MIGRAINE AND EPISODIC ATAXIA TYPE 2: A CLINICAL, GENETIC, NEUROTOLOGIC, AND MAGNETIC RESONANCE SPECTROSCOPY STUDY

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Academic Dissertation

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LIST OF ORIGINAL PUBLICATIONS

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I Harno H, Hirvonen T, Kaunisto MA, Aalto H, Levo H, Isotalo E, Kallela M, Kaprio J, Palotie A, Wessman M, Färkkilä M. Subclinical vestibulocerebellar dysfunction in migraine with and without aura. *Neurology 61:1748-1752, 2003*

II Harno H, Kaunisto MA, Levo H, Aalto H, Isotalo E, Liukkonen E, Kallela M, Palotie A, Wessman M, Färkkilä M, Hirvonen T. Abnormal oculomotor and postural findings in a family with familial hemiplegic migraine type 2. (Submitted)

III Kaunisto MA, Harno H, Kallela M, Somer H, Sallinen R, Hämäläinen E, Miettinen P, Vesa J, Orpana A, Palotie A, Färkkilä M, Wessman M. Novel splice site *CACNA1A* mutation causing episodic ataxia type 2. *Neurogenetics 5:69-73, 2004*

IV Harno H, Heikkinen S, Kaunisto MA, Kallela M, Häkkinen A-M, Wessman M, Färkkilä M, Lundbom N. Decreased cerebellar total creatine in episodic ataxia type 2: a ^{1}H MRS study. *Neurology 64:542-544, 2005*

V Harno H, Hirvonen T, Kaunisto MA, Aalto H, Levo H, Isotalo E, Kallela M, Somer H, Palotie A, Wessman M, Färkkilä M. Acetazolamide improves neurotological abnormalities in a family with episodic ataxia type 2 (EA-2). *J Neurol 251:232-234, 2004*

ABBREVIATIONS

ATP1A2	gene coding for Na^+, K^+ -ATPase alpha2 subunit
ATP	adenosine triphosphate
BM	basilar-type migraine
BPPV	benign paroxysmal positional vertigo
CACNA1A	gene coding for P/Q-type calcium channel subunit alpha1A
$Ca_v2.1$	P/Q type (calcium channel)
cGMP	cyclic guanosine monophosphate
CGRP	calcitonin gene-related peptide
Cho	choline containing compounds
CI	confidence interval
CNS	central nervous system
Cr	creatine
CSD	cortical spreading depression
EA1	episodic ataxia type 1
EA2	episodic ataxia type 2
EEG	electroencephalogram
ENG	electronystagmography
FHM	familial hemiplegic migraine
FHM1	familial hemiplegic migraine type 1
FHM2	familial hemiplegic migraine type 2
H	proton
5-HT	5-hydroxytryptamine
IHS	International Headache Society
Lac	lactate
MA	migraine with aura
MO	migraine without aura
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAA	N-acetylaspartate
NO	nitric oxide
PCR	polymerase chain reaction
PCr	phosphocreatine
Pi	inorganic phosphate
ppm	parts per million
RF	radio frequency
SCA6	spinocerebellar ataxia type 6
SD	standard deviation
SFEMG	single-fiber electromyography
VOG	video-oculography

ABSTRACT

Oculomotor and other neurotologic dysfunction in migraine is evident, but results are not consistent. About 40% of familial hemiplegic migraine type 1 (FHM1) patients show permanent cerebellar signs, whereas familial hemiplegic migraine type 2 (FHM2) patients show normal neurologic status interictally. Episodic ataxia type 2 (EA2) patients can develop permanent cerebellar signs in the course of the disease and show pronounced nystagmus interictally before any other cerebellar signs are detectable. In this study, we examined quantitatively oculomotor function and other neurotologic findings in episodic ataxia type 2 (EA2) with a novel *CACNA1A* splice site mutation, in the common types of migraine, and in familial hemiplegic migraine type 2 (FHM2).

