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A novel *ATP1A2* gene mutation in an Irish Familial Hemiplegic Migraine kindred

Disclosure

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Abstract:

OBJECTIVE: We studied a large Irish Caucasian pedigree with Familial Hemiplegic Migraine with the aim of finding the causative gene mutation.

BACKGROUND: Familial Hemiplegic Migraine is a rare autosomal dominant subtype of migraine with aura, which is linked to 4 loci on chromosomes 19p13, 1q23, 2q24 and 1q31. The mutations responsible for hemiplegic migraine have been described in the *CACNA1A* gene (chromosome 19p13), *ATP1A2* gene (chromosome 1q23) and *SCN1A* gene (chromosome 2q24).

METHODS: We performed linkage analyses in this family for chromosome 1q23 and performed mutation analysis of the *ATP1A2* gene.

RESULTS: Linkage to the FHM2 locus on chromosome 1 was demonstrated. Mutation screening of the *ATP1A2* gene revealed a G to C substitution in exon 22 resulting in a novel protein variant, D999H, which co-segregates with FHM within this pedigree and is absent in 50 unaffected individuals. This residue is also highly conserved across species.

CONCLUSIONS: We propose that D999H is a novel Familial Hemiplegic Migraine *ATP1A2* mutation.

Keywords: *ATP1A2* gene mutation; familial hemiplegic migraine

Abbreviations

FHM	Familial Hemiplegic Migraine
MA	Migraine with Aura
FHM1	Familial Hemiplegic Migraine Type 1
FHM2	Familial Hemiplegic Migraine Type 2
FHM3	Familial Hemiplegic Migraine Type 3
MO	Migraine without Aura
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
CEPH	The Centre d'Etudes du Polymorphisme Humaine
UCSC	University Of California, Santa Cruz
NCBI	The National Center for Biotechnology Information

Introduction

Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura (MA), which is classified by the International Classification of Headache Disorders as a familial migraine with aura that includes motor weakness.[1] The worldwide prevalence of hemiplegic migraine is unknown. However, a Danish epidemiological study revealed a prevalence of 0.01%, with both familial and sporadic forms of this condition occurring with equal frequency.[2]

There are four genetic variants of FHM described. FHM1 (MIM 141500), which is responsible for approximately 50% of all FHM families,[3] is linked to chromosome 19p13 [4] and is caused by mutations in the *CACNA1A* gene which encodes for the α1A subunit of the P/Q-type neuronal calcium channel, $Ca_v2.1$.[5] FHM2 (MIM 602681) accounts for 20-30% of known FHM families,[6] and is due to mutations in the *ATP1A2* gene, which is located on chromosome 1q23.[7] The *ATP1A2* gene encodes the α2 subunit of Na^+, K^+ - ATPase, a plasma membrane enzyme that counter-transports Na^+ and K^+ across cell membranes.[8] A third locus of familial hemiplegic migraine (FHM3) has recently been described on chromosome 2q24 (MIM 609634).[9] A missense mutation in the neuronal voltage-gated sodium channel gene, *SCN1A*, was found in three FHM families in which *CACNA1A* and *ATP1A2* gene mutations were excluded.[9] In addition to the above established FHM loci, a fourth locus (MIM 607516) is described in an American family (of German / Native American origin) linked to chromosome 1q31.[10] No responsible gene has yet been reported in this locus.

Clinical manifestations of FHM usually occurs in the first two decades of life.[11] Presentation may include migraine or non-migraine headaches with motor, visual,

sensory auras and speech dysphasia.[1] In addition, fever,[12] confusion, coma,[11,13-17] seizures,[3,11,13,15-19] transient or permanent cerebellar signs;[11,16,17,19-21] and rarely, mental retardation can occur.[12,15,17,19,22] Permanent cerebellar signs are more common in FHM1,[11,21] while seizures are a more frequent feature in FHM2.[3,11,13,15-19] In this study, we investigated an Irish family with clinical features of FHM, determined linkage to the FHM2 locus and described a novel mutation in the *ATP1A2* gene.

