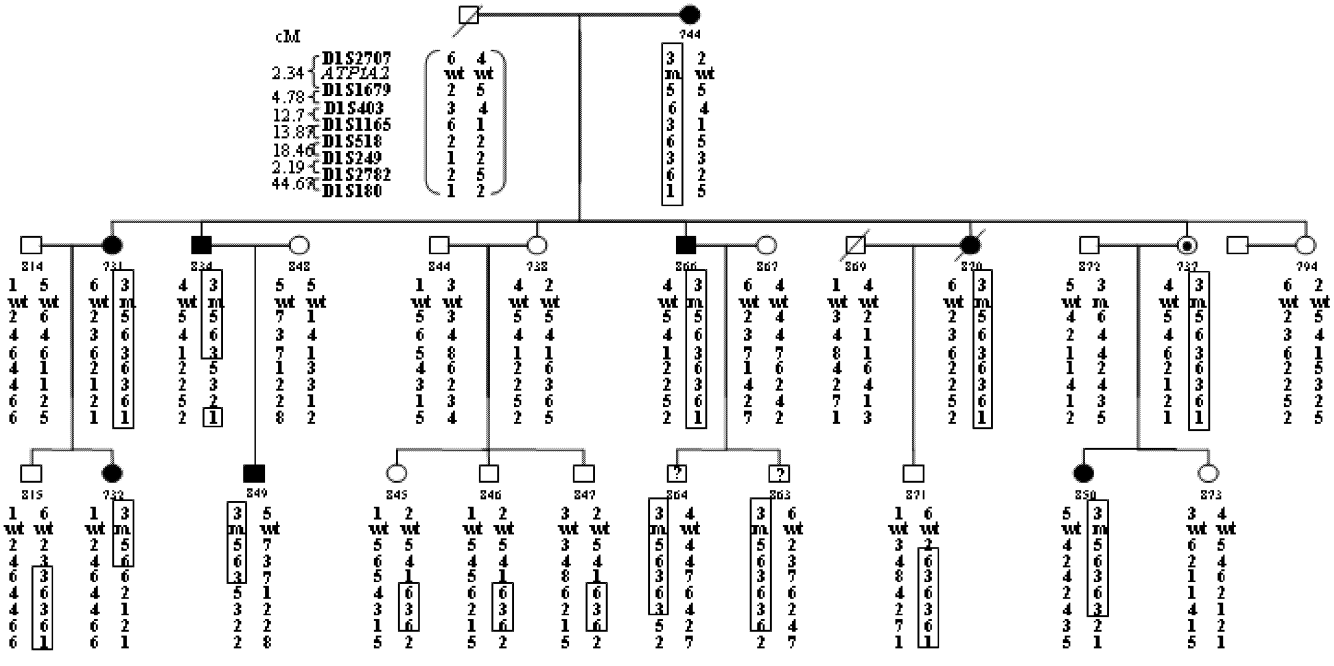

Fig. 1 Chromosome 1q haplotypes and inheritance of *ATP1A2* G901A. In the pedigree of the FHM2 family, *squares* stand for males, *circles* for females, *crossed out* symbols for deceased individuals, *shaded symbols* for subjects with FHM, and the *partially shaded symbol* for the obligate carrier 737. Individuals 864 and 863 were teenagers at the time of the first examination but refused further contact and were therefore classified as unknown with respect to FHM (indicated by a *?* sign). The haplotypes for the indicated chromosome 1q markers and the mode of inheritance of the G901A base change are given

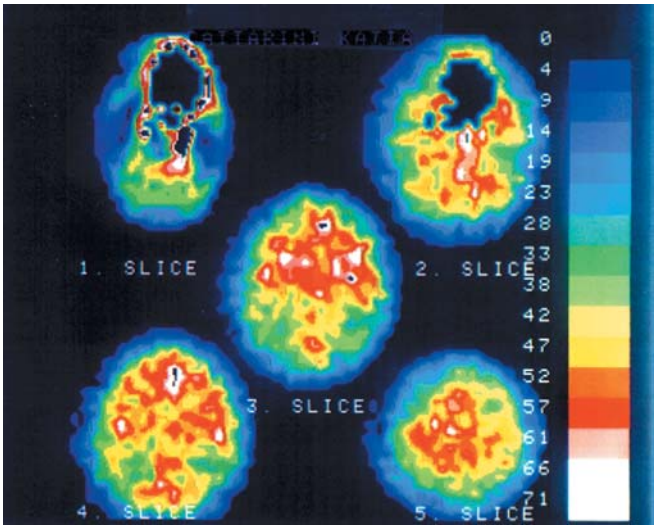

Fig. 2 Single-photon emission computerized tomography image of patient 732. The image was taken during an attack. Crossed cerebellar diaschisis with hypoperfusion of the left cerebellar hemisphere (*slice 1*) and of the right frontal area (*slice 5*) is shown (courtesy of Dr. Patrizia Pantano)

