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SHORT COMMUNICATION

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A novel missense *ATP1A2* mutation in a Finnish family with familial hemiplegic migraine type 2

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Abstract Familial hemiplegic migraine (FHM), a rare autosomal dominant subtype of migraine with aura, has been linked to two chromosomal loci, 19p13 and 1q23. Mutations in the Na^+,K^+-ATPase $\alpha 2$ subunit gene, *ATP1A2*, on 1q23 have recently been shown to cause familial hemiplegic migraine type 2 (FHM2). We sequenced the coding regions of this gene in a Finnish chromosome 1q23-linked FHM family with associated symptoms such as coma and identified a novel A1033G mutation in exon 9. This mutation results in a threonine-to-alanine substitution at codon 345. This residue is located in a highly conserved N-terminal region of the M4–5 loop of the Na^+,K^+-ATPase. Furthermore, the T345A mutation co-segregated with the disorder in our family and was not present in 132 healthy Finnish control individuals. For these reasons it is most likely the FHM-causing mutation in this family.

Keywords Familial hemiplegic migraine · Linkage · DNA sequence analysis · Na^+-K^+-exchanging ATPase · Missense mutation

Introduction

Familial hemiplegic migraine (FHM) is a genetically heterogeneous, rare autosomal dominant form of migraine characterized by aura including some degree of hemiparesis [1]. In severe cases, associated clinical features

such as progressive cerebellar ataxia, coma, fever, and/or epileptic seizures have been reported [2]. Two chromosomal loci, 19p13 (*FHM1*) and 1q21–23 (*FHM2*), have been linked to this disease [2, 3]. Missense mutations in two genes, *CACNA1A* encoding the α1 subunit of the voltage-gated $Ca_v2.1$ (P/Q type) calcium channel and *ATP1A2* encoding the α2 subunit of Na^+,K^+-ATPase, have been described [4, 5]. Only nine FHM2 families with linkage to 1q21–23 [2, 6, 7, 8, 9] and four *ATP1A2* mutations [5, 9] have been published to date.

The function of the Na^+,K^+-ATPase is to transport Na^+ ions out and K^+ ions into the cell. It is an electrogenic E1-E2 type ion pump that generates the ion gradients that maintain cell volume and provide the driving force for nutrient and neurotransmitter uptake as well as the propagated action potential [10, 11]. Na^+,K^+-ATPase is usually composed of two subunits, a catalytic α subunit and a modulatory β subunit. Four different α subunits have been identified and are expressed in a tissue- and development-specific manner [10]. The α1 isoform is ubiquitously expressed, unlike the α2 isoform that is mainly expressed in skeletal muscle, heart, and brain, especially astrocytes [12]. In mouse central nervous system the α2 isoform is widely expressed in neurons throughout the brain at the time of birth [12]. In the present study, we screened the *ATP1A2* gene for mutations in a previously undescribed chromosome 1q23-linked Finnish FHM family.

Subjects and methods

Family details

Clinical data were available on 28 family members, including 5 spouses, all of whom gave their informed consent to the study. Diagnoses of all individuals were made according to the IHS criteria [1]; 15 subjects (including all those reporting FHM attacks) were clinically examined and the remaining 13 subjects were interviewed by phone. Altogether 11 family members were diagnosed with FHM (Fig. 1). Three subjects had migraine without aura (MO) and one subject non-migrainous headache; 27 family members donated a blood sample for DNA studies. The ethics committee of the Helsinki University Central Hospital approved the study protocol.

Linkage analyses

Linkage of the disease to the 19p13 *CACNA1A* locus was tested using microsatellite markers D19S221, D19S1150, and D19S226 and to the 1q21–23 area using markers D1S514, D1S2343, D1S2346, D1S305, D1S2635, D1S2707, D1S2705, D1S1679, D1S2768, and D1S2844. Genotyping was performed using the ALFexpress sequencer (Amersham Biosciences, Uppsala, Sweden) as previously described [13]. Two-point linkage analyses were carried out by the LINKAGE program [14] assuming autosomal dominant inheritance, an incomplete penetrance of 0.8, a disease gene frequency of 0.0001, and no phenocopies. Subjects with MO or headache other than migraine were considered unaffected. Clinically unaffected family members under the age of 20 years were classified as unknown.