Materials and methods

FHM family

A large Irish family with autosomal dominant familial hemiplegic migraine was recruited (Figure 1). A total of 37 family members from the latter three generations were interviewed and examined by one of the authors (DF). Initially, two FHM probands (Figure 1: IV:6, IV:33) belonging to two large FHM families, were separately diagnosed. It was subsequently discovered that these two families were in fact related, yielding one large kindred. There are a total of thirty family members affected with FHM, of which twenty-one are still alive. Of these, nineteen participated in this study, one declined participation and another was living abroad. The histories of the deceased family members affected with FHM were obtained from relatives' historical accounts. The majority of relatives recognized the symptoms of hemiplegic migraine, as it is common within the family. In addition to the 19 participants with FHM, 4 family members who have migraine without aura (MO) (Figure 1: IV:3, IV:19, IV:39, V:7), three family members who have typical migraine with aura (MA) but without motor weakness (Figure 1: IV:10, V:38, V:53) and 12 unaffected family members also participated in this study. The diagnosis of migraine was based on the International Classification of Headache Disorders.[1] All clinical diagnoses of FHM and other types of migraine in this family were made prior to genetic testing. The majority of the FHM subjects also have migraine with aura attacks without the motor weakness and some of

them also suffer from MO at other times (Fig 1). Brain imaging was performed only in subjects attending the hospital or the migraine clinic as patients and was not routinely prescribed as part of this research.

All 19 FHM subjects described sensory and motor auras during their hemiplegic migraine attacks. All except three of these subjects also had visual auras (Figure 1; V:6, V:40, V:54) and all except three individuals reported speech dysphasia during their migraine attacks (Figure 1; V:6, V:40, V:54). In addition, all FHM subjects except V:6 and V:56 (Figure 1) had accompanying dysarthria, gait ataxia and upper limb clumsiness during their hemiplegic migraine attacks.

Additional clinical features found in the FHM subjects include epilepsy and mental retardation in subject V:40 (Figure 1), single episodes of childhood febrile convulsions in two subjects (Figure 1; IV:6, IV:12) and childhood seizures in three subjects (Figure 1; III:2, V:2, V:6). Individual V:40 has complex partial seizures with secondary generalisation. Subject III: 2 is 77 years old and could only give a retrospective account of generalized tonic-clonic seizures in childhood. No previous medical records were available. Both V:2 and V:6 had absence seizures in childhood and historical accounts were obtained from their parents.

Mild cerebellar signs including subtle intention tremor and nystagmus were found in five subjects (Figure 1; IV:2, IV:20, IV:30, IV:33, V:2) in between migraine attacks. These findings were noted incidentally on clinical examination. Apart from their migraine attacks, these subjects were otherwise asymptomatic. With the exception of IV:33 (Figure 1), who had an MRI brain scan because she was a migraine clinic patient, none of the other subjects had brain imaging. The brain MRI was normal in IV:33. In particular, there were no cerebellar white matter changes or atrophy. Additional functional studies of cerebellar dysfunction on these FHM subjects were not performed.

Our study methods are in accordance with the Declaration of Helsinki and were approved by the Clinical Research Ethics Committee. Blood samples from affected and unaffected family members were taken after informed consent was obtained.

In addition, 50 Irish Caucasian control subjects were recruited in whom migraine and epilepsy phenotypes were excluded. Peripheral blood samples were obtained from these subjects after informed consent was obtained.

Linkage analyses

Genomic DNA was extracted from peripheral blood lymphocytes using phenol-chloroform method or commercially available DNA extraction kits (Qiagen). DNA was then amplified by polymerase chain reaction (PCR) using fluorescently labeled primer pairs for microsatellite markers flanking the chromosomal regions of interest. The markers studied were D1S2635, D1S2707 and D1S2705 for the FHM2 locus on chromosome 1q23. All primer sequences are available on the UCSC human genome browser database. Individuals were genotyped by analyzing the amplified PCR products using the GENESCAN software on the ABI 310 automated DNA analyzer (Applied Biosystems, Foster City, USA). Linkage analysis was then performed using the FASTLINK software program. Information for allele frequencies was obtained from the CEPH database. The disease model used was autosomal dominant inheritance with 80% penetrance [13] and the disease gene frequency was taken to be to be 0.0001.[2]

Mutation Screening

For the FHM family, all exons of the *ATP1A2* gene were screened by PCR amplification of genomic DNA from two affected subjects (Figure 1: III:2, IV:6) and two unaffected subjects (Figure 1: III:3, IV:21). The genomic structure was available from the UCSC Human Genome browser database. Details of the intron-exon structures and primer sequences are available on request. Amplified PCR products of all exons were purified (Jetquick Spin Columns, Genomed)

and cycle-sequenced using a dye terminator kit (Applied Biosystems). Sequence analysis was then performed using the ABI 310 automated sequencer.