The neurotologic results for common types of migraine (36 patients and 43 controls) indicate that oculomotor dysfunction originates from the vestibulocerebellum for both migraine with aura (MA) and migraine without aura (MO). In video-oculography (VOG), abnormal nystagmus occurred in migraine subjects, but not in controls. Additionally, in migraine, increased body sway in posturography and decreased accuracy in saccadic tests suggested subclinical vestibulocerebellar dysfunction.

To our knowledge, quantitative oculomotor tests have not previously been performed on FHM2 patients. They usually show normal neurologic status interictally, as was the case with our FHM2 patients. However, the oculomotor tests on nine FHM2 patients suggested subclinical changes in postural control and in oculomotor function.

EA2 patients have well-characterized eye-movement abnormalities. They localize to the vestibulocerebellum with gaze-evoked, rebound, or downbeat nystagmus. We studied the effect of acetazolamide on baseline oculomotor and postural dysfunction in three EA2 family members on acetazolamide. Recordings were made with and without medication. After a wash-out period, we showed that with acetazolamide, interictal oculomotor and postural function in these patients improved.

Proton (^{1}H) magnetic resonance spectroscopy (MRS) of the brain in EA2 has shown high lactate peaks, but no changes in N-acetylaspartate (NAA) or in other ^{1}H MRS metabolite ratios. We studied nine mostly nonataxic EA2 family members with a specific *CACNA1A* mutation by ^{1}H MRS to evaluate metabolite changes in the cerebellar hemispheres, the vermis, and the thalamus. These EA2 patients, showing no atrophy in the cerebellum in MRI, showed decreased total creatine (tCr) in the cerebellar hemispheres and in the vermis. These results suggest cerebellar Purkinje cell dysfunction in EA2, possibly reflecting early signs of *CACNA1A* dysfunction.

In summary, this study suggests that MA and MO share similar abnormalities in interictal neurotologic dysfunction that may reflect central vestibular and cerebellar etiology. The results for FHM2 with subclinical abnormalities in neurotologic testing shared similar features with MA and MO as to defective oculomotor function. EA2 patients showed improved oculomotor function from baseline dysfunction with acetazolamide. Significantly decreased cerebellar tCr may reflect, in EA2, the first signs of intrinsic dysfunction of a *CACNA1A* mutation.

REVIEW OF THE LITERATURE

1. Clinical characteristics of migraine and episodic ataxia type 2

1.1. Migraine with and without aura

Migraine is a common neurological disorder, which affects up to 6% of men and 18% of women in the general population (Lipton and Stewart, 1998, Launer et al., 1999). Anyone can have an occasional migraine attack, if the triggering factors are strong enough, but migraine attacks must be recurrent before one is defined as a migraine patient. Common types of migraine include migraine with typical aura (MA) and migraine without aura (MO) according to International Headache Society criteria (IHS) (International Headache, 1988, Headache Classification Subcommittee of the International Headache, 2004). Migraine attacks are characterized by a unilateral throbbing headache of moderate to severe intensity, accompanied by nausea or vomiting, or photophobia and phonophobia. Physical exertion typically worsens the symptoms. About 20% of migraineurs have aura symptoms (MA) preceding a headache which usually starts within an hour after the aura has occurred. In MA, a visual aura with fortification spectrum is the most common aura type, with hemisensoric symptoms next in frequency. Less frequent are speech disturbances and unilateral weakness. Migraine attacks usually last from 4 to 72 hours.

Common symptoms among migraineurs are vertigo and dizziness (Baloh, 1997). Recently, migrainous vertigo was evaluated to be the most common cause of spontaneous recurrent vertigo (Neuhauser and Lempert, 2004), but is not presently included in the IHS criteria (Headache Classification Subcommittee of the International Headache, 2004). Migrainous vertigo presents with attacks of spontaneous or positional vertigo that last from seconds to days (Neuhauser et al., 2001). At least one of the following migrainous symptoms should occur at least in two vertiginous attacks: photophobia, phonophobia, migrainous headache, or visual or other auras. Migraine can cause positional vertigo as well, mimicking benign paroxysmal positional vertigo (BPPV). Compared to BPPV, migrainous vertigo presents shorter-lasting episodes, frequent recurrences, manifestation early in life, migrainous symptoms during positional vertigo, and atypical positional nystagmus (von Brevern et al., 2004).

