
Research Submission

Migrainous Vertigo: Mutation Analysis of the Candidate Genes *CACNA1A*, *ATP1A2*, *SCN1A*, and *CACNB4*

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Background.—Migrainous vertigo (MV) is increasingly recognized as a common cause of episodic vertigo. MV displays several clinical similarities with familial hemiplegic migraine (FHM) and episodic ataxia type 2 (EA-2), which have been linked to mutations in 3 genes, *CACNA1A*, encoding a neuronal calcium channel α subunit, *ATP1A2*, encoding a catalytic subunit of a Na^+/K^+ -ATPase, and most recently the voltage-gated sodium channel *SCN1A*. The present study explored the hypothesis that mutations in *CACNA1A*, *ATP1A2*, *SCN1A*, and the calcium channel β_4 subunit *CACNB4* confer susceptibility to MV.

Methods.—Mutation analysis of the coding exons and exon/intron junctions of *CACNA1A*, *ATP1A2*, *SCN1A*, and *CACNB4* was performed in 14 unrelated MV patients by conformation sensitive gel electrophoresis and automated sequence analysis.

Results.—Analysis of the 4 candidate genes in the 14 MV patients resulted in the identification of a total of 26 sequence variants. The silent substitution D29D in *CACNB4* was observed in 2 MV patients and was not present in 46 ethnically matched control DNA samples. The remaining variants were also observed in control DNA samples and the allele frequencies of variants that resulted in amino acid substitutions were not significantly different between patients and controls.

Conclusions.—Based on this group of patients there is no evidence that the genes causing FHM and EA-2 represent major susceptibility loci for MV.

Key words: migrainous vertigo, calcium channel, *CACNA1A*, *CACNB4*, *ATP1A2*, *SCN1A*

Abbreviations: MV migrainous vertigo, FHM familial hemiplegic migraine, EA2 episodic ataxia type 2, PCR polymerase chain reaction, CSGE conformation sensitive gel electrophoresis

(*Headache* 2006;46:1136-1141)

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Accepted for the publication March 23, 2006.

Migrainous vertigo (MV) is one of the most common causes of recurrent spontaneous vertigo and is increasingly recognized among neuro-otologists and migraine specialists.¹⁻³ The concept of MV as a vestibular syndrome that is causally linked to migraine is supported by case-controlled studies, which demonstrated that migraine is more common in patients with vertigo compared to age- and sex-matched controls⁴ and that vertigo is more common in migraineurs than in a control group.⁵

The pathophysiology of MV is still a matter of speculation. A channelopathy appears to be the most

promising hypothesis due to the clinical overlap between MV and the paroxysmal disorders familial hemiplegic migraine (FHM) and episodic ataxia type 2 (EA-2), which result from mutations in the calcium channel gene *CACNA1A*.⁶ Hemiplegic migraine and EA-2 are often associated with vertigo⁷⁻⁹ and approximately 50% of EA-2 patients experience migraine, often fulfilling the criteria for basilar migraine.⁸ In addition, some MV patients are responsive to acetazolamide, which is also effective in the treatment of EA-2.¹⁰ Kim et al (1998) failed to identify *CACNA1A* mutations in 9 MV patients.¹¹ However, *CACNA1A* is still an attractive candidate gene for MV.

FHM is also associated with mutations in the $\alpha 2$ subunit of the Na^+/K^+ pump, *ATPIA2*,¹² and the voltage gated sodium channel alpha subunit *SCN1A*,¹³ raising the possibility that these genes may also contribute to MV. No *ATPIA2* mutations were identified in patients with common forms of migraine;¹⁴ however, a role for this gene in MV has not been examined. *SCN1A* dysfunction also underlies several related subtypes of human idiopathic epilepsy.^{15,16}

Although not previously implicated in migraine, we hypothesized that the calcium channel β_4 subunit gene, *CACNB4*, is also a candidate for MV. *CACNB4* modulates the biophysical properties of *CACNA1A* and similar phenotypes are observed in mice that carry mutations in either *CACNB4* or *CACNA1A*,¹⁷ suggesting that defects in either gene could potentially lead to a similar disorder. *CACNB4* mutations have been previously reported in patients with episodic ataxia and epilepsy.¹⁸

In this report, we present the results from the mutation analysis of 4 candidate genes in 14 MV patients. The candidate genes were *CACNA1A*, *ATPIA2*, *SCN1A*, and *CACNB4* on human chromosomes 19p13, 1q21-q23, 2q24, and 2q22-q23, respectively.