Once the segregating variant was identified, DNA from 50 Irish control subjects was screened for the putative mutation in exon 22 using restriction enzyme analysis. PCR amplification was carried out using the primers 5'AGAAGAGGCTGTTGGAAGAAGACA and 5'GCAGGAACCAGTAGTGGGAGTGGA. Amplified PCR products were incubated overnight at 37˚C with the restriction enzyme *NlaIII*. The presence of the variant adds a restriction site in the 583 bp fragment; the digestion produces 5 DNA fragments for the wild type allele (18, 26, 74, 112 and 353 bp) and 6 DNA fragments for the mutant alleles (18, 26, 74, 112, 135 and 218 bp).

Results

Linkage analyses

Linkage to chromosome 1q23 was established with all three microsatellite markers achieving significant LOD scores (Table 1). The most significant LOD score was +5.6 for D1S2705 at $\theta=0$.

Mutation screening

The nucleotide 2995 G → C substitution in exon 22 of the *ATP1A2* gene was found to be disease segregating within this FHM family (Figure 2) and is present in all subjects affected with hemiplegic migraine. This point mutation results in the replacement of the acidic aspartic acid residue with a basic histidine at position 999 in the Na^+, K^+ - ATPase protein (D999H). This mutation was not found in the 100 control chromosomes that were screened.

In addition, examination of all the coding sequence of the *ATP1A2* gene did not detect other mutations including those that have been previously described in the literature (Figure 2).[7,14,16-18,23-26]

Discussion

We describe a novel missense mutation, D999H, in the *ATP1A2* gene in an Irish FHM pedigree. This is the first *ATP1A2* gene mutation to be described in an Irish FHM family. There are 20 known *ATP1A2* gene mutations causing Familial Hemiplegic Migraine (Figure 2), of which 18 are missense mutations.[7,14,16-18,23-26]

The *ATP1A2* gene encodes for the α2 subunit of the Na^+, K^+- ATPase plasma membrane enzyme, which couples ATP hydrolysis to counter-transport Na^+ and K^+ across cell membranes. The Na^+, K^+ - ATPase protein is composed of three heteromeric subunits, the α catalytic subunit, the β regulatory subunit and the γ subunit (function unknown).[8] Of the α subunits, the α1 subunit is ubiquitously expressed whilst the α2 subunit is expressed in muscle, adipose tissue and central nervous tissue, particularly astrocytes.[8,27] This α2 subunit is composed of an N-terminal region containing four membrane-spanning domains (M1-4) and a C-terminal region containing six membrane-spanning domains (M5-10), which are linked by a large intracellular loop. This large M4-5 loop contains critical functional sites and undergoes major conformational changes during the enzymatic cycle.[28] Eleven of the 20 known *ATP1A2* gene mutations causing FHM2 are located within this loop (T345A, T376M, R593W, A606T, G615R, R689Q, E700K, D718N, M731T, L764P and V628M; see Figure 2).[7,14,16-18,24,25,29] The D999H variant we have identified is located in the M10 trans-membrane domain, which is situated in the C-terminus cation transporting ATPase domain(Figure 2).[17] It is adjacent to the P979L mutation, which was associated with interictal mild cerebellar signs (mild dysarthria and nystagmus) in affected individuals.[17] Interestingly, only the P979L and G301R *ATP1A2* gene mutations were reported to cause interictal cerebellar signs in FHM.[16,17] No functional studies were

published on these mutations, although the G301R mutation is also located on a trans-membrane protein (Figure 2; M3). With the exception of the W887R mutation, no functional studies were published for the other FHM2 causing *ATP1A2* gene mutations located within the C-terminal region. Functional studies of the W887R mutation have shown a loss of function effect of the Na^+, K^+-ATPase protein and have demonstrated that mutated alleles caused functional haplo-insufficiency of the β subunit binding site within this domain.[7] As the Na^+, K^+-ATPase exchanges intracellular Na^+ for extracellular K^+, loss of the Na^+, K^+-ATPase function results in raised extracellular K^+ which facilitates cortical spreading depression,[30] a mechanism postulated to cause migraine aura.[31] This ATPase dysfunction also results in raised intracellular Na^+, which will increase intracellular Ca^{2+} levels as a result of a decrease in Na^+-Ca^{2+} exchange. This raised intracellular Ca^{2+} also facilitates cortical spreading depression.[32] Functional studies on *CACNA1A* gene mutations responsible for FHM also supports the effect of raised intracellular calcium in the pathogenesis of migraine aura.[33] Other *ATP1A2* gene mutations that have also demonstrated a loss of function effect of the Na^+, K^+-ATPase protein include R593W, G615R, L764P and D718N.[7,17,25,26]