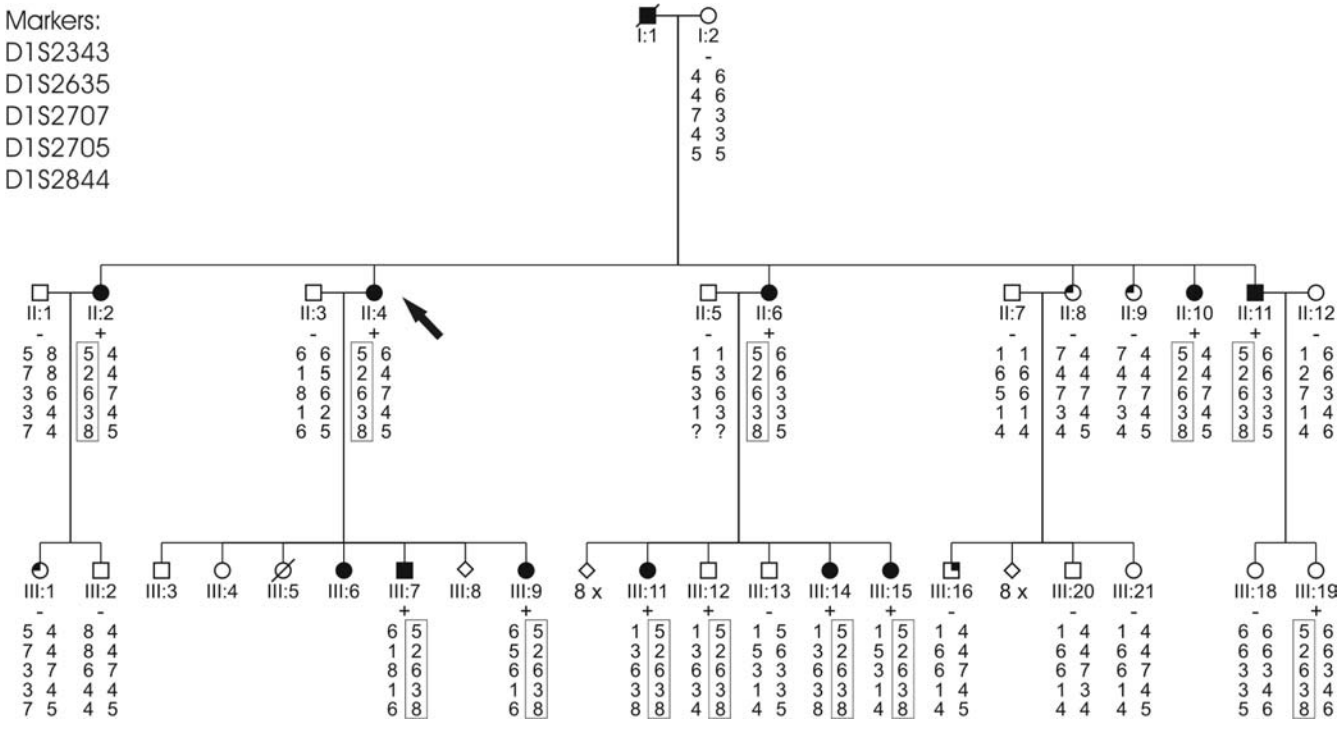

Fig. 1 Pedigree of the FHM2 family. The *arrow* indicates the proband. Affected individuals are represented by *blackened symbols* and individuals with migraine without aura or non-migrainous headache by symbols with the *upper-left or upper-right quarter in black*, respectively. Genotypes for 5 microsatellite markers from chromosome 1q22–24 are shown and the disease haplotype is *boxed*. The T345A mutation co-segregated with all affected family members. Presence of mutation is indicated by + sign and the absence of mutation by –

Mutation screening

All 23 exons of the *ATP1A2* gene were amplified by PCR using 21 sets of primers in the proband (II-4). Primer sequences are available upon request. PCR products were analyzed by cycle sequencing using the BigDye Terminator Ready Reaction Kit (Applied Biosystems, Foster City, Calif., USA) and an ABI3700 sequencer (Applied Biosystems). All PCR products were sequenced completely in both forward and reverse directions. GenBank contig NT_079484 was used as a reference sequence (http://www.ncbi.nlm.nih.gov/Genbank/index.html).

Minisequencing was used to demonstrate the segregation of the identified mutation within the family and to exclude the presence of the mutation in 132 unrelated healthy voluntary blood donors of Finnish origin. The procedure is described in detail elsewhere [15]. The area of interest in exon 9 was amplified by PCR using biotin-labeled intronic F-primer 5′-GCCACGGTCTAGGGTAAGG-3′ and exonic R-primer 5′-TCATGGATTTGGTTGTCGA-3′. The biotinylated DNA template was immobilized in a streptavidin-coated microwell and a detection primer 5′-CGTGCCATGCGC-TTGGCTG-3′ elongated with a single ^{3}H-labeled nucleotide, either dTTP (wild type) or dCTP (mutated sequence).