BPPV and Meniere's disease occur often among migraineurs, but the pathophysiological link is as yet unknown. Migraine presents three times as often in patients with BPPV of unknown cause as in those with BPPV secondary to trauma (Ishiyama et al., 2000). The lifetime prevalence of migraine is increased in patients with Meniere's disease. Up to 45% of patients with Meniere's disease experience migrainous symptoms (migrainous headache, photophobia, aura symptoms), which suggests a pathophysiologic link between these diseases. On the other hand, current diagnostic criteria may not differentiate between Meniere's disease and migrainous vertigo with sufficient accuracy (Radtke et al., 2002).

1.2. Familial hemiplegic migraine (FHM)

Familial hemiplegic migraine (FHM) is a rare, autosomal dominant form of MA that includes motor weakness and at least one first- or second-degree relative with similar symptoms (International Headache, 1988, Headache Classification Subcommittee of the International Headache, 2004). The aura can be prolonged for several days or weeks and outlast the headache phase. Usually the aura symptoms follow each other in succession, with visual disturbances, hemiparesthesia, and dysarthria or dysphasia often preceding and accompanying hemiparesis (Ducros et al., 2001, Gardner et al., 1997). Basilar-type migraine symptoms (e.g., vertigo, loss of balance, diplopia, bilateral visual symptoms switching from one side to the other, and drop attacks, as well as bilateral paresis or paresthesia or both) often occur last in the aura succession. FHM and basilar migraine show overlapping clinical phenotypes (Haan et al., 1995); basilar migraine symptoms are present in up to 68% of FHM patients (Thomsen et al., 2002), but some FHM patients have atypical attacks with confusion, decreased consciousness, fever, aseptic meningeal reaction, or seizures (Ducros et al., 1997, Gardner et al., 1997, Echenne et al., 1999, Kors et al., 2001, Marconi et al., 2003), with minor head trauma at times provoking attacks (Gardner et al., 1997, Ducros et al., 2001, Vanmolkot et al., 2003, Kors et al., 2001). Cerebral or coronary angiography can also trigger an attack (Ducros et al., 2001). Interestingly, some FHM patients may present with the common types of migraine attacks, as well (Ducros et al., 2001, Thomsen et al., 2002). This supports the hypothesis that FHM may be part of the migraine spectrum and can be used as a model to study the complex genetics and pathophysiology of the common types of migraine.

FHM has been divided into type 1 (FHM1) and type 2 (FHM2) on a genetic basis. Clinically it is difficult to separate the disorders. Cerebellar signs divide FHM1 families into pure FHM families (80% of the families) and FHM with cerebellar signs (20%) (Ducros et al., 2001). FHM2 families have shown no cerebellar signs (Marconi et al., 2003) except for one family of Italian origin (Spadaro et al., 2004). A few sporadic hemiplegic migraine patients also show cerebellar signs (Ducros et al., 1999, Vahedi et al., 2000). In FHM1, minor head trauma can cause cerebral edema or even fatal coma (Kors et al., 2001). Mild to moderate mental retardation has occurred in two Italian FHM2 families (Marconi et al., 2003) and in a British FHM1 family (Kors et al., 2003b).

1.3. Episodic ataxia type 2 (EA2)

Two forms of familial episodic ataxia exist: episodic ataxia type 1 with periodical ataxia and interictal myokymia (EA1), while episodic ataxia type 2 (EA2) shows bouts of vertigo, ataxia, dysarthria, nystagmus, nausea, vomiting, and fatigue. EA2 attacks commonly last from half an hour to two hours, whereas the bouts in EA1 are brief, lasting only minutes (Baloh and Winder, 1991, Ophoff et al., 1996). As part of the attack spectrum, about half the EA2 patients have migraine headache, and some present with hemiplegia (Baloh et al., 1997, Jen et al., 2001, Jen et al., 2004). Some have reported epilepsy (Jouvenceau et al., 2001, Jen et al., 2004). Attacks are most often triggered by physical exercise or emotional