Of the 19 FHM subjects within this family, four have histories of seizures and two have had histories of febrile convulsions. Seizures have been described more frequently in FHM2 families (9 of 15) than FHM1 families (5 individuals in 35 families). Also of interest is that five FHM subjects within this family demonstrated subtle interictal cerebellar signs (Table 1). These findings were noted incidentally on routine clinical

examination and confirmed by a senior neurologist. However, further functional assessment of cerebellar dysfunction and MRI brain imaging were not routinely performed. Only one subject, who attended the migraine clinic as a patient, had a MRI brain scan, which was normal. Cerebellar signs occurring during hemiplegic migraine attacks have been reported in FHM2 families.[16] However, permanent cerebellar signs persisting between migraine attacks are uncommon in FHM2.[11,21] Only 9 individuals in all of the FHM2 families reported to date, exhibited interictal cerebellar signs.[16,17] Another family with possible linkage to chromosome 1q21-23 also exhibited interictal cerebellar signs in the form of gaze-evoked nystagmus and scanning speech in three family members.[19]

Within our pedigree, only one family member with the *ATP1A2* D999H mutation did not have the clinical phenotype of FHM (Figure 1: IV:37). This finding is consistent with incomplete penetrance as previously described for FHM2.[13]

It has been suggested that typical MA and FHM are part of a spectrum of migraine disorders associated with auras.[11] One study found two novel *ATP1A2* mutations in MA and MO,[34] while another study failed to find any significant variants.[35] We did not detect any *ATP1A2* mutation in family members who do not have FHM but who suffer from either MA or MO. Our findings do not support *ATP1A2* mutations as the pathogenesis of MA or MO.

The D999H mutation co-segregates with FHM within this family and was present in all the family members affected with hemiplegic migraine. This mutation was also excluded

in 50 control subjects and is located in a residue that is highly conserved across different species (Figure 3). These findings are highly suggestive that D999H is the novel causative *ATP1A2* mutation for FHM in this Irish pedigree.

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Electronic-Database Information

URLs for data presented herein are as follows:

Mendelian Inheritance in Man (MIM) data were retrieved from the OMIM database: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

UCSC Genome Bioinformatics: http://genome.ucsc.edu

CEPH database: www.cephb.fr

Marshfield Genetic Maps: http://research.marshfieldclinic.org/genetics/

NCBI Conserved Domain Search: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=cdd

REFERENCES

1. Olesen J, Steiner TJ. The International classification of headache disorders, 2nd edn (ICDH-II). *J Neurol Neurosurg Psychiatry*. 2004;75, 808-11.
2. Thomsen LL, Eriksen MK, Roemer SF, Andersen I, Oestergaard E, Keiding N, et al. An epidemiological survey of hemiplegic migraine. *Cephalalgia*. 2002;22, 362-375.
3. Kors EE, Melberg A, Vanmolkot KR, Kumlien E, Haan J, Raininko R, Flink R, Ginjaar HB, Frants RR, Ferrari MD, van den Maagdenberg AM. Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new CACNA1A mutation. *Neurology*. 2004;63, 1136-7.
4. Joutel A, Bousser MG, Biousse V, Labauge P, Chabriat H, Nibbio A, Maciazek J, Meyer B, Bach MA, Weissenbach J, et al. A gene for familial hemiplegic migraine maps to chromosome 19. *Nat Genet*. 1993;5, 40-5.
5. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen GJ, Hofker MH, Ferrari MD, Frants RR. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell*. 1996;87, 543-52.
6. Estevez M, Gardner KL. Update on the genetics of migraine. *Hum Genet*. 2004;114, 225-35.
7. De Fusco M, Marconi R, Silvestri L, Atorino L, Rampoldi L, Morgante L, Ballabio A, Aridon P, Casari G. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet*. 2003;33, 192-6.
8. Mobasheri A, Avila J, Cozar-Castellano I, Brownleader MD, Trevan M, Francis MJ, Lamb JF, Martin-Vasallo P. Na+, K+-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. *Biosci Rep*. 2000;20, 51-91.
9. Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, van den Maagdenberg AM, Pusch M, Strom TM. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet*. 2005;366, 371-7.
10. Gardner K, Barmada MM, Ptacek LJ, Hoffman EP. A new locus for hemiplegic migraine maps to chromosome 1q31. *Neurology*. 1997;49, 1231-8.
11. Ducros A, Denier C, Joutel A, Cecillon M, Lescoat C, Vahedi K, Darcel F, Vicaut E, Bousser MG, Tournier-Lasserve E. The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. *N Engl J Med*. 2001;345, 17-24.
12. Kors EE, Haan J, Giffin NJ, Pazdera L, Schnittger C, Lennox GG, Terwindt GM, Vermeulen FL, Van den Maagdenberg AM, Frants RR, Ferrari MD. Expanding the phenotypic spectrum of the CACNA1A gene T666M mutation: a description of 5 families with familial hemiplegic migraine. *Arch Neurol*. 2003;60, 684-8.
13. Ducros A, Joutel A, Vahedi K, Cecillon M, Ferreira A, Bernard E, Verier A, Echenne B, Lopez de Munain A, Bousser MG, Tournier-Lasserve E. Mapping of

