The evolving genetic and pathophysiological spectrum of migraine

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CACNA1A mutation linking hemiplegic migraine and alternating hemiplegia of childhood

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Cephalalgia 2008;28:887–891.

Abstract

Familial hemiplegic migraine (FHM) and alternating hemiplegia of childhood (AHC) are severe neurological disorders that share clinical features. Therefore, FHM genes are candidates for AHC. We performed mutation analysis in the *CACNA1A* gene in a monozygotic twin pair with clinical features overlapping with both AHC and FHM and identified a novel *de novo CACNA1A* mutation. We provide the first evidence that a *CACNA1A* mutation can cause atypical AHC, indicating an overlap of molecular mechanisms causing AHC and FHM. These results also suggest that *CACNA1A* mutation scanning is indicated in patients with a severe neurological phenotype that includes paroxysmal (alternating) hemiplegia.

Introduction

Alternating hemiplegia of childhood (AHC) and familial hemiplegic migraine (FHM) are clinically very similar disorders.[1,2] AHC is typically characterized by episodes of alternating hemiplegia or quadriplegia and progressive neurological features beginning before the age of 18 months.[1] FHM is a rare subtype of migraine with aura associated with hemiparesis and in some cases ataxia, mental retardation, movement disorders or other neurological abnormalities.[3] For FHM, three genes have been identified: *CACNA1A*, *ATP1A2* and *SCN1A*, which all play a role in ion transport.[4-6]

Except for one Greek family with atypical AHC and an *ATP1A2* mutation, no genes have been identified for AHC.[7,8] Here we describe a monozygotic twin pair suffering from a complex phenotype of early-onset ataxia, alternating hemiplegia, epilepsy, migraine-like attacks and mental retardation, which clinically overlaps with both AHC and FHM. We identified a novel *de novo CACNA1A* mutation in both patients, confirming a genetic overlap between AHC and FHM.

Subjects and methods

Subjects

Standardized criteria were used for the diagnosis of FHM[3] and AHC.[1] All subjects gave informed consent. This study was approved by the local ethics committee of the University of Greifswald.

Genetic analysis

Genomic DNA was isolated from peripheral leucocytes using a standard salting out extraction method.[9] The 47 coding exons and adjacent sequences of the *CACNA1A* gene were scanned for mutations by direct sequencing. In brief, all exons were amplified by polymerase chain reaction, using genomic DNA as a template. Direct sequencing was done by Cycle Sequencing (Prism Big Dye Terminators Cycle Sequencing kit; Applied Biosystems, Foster City, CA, USA) using the dideoxy termination method and an ABI3700 automated sequencer (Applied Biosystems). One hundred healthy controls were screened by direct sequencing. Detailed information is available from the authors upon request.

Results

The monozygotic German twin brothers, now aged 17 years, were spontaneously born at term after an uneventful pregnancy. Their complex clinical features are summarized in the Table 1. One twin brother (patient I in Table 1) was severely affected and had a delayed psychomotor development. He is still not able to walk without support and is not able to speak. Between the ages of 2 and 7 years he suffered from generalized atonic seizures up to three times daily, often followed by unconsciousness lasting from seconds to several hours. Subsequently, he developed mental and psychomotor regression with ataxic and athetotic limb movements. At age 11 years, when he was hospitalized because of severe constipation and abdominal pain, episodes of abrupt stops in movement, tachycardia and swallowing automatisms were observed. Ictal EEG recordings showed mainly occipitotemporal bilateral synchronic sharp–slow wave activity (not shown). At the age of 12 years he experienced a period of alternating hemiplegia, starting on the left side and accompanied by fever. Cerebral magnetic resonance imaging (MRI) showed ictal right cortical swelling (Fig. 1). After 12 days the left-sided symptoms resolved, but there after right-sided weakness occurred, which lasted for days. EEG revealed slow wave activity over the left hemisphere (data not shown). As he is still not able to express himself, the presence and severity of migraine symptoms (headache, phonophobia, photophobia, nausea) are difficult to assess. At present, he is tetraspastic with athetotic and ataxic movements, and has intermittent convergent strabismus.