METHODS

Patients.—Patients with MV were included according to previously published, modified criteria:⁴

1. Episodic vestibular symptoms of at least moderate severity (rotational vertigo, other illusionary self or object motion, positional vertigo).

2. Migraine according to the diagnostic criteria of the International Headache Society (IHS).¹⁹
3. At least 1 of the following migrainous symptoms during at least 5 vertiginous attacks: migrainous headache, photophobia, phonophobia, visual or other auras.
4. Other causes ruled out by appropriate investigations.

Four male and 10 female unrelated MV patients (9 German, 4 Turkish, and 1 Bosnian) were enrolled in the study. Appropriate institutional informed consent was obtained from all participants. A detailed medical history was obtained from each patient and a comprehensive neurologic and neurotologic examination was performed.

Clinical Features.—The age of onset of MV ranged from 15 to 61 years. The duration of an attack of MV was seconds to minutes in 4 patients, up to several hours in 5 patients, and up to several days in 5 patients (Table 1). Most patients reported spontaneous attacks of vertigo but 2 had isolated recurrent episodes of positional vertigo (patients 13 and 14, Table 1). All patients except for patients 7 and 11 reported that vertigo had repeatedly occurred together with headaches. Patients 7 and 11 experienced the migrainous symptom of photophobia during vertiginous attacks. Patients 5 and 7 reported first-degree relatives with episodic vertigo and headaches who were, however, unavailable for consultation.

Interictal neurologic findings were unremarkable in all except four patients. Patient 5 showed transient downbeating nystagmus in the head hanging position, patient 9 had congenital pendular nystagmus and comittant strabism, patient 10 displayed horizontal head shaking nystagmus and patient 12 had unilateral gaze evoked nystagmus. Caloric testing was performed in 12 patients and was normal in all except patient 7 who displayed bilateral caloric hyporesponsiveness. This finding in patient 7 was likely to be a false positive result due to the absence of clinical signs of bilateral vestibulopathy. Audiometry was available from 13 patients, 11 of whom had normal results. In 2 patients with a longstanding history of nonfluctuating hearing loss, audiometry showed mild to moderate bilateral sensorineural hearing loss (patient 10) and moderate

Table 1.— Clinical Features of MV Patients

Patient	Sex/ Age	Age at Migraine/ MV Onset	Migraine Syndrome	Duration of MV Episodes	Frequency (Episodes/Year)	Vestibular Syndrome
1	F/47	20/30	MoA	Hours	6	CV, HMI
2	F/37	16/20	MoA	Minutes	24	CV, HMI
3	M/41	38/15	MoA	Days	48	CV, PV
4	F/52	20/20	MoA	Hours/days	6	CV
5	F/47	41/41	MoA	Seconds-days	24	CV, HMI
6	F/61	18/43	MoA	Days	1	CV, HMI
7	M/40	20/38	MoA	Hours	12	CV, PV
8	F/64	13/54	MoA	Minutes	36	CV
9	F/37	33/35	MoA	Seconds-minutes	60	CV, PV
10	F/41	20/28	MA	Seconds-hours	48	CV, PV
11	M/64	20/61	MoA	Minutes	72	CV, PV
12	M/42	16/40	MA	Minutes-hours	12	CV
13	F/67	45/45	MoA	Hours	6	PV
14	M/60	20/53	MoA	Days	1	PV

MoA = migraine without aura; MA = migraine with aura; CV = constant vertigo; PV = positional vertigo; HMI = vestibular head motion intolerance.

bilateral high-frequency hearing loss compatible with presbycusis (patient 13). Cerebral imaging with MRI or CT was performed in 10 patients and was unremarkable in all of them.