a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity. *Ann Neurol*. 1997;42, 885-90.
14. Kaunisto MA, Harno H, Vanmolkot KR, Gargus JJ, Sun G, Hamalainen E, Liukkonen E, Kallela M, van den Maagdenberg AM, Frants RR, Farkkila M, Palotie A, Wessman M. A novel missense ATP1A2 mutation in a Finnish family with familial hemiplegic migraine type 2. *Neurogenetics*. 2004;5, 141-6.
15. Marconi R, De Fusco M, Aridon P, Plewnia K, Rossi M, Carapelli S, Ballabio A, Morgante L, Musolino R, Epifanio A, Micieli G, De Michele G, Casari G. Familial hemiplegic migraine type 2 is linked to 0.9Mb region on chromosome 1q23. *Ann Neurol*. 2003;53, 376-81.
16. Spadaro M, Ursu S, Lehmann-Horn F, Liana V, Giovanni A, Paola G, Frontali M, Jurkat-Rott K. A G301R Na+/K+ -ATPase mutation causes familial hemiplegic migraine type 2 with cerebellar signs. *Neurogenetics*. 2004;5, 177-85.
17. Jurkat-Rott K, Freilinger T, Dreier JP, Herzog J, Gobel H, Petzold GC, Montagna P, Gasser T, Lehmann-Horn F, Dichgans M. Variability of familial hemiplegic migraine with novel A1A2 Na+/K+-ATPase variants. *Neurology*. 2004;62, 1857-61.
18. Vanmolkot KR, Kors EE, Hottenga JJ, Terwindt GM, Haan J, Hoefnagels WA, Black DF, Sandkuijl LA, Frants RR, Ferrari MD, van den Maagdenberg AM. Novel mutations in the Na+, K+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. *Ann Neurol*. 2003;54, 360-6.
19. Cevoli S, Pierangeli G, Monari L, Valentino ML, Bernardoni P, Mochi M, Cortelli P, Montagna P. Familial hemiplegic migraine: clinical features and probable linkage to chromosome 1 in an Italian family. *Neurol Sci*. 2002;23, 7-10.
20. Harno H, Hirvonen T, Kaunisto MA, Aalto H, Levo H, Isotalo E, Kallela M, Kaprio J, Palotie A, Wessman M, Farkkila M. Subclinical vestibulocerebellar dysfunction in migraine with and without aura. *Neurology*. 2003;61, 1748-52.
21. Battistini S, Stenirri S, Piatti M, Gelfi C, Righetti PG, Rocchi R, Giannini F, Battistini N, Guazzi GC, Ferrari M, Carrera P. A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. *Neurology*. 1999;53, 38-43.
22. Kors EE, Terwindt GM, Vermeulen FL, Fitzsimons RB, Jardine PE, Heywood P, Love S, van den Maagdenberg AM, Haan J, Frants RR, Ferrari MD. Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. *Ann Neurol*. 2001;49, 753-60.
23. Pierelli F, Grieco GS, Pauri F, Pirro C, Fiermonte G, Ambrosini A, Costa A, Buzzi MG, Valoppi M, Caltagirone C, Nappi G, Santorelli FM. A novel ATP1A2 mutation in a family with FHM type II. *Cephalalgia*. 2006;26, 324-8.
24. Riant F, De Fusco M, Aridon P, Ducros A, Ploton C, Marchelli F, Maciazek J, Bousser MG, Casari G, Tournier-Lasserve E. ATP1A2 mutations in 11 families with familial hemiplegic migraine. *Hum Mutat*. 2005;26, 281.
25. Vanmolkot KR, Kors EE, Turk U, Turkdogan D, Keyser A, Broos LA, Kia SK, van den Heuvel JJ, Black DF, Haan J, Frants RR, Barone V, Ferrari MD, Casari G, Koenderink JB, van den Maagdenberg AM. Two de novo mutations in