The proband's mother, 731, a 53-year-old woman, had migraine attacks with visual aura associated with nausea, vomiting, and photophobia from the age of 23 years. These episodes had a frequency of one attack every 10–15 days and a duration of 3–24 h or more. From the age of 25 years, the migraine attacks were preceded by right or left hemiparesis and/or hemihypoaesthesia with aphasia, if the right side was involved. Then a confusional state arose, followed by severe migraine with nausea, vomiting, and dizziness persisting for 4–48 h. Neurological symptoms sometimes outlasted the pain. Several attacks were heralded by tonic-clonic seizures and associated with an elevated temperature of up to 40°C, drowsiness, and torpor, and required admission to hospital. On these occasions, the erythrocyte sedimentation rate and leukocytes were normal, blood and cerebrospinal fluid culture provided negative results, and no other signs of bacterial or viral infection were present. CT and magnetic resonance imaging (MRI) scans were always normal, except on one occasion when a diffuse edema was present and cerebellar signs were reported. In the acute phase, EEG showed a slow delta, theta activity and spikes contralateral to the hemiparesis. The interictal neurological examinations were always in the normal range. Treatment with 100 mg phenobarbital daily decreased the frequency of complicated hemiplegic migraine attacks to one every 3 years, but did not decrease the rate of "normal" attacks. Acetazolamide was not effective in decreasing the attack frequency.

The proband's maternal grandmother, 744, is an 80-year-old woman. She had migraine attacks since her youth, although she could not tell the age of onset exactly. When she was 49 years old, episodes began with gait unsteadiness and occasional falls, which were sometimes followed by migraine attacks with nausea and vomiting.

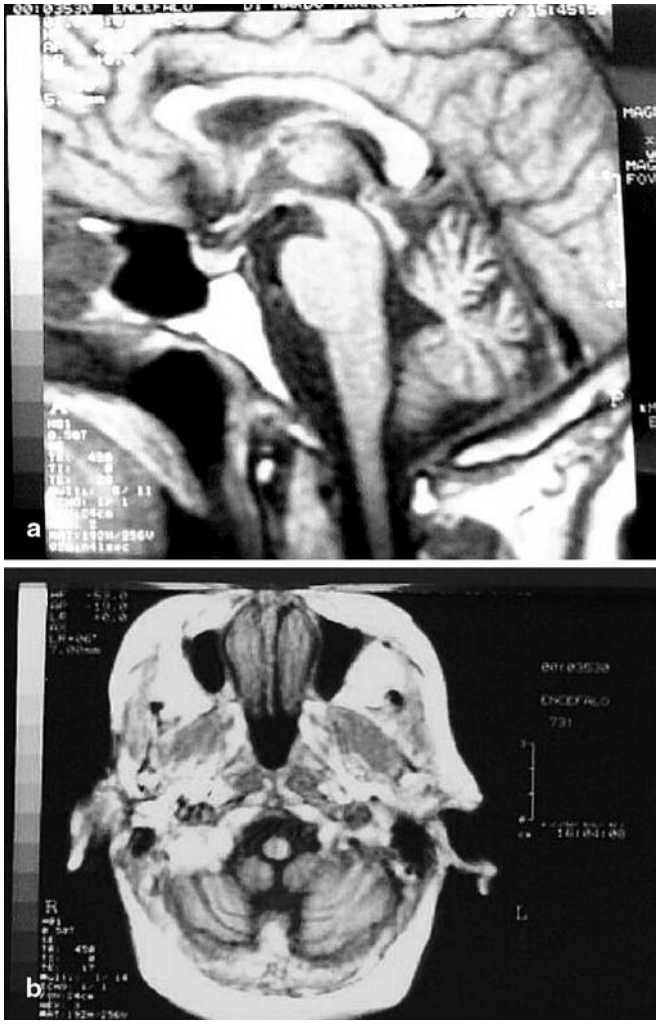

Fig. 3 Magnetic resonance imaging of patient 744. T1-weighted images on sagittal (**a**) and axial (**b**) planes showing cerebellar hemisphere and vermis atrophy