stress. Sometimes heat, coffee, small amounts of alcohol, or bright sunshine predispose to attacks (Subramony et al., 2003). The onset of the episodes is usually before age 20, whereas possible progressive cerebellar ataxia usually appears much later (Baloh et al., 1997, Harding, 1993). Spinocerebellar ataxia type 6 (SCA6), a late onset dominantly inherited ataxia syndrome, has clinical features — progressive cerebellar ataxia, oculomotor dysfunction, and ictal headaches or nausea (Ying et al., 2001, Sinke et al., 2001) — that may overlap with those of EA2 and FHM. SCA6 may present, although rarely, with episodes of dizziness and ataxia before permanent cerebellar signs. EA2 and SCA6 may reflect the same disorder with high phenotypic variability (Jen et al., 1998, Jodice et al., 1997).

In EA2 patients, neurological examination and MRI can range from normal findings to cerebellar ataxia plus atrophy (Baloh and Winder, 1991, Baloh et al., 1997). Moreover, variation in clinical phenotype appears between and within families (Van Den Maagdenberg et al., 2002).

2. Genetics and pathophysiology of migraine and EA2

2.1. Genetics of migraine

Family, twin, and genetic studies have shown that migraine etiology is complex, with multiple interacting genetic and environmetal factors (Russell and Olesen, 1995, Ulrich et al., 1999, Gervil et al., 1999). For example, a large population-based study shows that first-degree relatives of probands with MA have a relative risk of 3.8 for developing MA, whereas their spouses have no increased risk (Russell and Olesen, 1995). For MO, the relative risk for first-degree relatives is lower (1.9). The complex genetics of migraine has hampered gene identification in the common types of migraine with conflicting results (Table 1). Association studies, such as those of the dopaminergic system (Del Zompo et al., 1998, Peroutka et al., 1997, Maude et al., 2001, Shepherd et al., 2002), the serotonergic system (Erdal et al., 2001, Burnet et al., 1997, Kotani et al., 2002), mitochondria (Takeshima et al., 2001), the endothelial system (Tzourio et al., 2001), and homocysteine-related genes (Kara et al., 2003), are examples of areas of interest in migraine genetics.

Table 1. Results of linkage studies in common types of migraine.

Chromosome		Population/ number of families	Disease
19p13	May et al., 1995	German/ 28 families	MA
	Nyholt et al., 1998	Australian/ 4 families	
	Jones et al., 2001	US/ 16 families	
	Terwindt et al., 2001	Dutch/ 36 families	
No linkage to 19p13	Hovatta et al., 1994	Finnish/ 4 families	
	Noble-Topham et al., 2002	Canadian/ 64 families	
	Brugnoni et al., 2002	Italian/ 12 families	
	Wieser et al., 2003	German/ 143 unrelated patients	
	Kaunisto et al., 2004	Finnish/72 families	
1q31	Lea et al., 2002	Australian/ one family	MA and MO
4q24	Wessman et al., 2002	Finnish/ 50 families	MA
4q21	Björnsson et al., 2003	Icelandic/ 103 families	MO
6p12.2-p21.1	Carlsson et al., 2002	Swedish/ one family	MO and MA
11q24	Cader et al., 2003	Canadian/ 43 families	MA
14q21.2-q22.3	Soragna et al., 2003	Italian/ one family	MO
Xq24-q28	Nyholt et al., 2000	Australian/ 2 families	MA and MO

MA, migraine with aura; MO, migraine without aura

2.2. Genetics of FHM

FHM is inherited as an autosomal dominant trait and has turned out to be genetically heterogeneous (Ducros et al., 1997, Carrera et al., 1999). Nowadays, FHM is divided into two subtypes based on genetic background: Type 1 (FHM1) with mutations in the *CACNA1A* gene in chromosome 19p13 (Ophoff et al., 1996, Ducros et al., 2001) and type 2 (FHM2) with mutations in the *ATP1A2* gene on chromosome 1q23 (De Fusco et al., 2003, Vanmolkot et al., 2003). Some families are not linked to these loci, however, which indicates that more loci for FHM must exist (Ducros et al., 1997).