the Na,K-ATPase gene ATP1A2 associated with pure familial hemiplegic migraine. *Eur J Hum Genet*. 2006;14, 555-60.
26. Vanmolkot KR, Stroink H, Koenderink JB, Kors EE, van den Heuvel JJ, van den Boogerd EH, Stam AH, Haan J, De Vries BB, Terwindt GM, Frants RR, Ferrari MD, van den Maagdenberg AM. Severe episodic neurological deficits and permanent mental retardation in a child with a novel FHM2 ATP1A2 mutation. *Ann Neurol*. 2006;59, 310-4.
27. Moseley AE, Lieske SP, Wetzel RK, James PF, He S, Shelly DA, Paul RJ, Boivin GP, Witte DP, Ramirez JM, Sweadner KJ, Lingrel JB. The Na,K-ATPase alpha 2 isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. *J Biol Chem*. 2003;278, 5317-24.
28. Moller JV, Juul B, le Maire M. Structural organization, ion transport, and energy transduction of P-type ATPases. *Biochim Biophys Acta*. 1996;1286, 1-51.
29. Vanmolkot KR, Kors EE, Turk U, Turkdogan D, Keyser A, Broos LA, Kia SK, van den Heuvel JJ, Black DF, Haan J, Frants RR, Barone V, Ferrari MD, Casari G, Koenderink JB, van den Maagdenberg AM. Two de novo mutations in the Na,K-ATPase gene ATP1A2 associated with pure familial hemiplegic migraine. *Eur J Hum Genet*. 2006;
30. Kraig RP, Ferreira-Filho CR, Nicholson C. Alkaline and acid transients in cerebellar microenvironment. *J Neurophysiol*. 1983;49, 831-50.
31. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. *Brain*. 1994;117 (Pt 1), 199-210.
32. Kunkler PE, Kraig RP. Calcium waves precede electrophysiological changes of spreading depression in hippocampal organ cultures. *J Neurosci*. 1998;18, 3416-25.
33. Tottene A, Fellin T, Pagnutti S, Luvisetto S, Striessnig J, Fletcher C, Pietrobon D. Familial hemiplegic migraine mutations increase Ca(2+) influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc Natl Acad Sci U S A*. 2002;99, 13284-9.
34. Todt U, Dichgans M, Jurkat-Rott K, Heinze A, Zifarelli G, Koenderink JB, Goebel I, Zumbroich V, Stiller A, Ramirez A, Friedrich T, Gobel H, Kubisch C. Rare missense variants in ATP1A2 in families with clustering of common forms of migraine. *Hum Mutat*. 2005;26, 315-21.
35. Jen JC, Kim GW, Dudding KA, Baloh RW. No mutations in CACNA1A and ATP1A2 in probands with common types of migraine. *Arch Neurol*. 2004;61, 926-8.
36. Terwindt GM, Ophoff RA, van Eijk R, Vergouwe MN, Haan J, Frants RR, Sandkuijl LA, Ferrari MD. Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. *Neurology*. 2001;56, 1028-32.
37. May A, Ophoff RA, Terwindt GM, Urban C, van Eijk R, Haan J, Diener HC, Lindhout D, Frants RR, Sandkuijl LA, et al. Familial hemiplegic migraine locus on 19p13 is involved in the common forms of migraine with and without aura. *Hum Genet*. 1995;96, 604-8.
38. Nyholt DR, Lea RA, Goadsby PJ, Brimage PJ, Griffiths LR. Familial typical migraine: linkage to chromosome 19p13 and evidence for genetic heterogeneity. *Neurology*. 1998;50, 1428-32.

39. Wessman M, Kallela M, Kaunisto MA, Marttila P, Sobel E, Hartiala J, Oswell G, Leal SM, Papp JC, Hamalainen E, Broas P, Joslyn G, Hovatta I, Hiekkalinna T, Kaprio J, Ott J, Cantor RM, Zwart JA, Ilmavirta M, Havanka H, Farkkila M, Peltonen L, Palotie A. A susceptibility locus for migraine with aura, on chromosome 4q24. *Am J Hum Genet*. 2002;70, 652-62.
40. Noble-Topham SE, Dyment DA, Cader MZ, Ganapathy R, Brown JD, Rice GP, Ebers GC. Migraine with aura is not linked to the FHM gene CACNA1A or the chromosomal region, 19p13. *Neurology*. 2002;59, 1099-101.
41. Lea RA, Curtain RP, Hutchins C, Brimage PJ, Griffiths LR. Investigation of the CACNA1A gene as a candidate for typical migraine susceptibility. *Am J Med Genet*. 2001;105, 707-12.
42. Hovatta I, Kallela M, Farkkila M, Peltonen L. Familial migraine: exclusion of the susceptibility gene from the reported locus of familial hemiplegic migraine on 19p. *Genomics*. 1994;23, 707-9.
43. Jones KW, Ehm MG, Pericak-Vance MA, Haines JL, Boyd PR, Peroutka SJ. Migraine with aura susceptibility locus on chromosome 19p13 is distinct from the familial hemiplegic migraine locus. *Genomics*. 2001;78, 150-4.
44. Monari L, Mochi M, Valentino ML, Arnaldi C, Cortelli P, De Monte A, Pierangeli G, Prologo G, Scapoli C, Soriani S, Montagna P. Searching for migraine genes: exclusion of 290 cM out of the whole human genome. *Ital J Neurol Sci*. 1997;18, 277-82.
45. Wieser T, Mueller C, Evers S, Zierz S, Deufel T. Absence of known familial hemiplegic migraine (FHM) mutations in the CACNA1A gene in patients with common migraine: implications for genetic testing. *Clin Chem Lab Med*. 2003;41, 272-5.
46. Brugnoni R, Leone M, Rigamonti A, Moranduzzo E, Cornelio F, Mantegazza R, Bussone G. Is the CACNA1A gene involved in familial migraine with aura? *Neurol Sci*. 2002;23, 1-5.