Table 1 *Clinical features of monozygotic twins compared with alternating hemiplegia of childhood (AHC) and familial hemiplegic migraine (FHM)*

	AHC	FHM1	Patient I	Patient II
Onset of (alternating) hemiplegic attacks	0–18 months*	> 2 years to adolescence†	12 years	10 years
Hemidystonic spells	+	-	-	-
Quadriplegia	+	-	+	-
Choreoathetosis	+	-	+	-
Ataxia	+	+	+	+
Nystagmus	+	+	-	-
Strabismus	+	-	+	+
Mental retardation	+	-	+	+
Autonomic symptoms	+	+	+	+
Positive effect of sleep	+	-	-	-
Epilepsy	+	+	+	+
Aura symptoms	-	+	NA	+
Migrainous headache	-	+	NA	+

*(+) or (-) indicates presence or absence of a symptom, respectively. NA indicates not applicable due to inability of verbal expression of the patient. *Typically alternating hemiplegia. †Typically non-alternating hemiplegia.*

The other twin brother (patient II in Table 1) was delivered shortly after his twin brother. Early infant psychomotor development was delayed. At the age of 1.5 years he was able to speak single words. Between the ages of 3 and 8 years he suffered twice a year from atonic episodes accompanied by loss of consciousness for 1 h, followed by ataxia. Ictal EEGs showed no signs of epilepsy (data not shown).

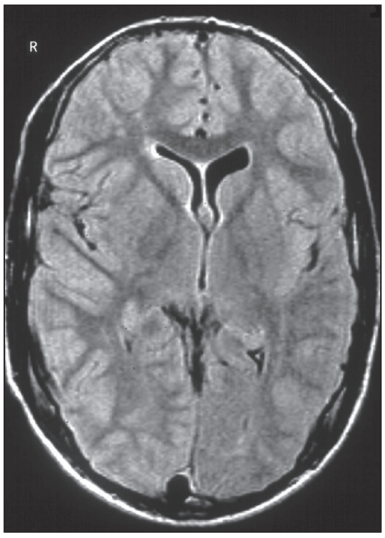

Figure 1 *Fluid-attenuated inversion recovery (FLAIR) image shows ictal diffuse cortical swelling of the right hemisphere of patient I.*

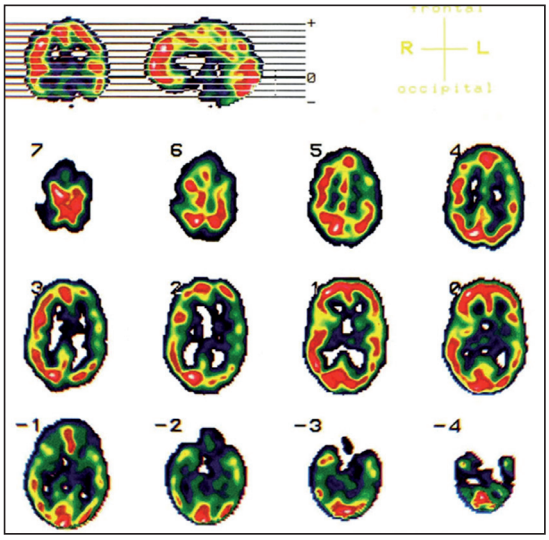

Figure 2 *Single photon emission computed tomography scan shows ictal left-sided hypoperfusion contralateral to the hemiparesis in the parietofrontal (severe) and temporal (mild) cortex of patient II.*

Since the age of 10 years he has experienced several episodes of right- or left-sided hemiplegia, and one episode with alternating hemiplegia for several days. One of the hemiplegic episodes was accompanied by reduced consciousness and fever and lasted several weeks. Cerebral single photon emission computed tomography showed ictal left-sided hypoperfusion in the parieto-fronto-temporal cortex (Fig. 2). At the age of 12 years, treatment with flunarizine was started, with doubtful effect. During hemiplegic attacks he complained of frontal throbbing headache with nausea, sometimes accompanied by phonophobia. Ophthalmic examination shows a strabismus. Except for the twin pair, no other family members suffer from attacks of hemiplegia, epilepsy or migraine. Monozygosity of the twins was confirmed and false paternity was excluded by genetic, multimarker analysis (data not shown). Extensive metabolic screening in both twins revealed no abnormalities. Cerebral MRI, showing diffuse ictal swelling in patient I (see above), was without severe abnormalities in either of the twins, and did not revealcerebellar atrophy.