FHM1

In 1996 Ophoff et al. found a missense mutation in the *CACNA1A* of chromosome 19p13 from four FHM families. *CACNA1A* encodes alpha 1A subunits of voltage-gated P/Q-type ($Ca_v2.1$) calcium channels in neurons, with an especially abundant expression in Purkinje cells of the cerebellum (Mori et al., 1991) (Figure 1). After 1996, at least 14 *CACNA1A* missense mutations were identified in 35 FHM families (Carrera et al., 1999, Ducros et al., 2001, Wada et al., 2002, Takahashi et al., 2002, Alonso et al., 2003, Kors et al., 2003b). T666M mutation of the *CACNA1A* has been the most frequent mutation associated with 17 FHM families (Ducros et al., 2001, Takahashi et al., 2002, Kors et al., 2003b). Kors et al. (2003b) described the clinical picture of five unrelated FHM families with a T666M mutation. They found remarkable clinical heterogeneity among the families: Three of the families showed cerebellar ataxia, one family showed attacks with confusion without hemiparesis, and another family, progressive cognitive dysfunction. About 20% of FHM1 patients present with cerebellar ataxia and cerebellar atrophy (Ducros et al., 2001).

CACNA1A mutations have been found in six sporadic hemiplegic migraine patients, as well (Carrera et al., 1999, Ducros et al., 2001, Terwindt et al., 2002, Kors et al., 2003a), most of them with cerebellar signs. However, in a study of Terwindt et al., (2002), most of these patients who were screened (25 of 27 patients) displayed no *CACNA1A* mutations.

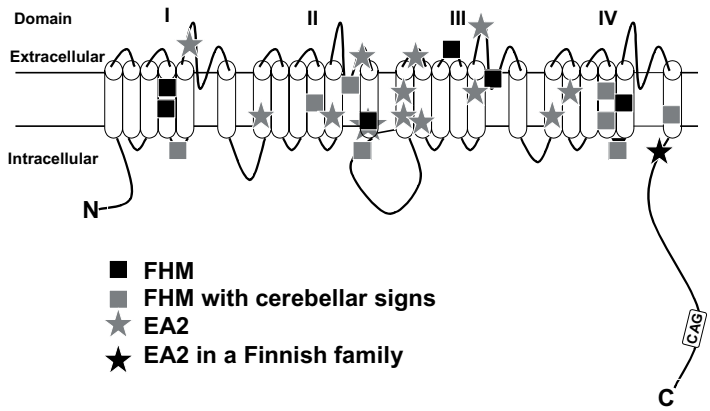


Figure 1. Structure of the alpha1A calcium channel subunit with some mutations indicated.

FHM2

DeFusco et al. (2003) were the first to report two *ATP1A2* missense mutations in chromosome 1q23 to cosegregate with FHM2. These were soon followed by Valmolkot et al. (2003) with a report of two novel missense mutations in the *ATP1A2* in two families with FHM. Interestingly, one of their families cosegregated partially with benign familial infantile convulsions. *ATP1A2* encodes the alpha2 subunit of the Na^+, K^+-ATPase, which is expressed mostly in the astrocytes (Moseley et al., 2003). FHM shows incomplete penetrance in both FHM1 and FHM2 (Ducros et al., 1995, 2001), possibly with FHM2 presenting lower penetrance than FHM1 (Ducros et al., 1997).

2.3. Genetic background of EA2

Episodic ataxia type 1 and type 2 are each inherited as an autosomal dominant trait, EA1 with mutations in a brain potassium channel (*KCNA1*) located on chromosome 12p, and EA2 with mutations in the *CACNA1A* on chromosome 19p13.