Table 1: Lod scores of microsatellite markers showing linkage to the FHM2 locus (chromosome 1q23).

		θ values					
Locus	**Markers**	**0.00**	**0.05**	**0.10**	**0.20**	**0.30**	**0.40**
FHM2	D1S2635	- 0.33	3.92	3.80	3.16	2.25	1.17
	D1S2707	3.50	3.26	2.99	2.36	1.63	0.76
	D1S2705	5.68	5.28	4.83	3.83	2.67	1.37

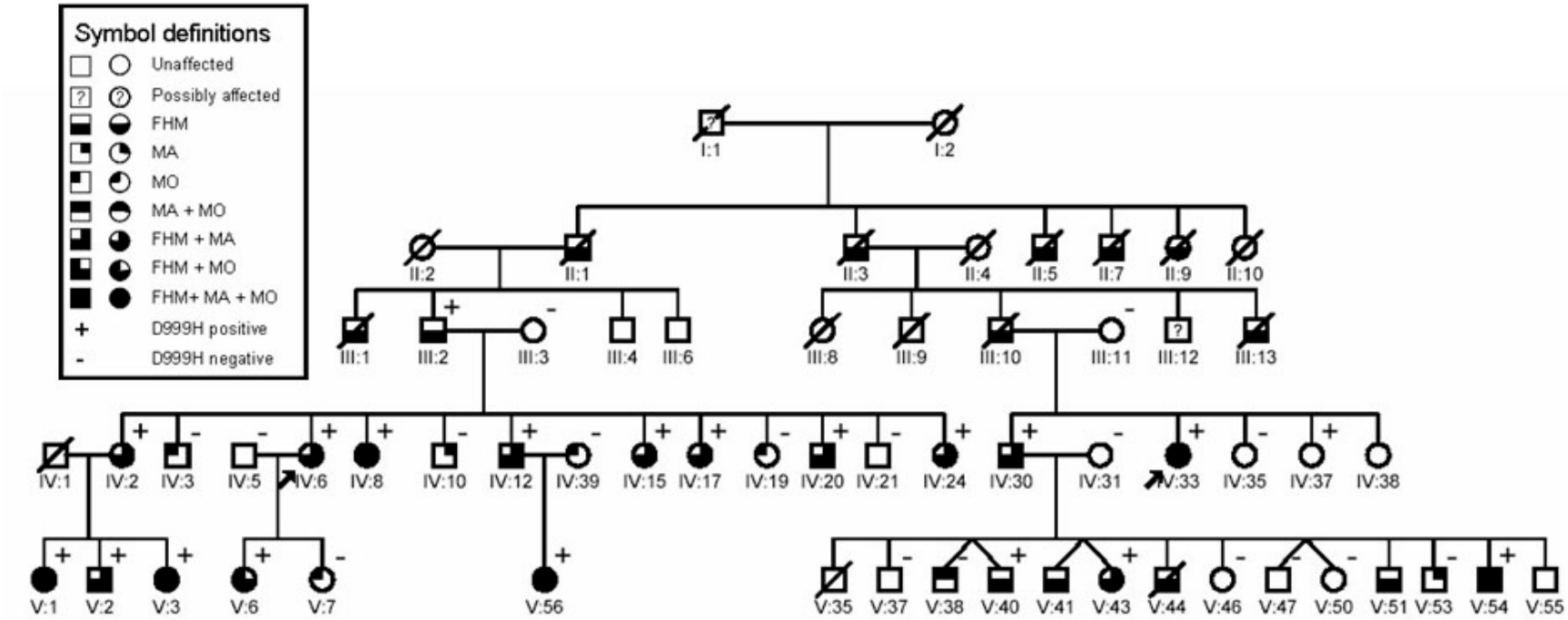

Figure 1: Irish FHM pedigree

Males are represented by squares and females by circles. Individuals with diagonal lines are deceased. Probands are indicated by arrows. The disease status is represented by the symbols indicated. Black lower half indicates FHM; black right upper quadrant indicates MA and black left upper quadrant indicates MO; some individuals have more than one type of migraine at different times. The + sign indicates presence of the D999H mutation and the – sign indicates absence of this mutation in the individuals who had mutation analysis.