At the age of 52 years, the attacks had a yearly frequency and were heralded by partial motor seizures followed by hemiparesis, hemihypoaesthesia of the limb involved in the seizure, migraine contralateral to paresis, aphasia when right hemiparesis occurred, nausea, vomiting, confusion, and dizziness. The neurological anomalies persisted from a few minutes to 36 h and then completely remitted. Migraine lasted from several days to a week. Some episodes of long-lasting hemiparesis required admission to hospital. CT scan and Doppler ultrasonography, performed at the time of more than one admission before her 65th year, were normal. In contrast, EEG in the acute phase showed slow rhythm and sporadic spikes on the hemisphere contralateral to the paresis. She always refused any drug treatment. The last neurological examination several years ago showed interictal horizontal and vertical nystagmus, mild gait ataxia, and intentional tremor in the finger-nose test. An MRI scan, performed when she was 72 years, showed a marked cerebellar atrophy (Fig. 3). Her relatives recently reported that, since

Table 2 Clinical features of the 8 affected family members (*MRI* magnetic resonance imaging, *EEG* electroencephalography, *CT* computed tomography, *SPECT* single-photon emission tomography, CSF cerebrospinal fluid, *NT* not tested, *CCD* crossed cerebellar diaschisis)

Individual	744	731	834	866	870	732	849	850
Age at last examination	72	52	43	42	50	28	10	30
Age at death (years)	–	–	–	–	53[a]	–	–	–
Onset of migraine (years)	Youth	23	29	21	30	8–9	6	?
Onset of hemiplegic aura (years)	52	25	29	21	34	12	6	26
Hemiplegic attacks								
Frequency	1–2/year	5–10/year	2/year	1–2/year	3–4/year	4–5/year	1/month	1/year
Duration of neurological signs	Minutes to days	4–48 h	Days	Days	Days	Hours to weeks	Days	10–30 min
Duration of headache	Days to week	Hours to days	Days	Days	Days	Days to weeks	Days	Minutes
Visual aura	-	+	-	-	-	+	+	+
Hemiparesis	+	+	+	+	+	+	+	+
Unilateral sensory signs	+	+	+	+	+	+	+	+
Dysphasia/aphasia	+	+	+	+	+	+	+	+
Nausea/vomiting	+	+	+	+	+	+	+	+
Coma/torpor	+	+	+	+	-	+	+	-
Seizure	Motor partial	Grand mal	Grand mal	Grand mal	-	Grand mal	-	-
EEG	Slow and spikes	Slow	Slow	Slow and spikes	Normal	Slow and spikes	Slow	NT
Elevated temperature	-	+	+	+	-	+	+	-
Cerebellar deficit	+	+	-	-	-	+	-	-
Confusional state	+	+	-	+	+	+	+	+
Brain MRI/CT scan	Normal	Edema	Normal	Normal	NT	Edema	Normal	NT
SPECT	NT	NT	NT	NT	NT	CCD	NT	NT
CSF	NT	Normal	Normal	Normal	NT	Normal	NT	NT
Interictal signs								
Gait ataxia	+	-	-	-	-	-	-	-
Dysmetria	+	-	-	-	-	-	-	-
Tremor	+	-	-	-	-	-	-	-
Nystagmus	+	-	-	-	-	-	-	-
MRI	Cerebellar atrophy	Normal	NT	NT	Normal	-	NT	Normal