CACNA1A mutations show remarkable genotype-phenotype correlation complexity (Kors et al., 2002, Jen and Geschwind, 2001). Currently at least 34 different mutations in the *CACNA1A* gene have been identified in 26 EA2 families and 11 sporadic cases (Ophoff et al., 1996, Denier et al., 1999, Yue et al., 1998, Jen et al., 1999, Scoggan et al., 2001, Jen et al., 2001, Van Den Maagdenberg et al., 2002, Matsuyama et al., 2003, Subramony et al., 2003, Jen et al., 2004). Early data associated *CACNA1A* missense mutations with FHM, and nonsense or splice site mutations with EA2. However, missense mutations (Friend et al., 1999, Denier et al., 2001, Guida et al., 2001, Jen et al., 2001, Van Den Maagdenberg et al., 2002, Jen et al., 2004), a deletion of three nucleotides (Denier et al., 1999), and a CAG repeat expansion (Jodice et al., 1997) have also caused EA2. A CAG repeat expansion usually causes SCA6, a late-onset ataxia (Zhuchenko et al., 1997), but a *CACNA1A* missense mutation has also been identified in a family with a SCA6 phenotype (with episodic features) (Yue et al., 1997). EA2 mutation carriers show highly variable penetrance and expression of the disorder (Denier et al., 1999). Furthermore, as more EA2 families have nowadays been identified, evidence shows that the type of mutation or the mutation location in the *CACNA1A* does not predict the clinical phenotype (Jen et al., 2004). Anticipation, i.e., onset of disease as being earlier and symptoms being worse in generations to come, has not been reported in EA2 families (Baloh et al., 1997, Van Den Maagdenberg et al., 2002, Subramony et al., 2003), except in one study (Teh et al., 1995).

2.4. Migraine mechanisms

Substantial knowledge exists about migraine pathophysiology once the attack has started, but what is not known is why and how the attack actually begins (Ferrari, 1998, Kors et al., 2003a, Silberstein, 2004). The aura is, according to the current view, caused by ”cortical spreading depression” (CSD) (Leao, 1944, Lauritzen, 1994). This is a depolarization wave that propagates across the cortex at 2 to 3-mm/min followed by a failure of brain-ion ho-

meostasis, efflux of excitatory amino acids from nerve cells, enhanced energy metabolism (Lauritzen, 1994), and transient increases in cortical blood flow followed by sustained flow decreases (Bolay et al., 2002). In migraine headache pathophysiology, the trigeminovascular system is pivotal (Figure 2). The activation of the trigeminal nucleus caudalis and the trigeminal afferent fibers that innervate large cerebral vessels, pial vessels, large venous sinuses, and dura mater causes perivascular release of powerful vasoactive neuropeptides. This leads to vasodilatation, neurogenic inflammation, central transmission of pain information, and headache (Ferrari, 1998, Kors et al., 2003a). Bolay et al. (Bolay et al., 2002) found, in an experimental study of CSD, a possible link between CSD and trigeminovascular activation in migraine with aura. Furthermore, in cats and rats, CSD induced a widespread release of nitric oxide (NO) (Read et al., 2000), which is a potent endogenous vasodilatator with multiple physiological actions. Increased cortical concentration of cyclic guanosine monophosphate (cGMP) (Read et al., 2001) and increased extracellular K^+ (Van Den Maagdenberg et al., 2004) may facilitate CSD, as well.