Determining allele frequencies for the identified *ATP1A2* polymorphisms

Allele frequencies in pooled DNA samples were estimated using the Homogenous MassExtend MassARRAY system and the Allelotyping software (Sequenom, San Diego, Calif., USA). Previous studies have shown that this method can be used to accurately estimate single nucleotide polymorphism (SNP) allele frequencies in pooled DNA samples [16, 17]. For the allele frequency determination we used a DNA pool constructed using equimolar amounts of DNA from 132 anonymous Finnish individuals and a Coriell Cell Repositories human variation DNA pool NA16129 consisting of 24 individuals belonging to different Asian, American Indian, and Pygmy populations. To obtain a more-accurate estimate of absolute allele frequencies, we also genotyped 15 individual DNA samples.

Assays were designed using the AssayDesign 2.0 software (Sequenom) and assay information is available from the authors upon request. The PCR and primer extension reactions were performed under standard conditions according to the manufacturer's instructions. For each assay, we performed 12 replicate PCR and extension reactions on the Finnish DNA pool samples and 4 replicates on the Coriell NA16129 DNA pool sample.

Mass spectra were analyzed and peak areas obtained using the MassARRAY Typer software (Sequenom). We included in the analysis only allele frequency data that was successfully collected from at least 4 of the 5 available raster positions. We used the ratio of the peak areas of the two alleles (k) obtained for heterozygote individuals to correct for possible skew in allele frequency estimation in the DNA pools. The formula used for the allele frequency estimate was p=(A/(A+kB)) where A and B are the peak areas of the two alleles in the pooled DNA sample [16]. For SNP IVS12–11C>G data on heterozygous individuals were not available and thus the frequency could not be adjusted.

Results

Clinical features

All 11 FHM patients showed hemisensoric aura with mild-to-moderate hemiparesis mostly affecting the upper extremity. Interictal neurological examinations were normal and thus all 11 patients fulfilled the clinical criteria of pure FHM. Migraine attacks usually started with gradually spreading hemisensoric aura. Hemiplegic aura was always accompanied by hemiparesthesias, dysarthria, or dysphasia, and often by visual symptoms. Of the 11 affected family members, 10 reported onset before the age of 15 years (range 5–31 years). The frequency of attacks varied from twice a month to once a year, and 2 individuals reported ceasing of attacks during their teens. Minor head trauma triggered attacks in 5 of the 11 patients and severe vomiting could last for days in 4 patients. Confusion and mild anxiety were common features during attacks in 5 subjects. In 4 of these patients a mild head trauma had triggered coma accompanied by fever that lasted from 2 days to 2 weeks. Hemiparetic symptoms could persist for as long as 2 weeks in some individuals. None of the patients had seizures.

Linkage and haplotype analysis

The two-point lod scores were negative for all three markers covering a 6-cM region surrounding *CACNA1A* on 19p13 (−4.65 for the *CACNA1A* intragenic marker D19S1150 at θ=0.001), suggesting a lack of involvement of this locus in our family. The family showed significant linkage to the 1q23 locus with the highest two-point lod score of 3.37 (θ=0.00) reached with markers D1S2343, D1S2635, D1S1679, and D1S2844. All FHM patients shared a common haplotype extending from marker D1S2343 to D1S2844.

Mutation screening

Sequencing of *ATP1A2* showed a heterozygous single nucleotide change A1033G in exon 9 (Fig. 2). This change replaces a threonine with an alanine residue at position 345 of the Na^+,K^+-ATPase. The T345A mutation co-segregated with the disease phenotype in the family. Of the 12 family members (Fig. 1) carrying the identified mutation, 10 had FHM attacks and 2 were asymptomatic at-risk subjects (aged 19 and 10 years) who had inherited the high-risk haplotype. The mutation was not present in 132 healthy control individuals tested. In addition to this disease-associated mutation, the proband was homozygous for 1 intronic polymorphism and heterozygous for 1 exonic silent polymorhism and 9 intronic polymorphisms (Table 1). Since the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) contained allele frequency information on only 1 of these 11 identified polymorphisms, we estimated the allele frequencies of these polymorphisms in a pooled DNA sample consisting of 132 Finnish individuals (the same control individuals that were studied for the presence of mutation). The mean and standard deviation of the allele frequency estimates are listed in Table 1. The minor allele frequency of the rarest polymorphism (IVS12–11C>G) was 7%.