[a] Deceased due to an intestinal neoplasia

Table 3 Pairwise LOD scores between the disease and migraine loci markers

Locus	cM	Marker	0.00	0.05	0.10	0.20	0.30	0.40
FHM1 (chromosome 19p13)	0	D19S221	$-\infty$	−3.48	−2.24	−1.05	−0.47	−0.15
	6	D19S226	$-\infty$	−2.89	−1.96	−1.01	−0.51	−0.20
FHM2 (chromosome 1q23)	0	D1S2707	3.6	3.3	3.0	2.3	1.6	0.7
		ATP1A2-G901A	3.6	3.3	3.0	2.3	1.6	0.7
	2.3	D1S1679	0.9	0.8	0.8	0.6	0.4	0.2
	7.1	D1S403	1.9	2.2	2.1	1.8	1.2	0.6
	19.8	D1S1165	−6.5	−0.3	3.3	0.6	0.5	0.2
FHM chromosome 1q31	33.7	D1S518	−14.4	−2.8	−1.6	−0.6	−0.2	0.0
(Gardner et al. [17])	52.2	D1S249	−8.3	−2.2	−1.5	−0.7	−0.4	−0.1
Migraine chromosome 1q31	54.4	D1S2782	−13.5	−3.1	−1.9	−0.8	−0.3	−0.1
(Lea et al. [32])	99.1	D1S180	−6.0	−0.2	0.1	0.4	0.3	0.1

the last neurological examination, she has continued to have episodes of ataxia intermingled with complicated migraine attacks. Her unsteadiness has progressively worsened, making her currently unable to walk. Screening for spinocerebellar ataxias (SCA) type 1, 2, 3, 6, 8, 10, and 14 (exon 4) mutations showed wild type alleles, excluding the presence of an alternative form of progressive ataxia in the patient.

An overview of the features of the 8 clinically affected family members is summarized in Table 2 (obligate carrier 737 is not included because unaffected). For all 8 individuals, the age at onset of migraine was less than 30 years, but in most non-hemipegic migraine began first, followed after a variable period of time by attacks with various neurological symptoms. Patients were not able to indicate particular stimuli triggering the attacks, except in one case, 849, in which an attack was precipitated by a minor head trauma. In 6 patients, neurological involvement was severe, including prolonged alterations of consciousness and elevated temperature, which abruptly initiated and ended with the attacks. In 5 of 8 patients, the attacks were heralded by seizures with EEG anomalies that promptly remitted after the acute phase. In 3 patients, proband 732, her mother 731, and her grandmother 744, paroxysmal cerebellar signs were present either during migraine attacks or as isolated episodes. Neuroimaging in the ictal phase was usually normal, except on a few occasions in which diffuse cerebral edema and a crossed cerebral-cerebellar diaschisis were documented. Interictally, with the exception of 744 showing the cerebellar signs described above, all patients were completely normal. None of the patients had hypertension or other risk factors for vascular brain disorders, supporting the idea that the observed cerebellar signs could be associated with the *ATP1A2* mutation described below.

Linkage analysis

For the LOD score calculation, the phenotype of subjects 863 and 864 who were seen only once when they were in their teens was considered to be unknown. Individual 737 was considered as an obligate carrier. Pair-wise analysis between the disease and D19S221 and D19S226 markers, defining a 6-cM interval that included the FHM1 *CACNA1A* locus, showed highly negative LOD scores (Table 3). A three-point linkage analysis with the clinical phenotype against the two markers yielded LOD scores within the 6-cM interval below −6, excluding linkage between the disease and the FHM1 region. The haplotype of the individuals showing cerebellar signs, 744, 731, and 732, were also present in several affected and unaffected family members without cerebellar signs (834, 870, 871, 737, and 794). This makes an involvement of *CACNA1A* in generating cerebellar symptoms highly unlikely. In contrast, linkage analysis for the FHM2 locus on chromosome 1q23 yielded significant positive LOD scores for D1S2707 and a G901A base change in *ATP1A2* (Table 3). The LOD scores remained significant (>3.0) even when reducing the penetrance down to 65%, a value much below the assumed penetrance, indicating the linkage to be reliable. In more-telomeric regions of chromosome 1q encompassing the more-distal FHM locus region [17] and a general migraine susceptibility locus [32], at least 2 affected individuals were recombinant in every marker of the telomeric region (732 and either 849 or 850) in addition to several unaffected individuals (Fig. 1). This does not support the involvement of a second locus on chromosome 1q in generating either the migraine phenotype of our family or the cerebellar signs.