Mutation Screening.—Blood samples were obtained and DNA was extracted using standard methods. The coding exons and exon/intron junctions of each candidate gene were amplified from genomic DNA by polymerase chain reaction (PCR) using previously described primers and conditions for *CACNA1A*,⁶ *ATPIA2*,¹² *SCN1A*,¹⁵ and *CACNB4*.¹⁸ The PCR products were incubated at 99°C for 10 min-

utes and then 68°C for 30 minutes. Samples were analyzed by conformation sensitive gel electrophoresis (CSGE) and visualized by ethidium bromide staining as previously described.¹⁸ PCR products that generated mobility variants on the CSGE gel were reamplified and purified using the QIAquick PCR purification kit. DNA sequencing with BigDye Terminator mix was carried out on an ABI 3100 automated sequencer.

RESULTS

Nonsynonymous Substitutions.—A total of 4 nonsynonymous substitutions were identified (Table 2). These were E918D and E993V in *CACNA1A* exon 19, G1105S in *CACNA1A* exon

Table 2.—Nonsynonymous Substitutions Identified in the *CACNA1A* and *SCN1A* Genes

Location	dbSNP ID	Nucleotide	Major/Minor Allele	Amino Acid Change	Frequency of Minor Allele in MV Patients	Frequency of Minor Allele in Controls
<i>CACNA1A</i>						
Exon 19	rs16022	2754	G/C	E918D	0.18	0.16
Exon 19	rs16023	2978	A/T	E993V	0.18	0.16
Exon 20	rs16027	3313	G/A	G1105S	0.11	0.11
<i>SCN1A</i>						
Exon 16	rs2298771	3199	A/G	T1067A	0.46	0.43

Nucleotide positions based on Genbank Accession X99897 (*CACNA1A*) and AB093548 (*SCN1A*). The translational start site of each gene is considered as nucleotide position 1.

Table 3.—Synonymous and Intronic Variants Identified in the *CACNA1A*, *ATPIA2*, *SCN1A*, and *CACNB4* Genes

Location	dbSNP ID	Nucleotide	Major/Minor Allele	Amino Acid
CACNA1A				
Intron 1	rs16003	IVS1 + 53	G/A	NA
Exon 6	rs16006	876	A/G	E292E
Exon 8	rs2248069	1182	A/G	E394E
Intron 8	rs2306348	IVS8 + 48	A/G	NA
Intron 8	rs16008	IVS8 – 31	G/A	NA
Exon 16	rs16016	2094	G/A	T698T
Exon 19	rs16025	3060	G/A	R1020R
Exon 23	rs16030	3867	T/C	F1289F
ATPIA2				
Intron 1	NA	IVS1 – 12 (ins TTCC)	-/TTCC	NA
Exon 9	rs1063125	1119	G/A	S373S
Intron 10	rs5778151	IVS10 + 50 (ins G)	-/G	NA
Exon 16	rs17846715	2259	C/T	A753A
Intron 21	NA	IVS21 – 27	A/C	NA
Intron 22	NA	IVS22 + 14	C/T	NA
SCN1A				
Intron 6	rs994399	IVS6 – 21	T/C	NA
Intron 7	NA	IVS7 + 21	T/C	NA
Intron 7	NA	IVS7 + 100	A/T	NA
Intron 8	NA	IVS8 – 8	NA	NA
Exon 9	rs7580482	1212	A/G	V404V
Intron 23	NA	IVS23 + 33	A/G	NA
Exon 25	NA	4731	T/C	N1577N
CACNB4				
Exon 2	NA	87	T/C	D29D

Nucleotide positions based on Genbank Accession X99897 (*CACNA1A*), NM000702 (*ATPIA2*), AB093548 (*SCN1A*), and NM001005747 (*CACNB4*). The translational start site of each gene is considered as nucleotide position 1. NA, not applicable.