Direct sequencing of all 47 exons of the *CACNA1A* gene revealed a heterozygous 5361 G>T substitution (Genbank Ac. no. X99897) in exon 33 in both twins. This point mutation resulted in an amino acid change from a valine to a phenylalanine at position 1696. The mutation is located within the transmembrane segment S5 of the fourth domain, which together with segment S6 forms the inner part of the pore of the $Ca_v2.1$-α1 subunit. The parents did not carry the mutation, indicating that V1696F is a *de novo* mutation. Screening of 100 subjects from the general Dutch population with no history of migraine or epilepsy was performed by sequence analysis of exon 33 and was negative.

Discussion

We have identified a novel *de novo CACNA1A* V1696F mutation in two monozygotic twin brothers with complex clinical features in part fulfilling the criteria of both AHC[1] and FHM[3] (Table 1). The presence of hemiplgia and other aura symptoms aswell as migrainous headache are in favour of FHM. Episodes of alternating hemiplegia, developmental delay, mental retardation, choreoathetotic movements, strabismus and chronic ataxia are supportive of AHC. The severity of the phenotype, interictal symptoms and the relatively young age at onset of these patients are, in particular, very rare for FHM1 and more common in AHC. As no positive effect of sleep was observed and the age at onset was after 18 months, we name this overlap syndrome 'atypical AHC'. Although both monozygotic twin brothers are genetically identical, their clinical symptoms and attack frequency vary in severity, with patient I being more severely affected.

Several lines of evidence indicate that V1696F is the disease-causing mutation in this family. Val^{1696} is highly conserved across multiple calcium channel homologues and across species (data not shown). The mutation was not identified in a large number of control chromosomes. The mutation occurred *de novo*, thus strengthening the evidence that it is the V1696F mutation that caused the disease. In a French FHM family another mutation affecting the same residue Val^{1696} (V1696I) caused hemiplegic migraine without cerebellar signs.[10] Finally, electrophysiological analysis of mutant $Ca_v2.1$-α1 containing Iso^{1696} has revealed that loss of the valine residue is associated with altered channel kinetics compatible with a phenotype of an increased Ca^{2+} influx.[11] Notably, change of Val^{1696} to a phenylalanine (V1696F) or an isoleucine (V1696I) causes phenotypes of different severity. Two out of three V1696I mutation carriers had hemiplegic migraine without cerebellar signs or other severe neurological features.[10] Both V1696F mutation carriers, however, had a very severe phenotype of atypical AHC. Apparently, substitution of the valine residue for a bulky phenylalanine in the transmembrane domain has a more dramatic effect on channel functioning.

Previously, sporadic patients with typical AHC have been scanned for mutations in the *CACNA1A* (FHM1), *ATP1A2* (FHM2) and *SLC1A3* genes, but no mutations were found.[12-14] Now, we present the first *CACNA1A* mutation in patients with atypical AHC. Previously an *ATP1A2* mutation was identified in a Greek family with atypical AHC, with a similar overlapping FHM/AHC phenotype.[7,8] Of note, both *CACNA1A* and *ATP1A2* are involved in ion transport, and mutations in these genes are predicted to increase concentrations of K^+ and glutamate in the synaptic cleft as a result of either increased neurotransmitter release (*CACNA1A* mutations) or impaired removal of K^+ and neurotransmitter (*ATP1A2* mutations).[15,16] Elucidating the molecular basis in this German family with complex clinical features strengthens the evidence for a common pathogenesis of FHM and AHC. Our results suggest that in severely affected paroxysmal (alternating) hemiplegic patients with mental retardation and an age at onset of alternating episodes beyond 18 months, *CACNA1A* mutation scanning is indicated.

Acknowledgements

The authors thank Professor N. Hosten and M. Kirsch, MD (Institute for Diagnostic Radiology, University of Greifswald) for providing Figures 1 and 2. This work was supported by grants of the Netherlands Organization for Scientific Research (NWO) (903-52-291, M.D.F, R.R.F.; Vici 918.56.602, M.D.F), The Migraine Trust (R.R.F., M.D.F.), the EU 'Eurohead' grant (LSHM-CT-2004-504837; M.D.F., R.R.F., A.M.J.M.v.d.M.), EU FP6 ENRAH (LSSM-CT-2005-516513; A.M.J.M.v.d.M., L.A.E.M.L.) and the Centre of Medical System Biology (CMSB) established by the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (NGI/NWO).

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