Mutation screening

Direct sequencing of the 23 *ATP1A2* exons in sample 744 yielded three base exchanges coding for amino acid substitutions: C244A coding for P82T, G901A coding for G301R, and T2774A coding for V925E. Of these three changes, only the G901A change was inherited with the phenotype, while the other two were both on the unaffected allele without recombinations (Fig. 1). The pattern of inheritance suggests at least 1 case of non-penetrance is present: the obligate carrier 737. Additionally, the 2 unclassified individuals, 863 and 864, who did not co-operate with further investigation may represent 2 cases of incomplete or non-penetrance, but based on their teenage age at first examination, we cannot completely exclude that they may have developed migraine in the mean time. In spite of this, G901A co-segregated with FHM in the family, generating two-point LOD scores >3 for a range

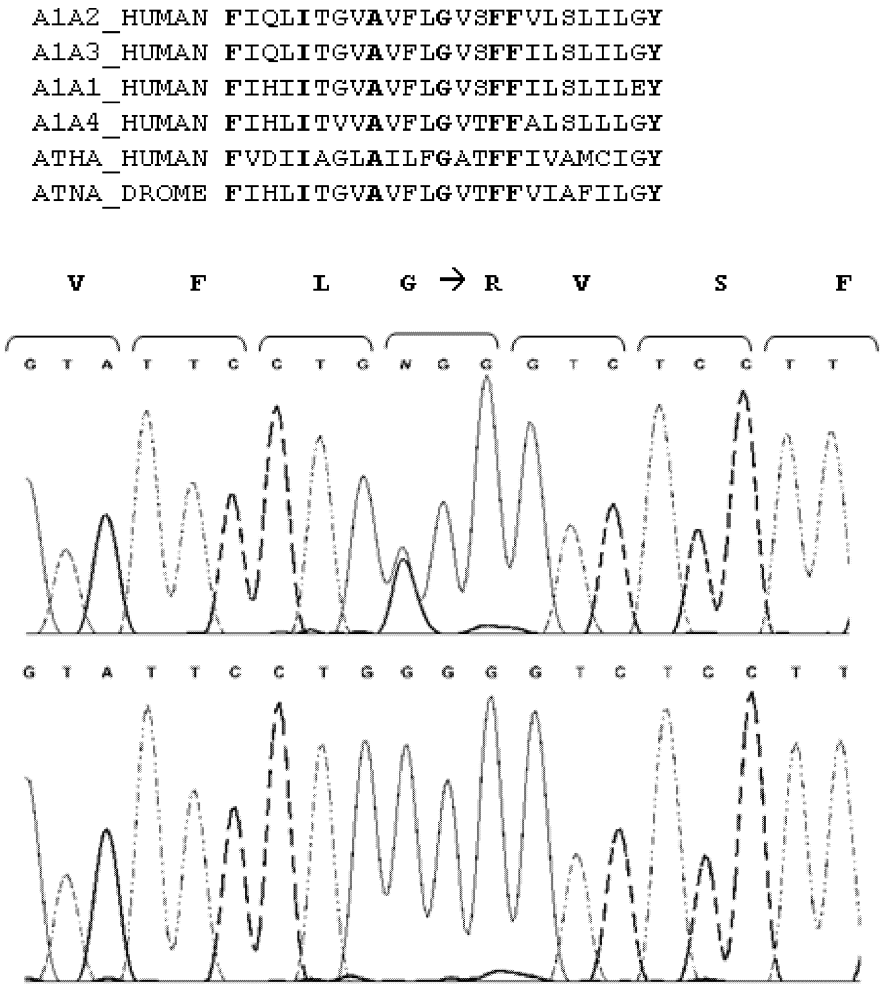

Fig. 4 *Top*: an amino acid alignment of several members of the human Na^+/K^+-ATPase family (A1A1-A1A4), a human H^+/K^+-ATPase (ATHA), and the sequence of the *Drosophila* ATPase (ATNA) of the area surrounding glycine301 is given. Identical residues are *bold*, G301 is *boxed*. *Bottom*: sequencing results of the sample 744 and a control in the area around the base G901 are indicated. The patient sample shows a heterozygous G to A base exchange of G901

of theta from 0 to 10 cM (Table 3). Additionally, protein alignments revealed that glycine301 is highly conserved within the family of Na^+/K^+-ATPases (Fig. 4) and our restriction analysis suggested that it is not present in 100 Italian and 79 Central European unrelated controls without migraine. The residue is part of a nine-amino acid stretch in transmembrane segment M3 of the protein that has been shown to be important for the dephosphorylation of homologous ATPases [35]. Therefore, several criteria supportive of disease causality are fulfilled for the G901A base change coding for G301R: conservation of the residue, co-segregation with the phenotype, absence in a large control sample of matched ethnic origin, and localization in a region of putative importance for protein function.