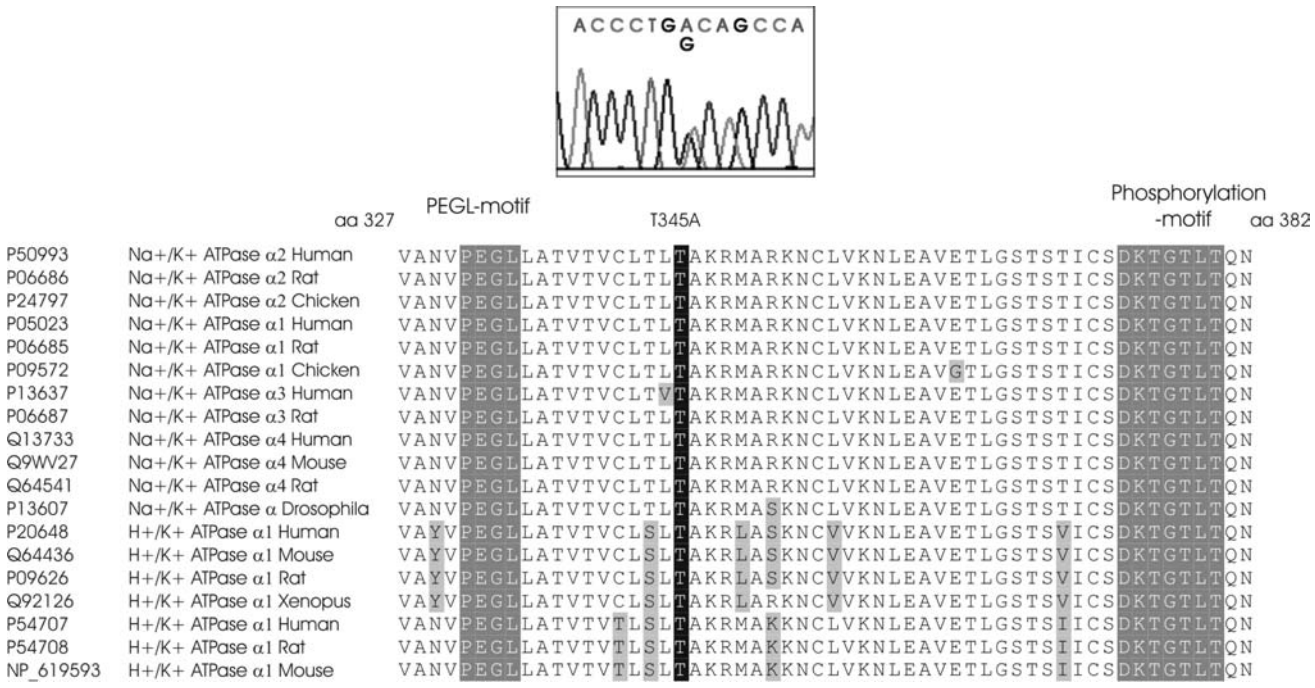

Fig. 2 Alignment of ATPase α-subunit amino acid sequences from different species over a highly conserved 40-amino acid region. This conserved region extends from a PEGL motif with an important role in energy transduction to the phosphorylated DKTGTLT motif. The electropherogram shows the A1033G mutation that results in Thr345Ala change in the middle of this region. The SwissProt accession numbers are indicated, except in the case of mouse H^+,K^+ -ATPase α1 subunit, where the GenBank accession number is shown. Amino acid numbering is presented according to the human *ATP1A2* and variations from this sequence are indicated by *light gray*

Table 1 *ATP1A2* polymorphisms found in the proband (*SNP* single nucleotide polymorphism, *NA* not applicable)

Polymorphism	Location	SNP ID dbSNP	Minor allele (Finns)	Minor allele frequency (p) in the general population[c]		Proband's genotype
				Finnish	Asian/American Indian/Pygmy	
IVS5+81T>A	Intron 5	rs2854248	A	0.41±0.031	0.52±0.052	T/A
IVS7+84T>A	Intron 7	rs7548116	T	0.44±0.070	0.53±0.004	T/A
IVS7–27C>A	Intron 7	rs2295623	A	NA[a]	NA[a]	C/A
IVS8+56G>A	Intron 8	rs6695366	G	0.20±0.026	0.41±0.033	A/A
IVS10+50insG	Intron 10	rs5778151	–	0.21±0.021	0.45±0.021	–/G
IVS12–11C>G	Intron 12	NA	G	0.07±0.018[b]	0.00[b]	C/G
C2159T (A753A)	Exon 16	NA	T	0.19±0.018	0.20±0.011	C/T
IVS20–20insC	Intron 20	NA	InsC	0.16±0.014	0.16±0.008	–/C
IVS21–47C>G	Intron 21	NA	C	0.23±0.027	0.21±0.008	C/G
IVS21–27G>C	Intron 21	NA	G	0.28±0.014	0.24±0.014	G/C
IVS22+14C>T	Intron 22	NA	T	0.18±0.023	0.16±0.005	C/T