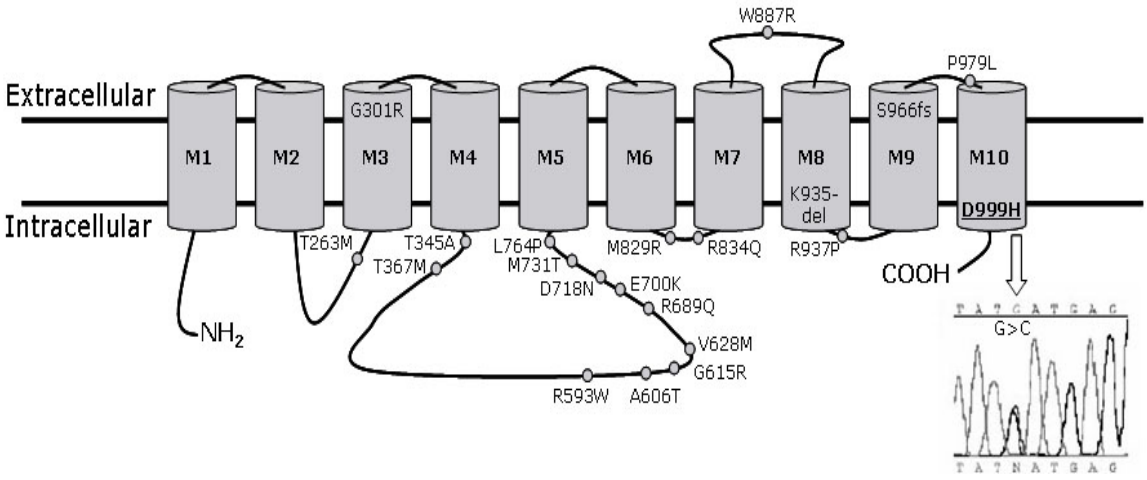

Figure 2: *ATP1A2* mutations on the Na^+, K^+ - ATPase protein

A diagram of the Na^+, K^+ - ATPase trans-membrane protein showing the locations of the various *ATP1A2* mutations which cause FHM. The D999H mutation, which is bold and underlined is located in the M10 transmembrane domain, is a result of a G→C shift on Exon 22 of the *ATP1A2* gene.

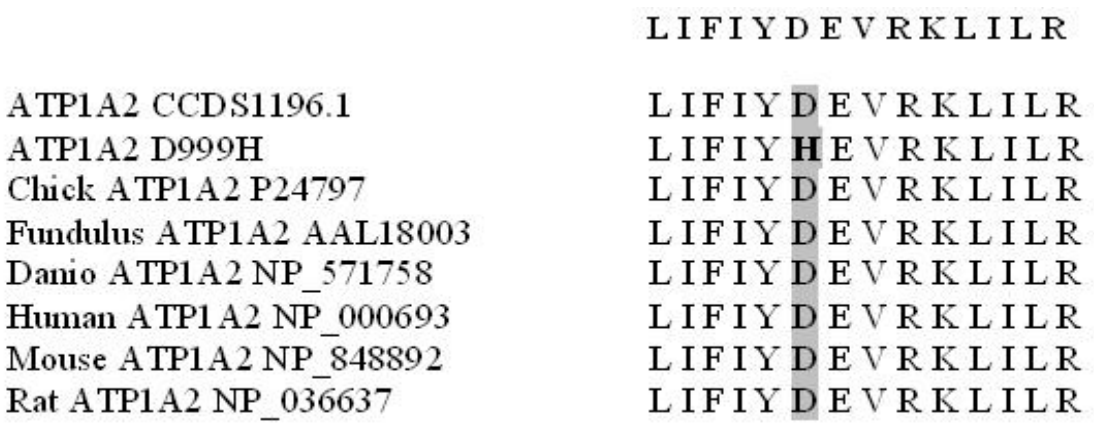

Figure 3: Local protein alignment of ATP1A2 sequences indicating the conservation of aspartic acid (D) at position 999.