Discussion

According to our LOD score calculations, the present family shows a significant linkage between FHM2 markers and the disease phenotype. Additionally, we have identified a G901A base change in *ATP1A2* coding for G301R in the Na^+/K^+-ATPase that fulfills several criteria for a disease-causing mutation. Therefore, we categorize this family as having FHM2. As such, it shows unusual clinical features not previously observed in FHM2. For example, 5 of 8 patients presented with two different patterns of hemiplegic migraine attacks, either simple or complicated. While the simple ones were typical migraine attacks with a preceding hemiplegic aura of short duration, the complicated attacks were characterized by seizures, elevated temperature, and various degrees of disturbance of consciousness. Partial or generalized seizures were not observed in any of these 5 patients outside of hemiplegic migraine attacks, which does not support a general epileptic susceptibility in the family. This is in contrast to previous reports of 12 subjects from two FHM2 families who had a history of seizures in their childhood without any relation to the hemiplegic migraine attacks [29, 30]. The recurrence of seizures during complicated attacks in the present family is also in contrast to a report of two individuals of an FHM2 family who experienced only one seizure during the first hemiplegic migraine attack and no more since [28]. Focal seizures were also reported in one FHM1 family, but only as a sporadic event during the course of a very severe episode [6] or in the first attack of a child from a genetically unclassified FHM family [36].

Another unusual feature of our family is the recurrent prolonged coma in 5 of the 6 patients with consciousness

abnormalities during the attacks. Although confusion and drowsiness have frequently been reported both in FHM1 and FHM2, coma occurring repeatedly has only been described in one patient [31]. Additionally, the lack of mental retardation is remarkable, as it has already been reported in several patients with FHM2 [21, 29].

In most patients, neuroimaging did not show indications of permanent brain lesions such as cerebellar atrophy, which is frequently seen in FHM1. However, an MRI scan of subject 744 with progressive ataxia clearly showed cerebellar hemisphere and vermis atrophy. During the attacks, transient changes could be found. Two subjects, 372 and 371, showed diffuse or unilateral cortical swelling and edema lasting several days or weeks, with complete subsequent remission. Similar findings have previously been reported in FHM1 patients [7, 26, 27], but not yet in FHM2. SPECT performed during an attack in patient 732 showed a crossed cerebellar diaschisis, which fits well with the observed clinical cerebellar signs. Additionally, it indicates that the perfusion deficits do not correspond to a single vascular territory supporting a neurogenic theory of pathogenesis of hemiplegic migraine rather than a vascular one. Therefore, the perfusion changes may be considered to be a secondary phenomenon, possibly due to a cytotoxic edema, as recently demonstrated by serial MRI with diffusion-weighted imaging [27]. Hypoperfusion of the affected cerebral hemisphere and crossed cerebellar diaschisis have been previously reported in a genetically unexamined child with probable FHM [36], however, a reduced flow confined to the affected cerebral hemisphere has only been documented in one FHM1 patient during a prolonged aura [10].

To date, the total absence of cerebellar involvement in FHM2 has been considered a decisive clinical feature distinguishing it from FHM1 [28]. However, 3 of our patients showed cerebellar symptoms such as nystagmus, gait ataxia, dysmetria, and dysarthria, either as part of the migraine attack (individuals 731 and 732) or as a permanent feature with progressive cerebellar atrophy (744). On closer scrutiny, signs indicative of cerebellar involvement have previously been reported in FHM2 patients in the form of interictal saccadic pursuit in 4 patients [17] or of cerebellar dysarthria with or without gaze-evoked nystagmus in 3 patients [37]. These signs, however, were not interpreted as belonging to FHM2 and other reasons for their presence were discussed, for example, alcohol or tranquilizer intake [37]. In our family, no other reasons for the cerebellar signs such as alcohol, drugs, *CACNA1A* or SCA mutations, or vascular brain disorders could be found, suggesting that these signs are part of the FHM2 phenotype in this family. Therefore, the presence of cerebellar involvement in general may not be a reliable criterion to distinguish FHM1 from FHM2, even if its frequency appears to be far lower in the former than the latter.

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