[a] Minor allele frequency 0.11 based on dbSNP database information
[b] Result not adjusted using information from heterozygous individuals
[c] Frequencies are mean ± SD

Discussion

We identified a novel missense mutation, T345A, in the *ATP1A2* gene encoding the Na^+,K^+-ATPase α2 subunit in a Finnish family with FHM. The Na^+,K^+-ATPase α2 subunit consists of a N-terminal part containing four membrane spanning domains (M1–4), followed by a large intracellular loop and a C-terminal region with six membrane spanning domains (M5–10). The large M4–5 loop undergoes major conformational changes during the enzymatic cycle and contains critical functional sites for nucleotide binding and phosphorylation [11, 18]. It is also a region critical for isoform-specific functions, that distinguish it from the ubiquitous abundantly expressed α1 isoform, including enzyme kinetics and regulation [18]. Threonine 345 is located at the beginning of this loop, in the middle of a highly conserved 40-amino acid sequence (Fig. 2) [11]. Interestingly, three of the four previously

published *ATP1A2* mutations are also located in this loop, although in the C-terminal end [5, 9].

Three different facts indicate that the T345A mutation most probably is a causative mutation in this family. Namely, the mutation is located in a functionally important and conserved region, it co-segregates with the disease in the family, and it was absent in the 132 control individuals. Although 11 other polymorphisms were identified in the proband's *ATP1A2* gene, they all were relatively common in the normal population, and thus could not be regarded as potential disease-causing mutations. The rarest polymorphism (IVS12–11C>G) has an estimated minor allele frequency of 7% in the Finnish population. Unfortunately, we were unable to adjust this result by compensating for possible allele skew using allele peak area ratio data from heterozygous individuals. However, we were able to see a clear allele frequency difference between the Finnish pool and the Asian/American Indian/Pygmy pool, in which the minor allele was totally absent.

The phenotype of the present FHM2 family resembles for the most part the few FHM2 families described to date [2, 6, 7, 8, 9]. Several of our FHM2 patients have experienced episodes of confusion or impaired consciousness for several days, fever, and mild-to-moderate hemiplegia after minor head trauma. Minor head trauma has also been shown to trigger attacks in several other FHM2 families [2, 6, 9]. After the finding that the R689Q mutation causes both FHM and benign familial infantile convulsions in a Dutch-Canadian family [9], of special phenotypic interest are the epileptic seizures described in several other FHM2 patients [2, 7, 8]. However, none of the patients in our family had a history of seizures.

The functional studies of the first two published FHM2 mutations, L764P and W887R, suggested that these mutations have a functional haploinsufficiency effect [5]. All published FHM2 mutations, including ours, have proven to be missense mutations. Further studies will clarify if all FHM2 mutations are missense and whether their common mechanism is haploinsufficiency. This mechanism is brought into focus by an *Atp1a2* knockout mouse that has been developed [12, 19]. The homozygous knockout mice died very soon after birth because of difficulties breathing and displayed selective neuronal apoptosis in the amygdala and the piriform cortex [12, 19]. In contrast, an increased contraction force of heart and skeletal muscle as well as augmented fear/anxiety behavior and enhanced neuronal activity in the amygdala and the piriform cortex after conditioned fear stimuli were reported in the heterozygous mutant mice [10, 19]. Interestingly, some of our patients showed confusion and anxiety during a FHM attack. It is tempting to speculate that these behavioral changes reflect the same pathophysiology.

Penetrance seems to be lower in FHM2 families than in FHM1 families [2]. In the present study, two symptomless children are carriers of the T345A mutation, but because of the variable age of onset of the disease it is still too early to tell whether they represent truly non-penetrant individuals. In addition to the incomplete penetrance, the linkage analysis of FHM is often complicated by the classification of subjects with only common types of migraine. Within FHM2 families, many MO patients or patients with migraine with only visual aura have been shown to carry the high-risk haplotype. However, in the present study, the 4 patients who had MO or non-migrainous headache were not mutation carriers. The identification of specific mutations, such as that presented, will eventually redefine the wide clinical spectrum of migrainous disorders. This has vast implications in a variety of clinical entities, such as hemiparesis, ataxia, seizures, coma, and eventually migraine with and without aura.

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