20 and T1067A in *SCN1A* exon 16. The minor alleles of the 2 *CACNA1A* exon 19 variants were observed together in 5 MV patients (18% allele frequency), suggesting that they are part of a single haplotype. The presence of at least 2 amino acid substitutions within this haplotype raised the possibility that it may be associated with altered channel function in MV patients. To test this hypothesis we PCR amplified and sequenced *CACNA1A* exon 19 from 46 control individuals that were negative for a history of MV. The controls were ethnically matched to the MV patients and comprised 40 German, 4 Turkish, and 2 Bosnian samples. The minor alleles of the *CACNA1A* exon 19 variants were detected at a frequency of 16% in the controls, indicating that this haplotype is unlikely to confer a highly penetrant susceptibility effect. Similar allele frequencies were also observed in MV patients and controls for the amino acid substitutions G1105S in *CACNA1A* and T1067A in *SCN1A* (Table 2).

Synonymous and Intronic Variants.—Twenty-two noncoding variants were identified; *CACNA1A* (8), *ATPIA2* (6), *SCN1A* (7), and *CACNB4* (1) (Table 3). The novel nucleotide substitution T87C in exon 2 of *CACNB4* was identified in 2 MV patients but was not observed in the control samples. This silent substitution does not change the amino acid sequence (D29D). All remaining variants were previously reported in the literature and were observed in control samples indicating that they are unlikely to be pathogenic.

COMMENTS

In this study, we examined a group of patients with recurrent vertigo that was temporally related to migraine symptoms. At present, there are no internationally approved criteria for MV. Vertigo is a common manifestation of basilar migraine.²⁰ However, the IHS diagnostic criteria for basilar migraine¹⁹ are rarely

fulfilled in MV because this requires at least 2 aura symptoms from the posterior circulation territory.²¹ Furthermore, the duration of an attack of MV can vary from seconds to several days, and as a result often differs from a typical migraine aura.^{4,21} Our group has proposed diagnostic criteria for MV based on a history of migrainous symptoms that are temporally related to recurrent vertigo.⁴ Recently, a prospective study showed that MV is most commonly accompanied by transient central vestibular dysfunction.²²

Taking into account the complex and heterogeneous pathophysiologic mechanisms underlying susceptibility to migraine disorders, the phenotypic dissection of specific migraine subtypes is a promising strategy to unravel core molecular pathways. Although the mechanism of vestibular dysfunction in MV is unknown, MV represents a suitable endophenotype to dissect phenotype-genotype relationships that are closer to the effect of the underlying susceptibility gene. The identification of the genes that underlie MV will provide important insights into core molecular pathways of this poorly understood migraine disorder and may have equally significant therapeutic implications.

The discovery that FHM is a channelopathy linked to at least 3 genes, *CACNA1A*⁶ and *ATP1A2*,¹² and *SCN1A*,¹³ led to speculation about the contribution of these genes to the more common forms of migraine. In patients with MV, a channelopathy could cause vestibular symptoms via several interfaces: (1) altered ion concentration in the endolymph could cause hair cell depolarization of the vestibular sensory epithelium; (2) calcium channel genes may affect neurotransmitter release in the vestibular system; and (3) altered calcium influx in neurons may provoke spreading depression like events.

In the present study, we identified several polymorphisms in the coding regions of *CACNA1A*, *ATP1A2*, *SCN1A*, and *CACNB4*. With the exception of the silent substitution D29D in *CACNB4*, all of the identified variants were also observed in unaffected controls. The 3 *CACNA1A* variants that resulted in amino acid substitutions were present at similar frequencies in patients and controls indicating that they are unlikely to contribute a highly penetrant susceptibility effect to the development of MV. Thus, if these

variants do confer susceptibility to MV then the size of the effect was too small to be detected in the present sample. These results confirm the findings of a previous study¹¹ and do not support a major role for these genes in MV.

Conflict of Interest: None

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