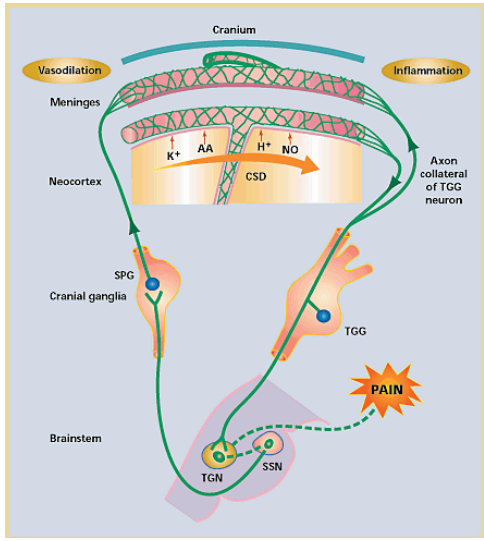


Figure 2. Trigeminovascular activation, where the activation of the trigeminal nucleus caudalis (TGN) and the trigeminal fibers cause perivascular release of vasoactive neuropeptides in large cerebral vessels and dura mater. This leads to vasodilatation, neurogenic inflammation, central transmission of pain information, and headache. Cortical spreading depression (CSD) may facilitate headache in migraine by releasing extracellular potassium ions (K+), hydrogen ions (H+), arachidonic acid (AA), nitric oxide (NO), etc. which in turn may depolarize perivascular trigeminal terminals that cause activation of the TGN in the brainstem. The activation of TGN caused by CSD produces vasodilatations of meningeal vessels through a pathway originating from the superior sagittal sinus (SSN) and reaching meningeal blood vessels via the sphenopalatine ganglion (SPG). The dashed lines between TGN, SSN, and regions generating the pain indicate that these connections are either unknown or have not been depicted. (Reprinted with permission of Nature's copyright from Iadecola C: From CSD to headache: a long and winding road. Nat Med 2002;8:110-112. http://www.nature.com/*)*
TGG = trigeminal ganglion; SPG = sphenopalatine ganglion; SSN = superior sagittal sinus

During a migraine attack, more than 70% of migraine patients develop cutaneous allodynia (exaggerated skin sensitivity) (Burstein et al., 2000). This suggests a transient increase in the responsiveness (i.e., sensitization) of central pain neurons in addition to irritation of meningeal perivascular pain fibers.

Vascular risk factors such as smoking, high blood preassure, or use of oral contraceptives seem, in migraine patients, to have more than a multiplicative effect on risk for ischemic stroke (Chang et al., 1999). Migraine has been suggested to be an independent risk factor

for stroke in women of childbearing age (Tzourio et al., 1995, Carolei et al., 1996, Merikangas et al., 1997), especially those with MA (Donaghy et al., 2002, Kruit et al., 2004). Possibly 20% to 40% of strokes in migrainous women develop directly from a migraine attack (Chang et al., 1999). Reports on patients with migrainous infarction suggest that the posterior circulation territory is most commonly affected (Hoekstra-van Dalen et al., 1996, Milhaud et al., 2001). In addition, recent data on the prevalence of brain infarction and white matter lesions among patients with MA and MO confirm the vulnerability of the posterior circulation territory, especially the cerebellum of MA patients (Kruit et al., 2004). The risk for deep white matter lesions increases with attack frequency in women with MA or MO (Kruit et al., 2004).

The periaqueductal gray matter is at the center of the descending antinociceptive neuronal network. Iron homeostasis in this periaqueductal gray matter in patients with migraine with and without aura and chronic daily headache is progressively impaired. Thus the periaqueductal gray matter presents a possible important role in migraine attack generation, potentially by the dysfunction of the trigeminovascular nociceptive system (Welch et al., 2001).

5-Hydroxytryptamine

The metabolism of 5-hydroxytryptamine (5-HT) is disturbed in migraine patients with reduced systemic 5-HT levels interictally and raised levels during migraine attacks. This phenomenon may reflect the failing self-defence mechanism (Ferrari et al., 1989). Acute antimigraine drugs, triptans, are $5\text{-HT}_{1B/1D}$ receptor agonists with an ability to inhibit release of neuropeptides from trigeminal sensory nerve fibers (Goadsby, 2000). Triptan therapy provides complete pain relief for many migraine patients, but not for others. The probability of consistent pain-free outcome increases if triptan therapy is timed to precede any signs of cutaneous allodynia (Burstein et al., 2004). The exact mechanism and site of action by which triptans abort migraine pain is unknown. In an animal model of intracranial pain, the analgesic action of sumatriptan appeared to exert its effects through presynaptic $5\text{-HT}_{1B/1D}$ receptors in the dorsal horn, with blockage of synaptic transmission in the axon terminals of peripheral trigeminovascular neurons and in the cell bodies of central trigeminovascular neurons (Levy et al., 2004).

Interestingly, P-type neuronal calcium channels in vitro mediate 5-HT release (Codignola et al., 1993), adding a possible predisposition to migraine attacks in patients with these dysfunctioning channels. In rats, P/Q-, N-, and L-type voltage-gated calcium channels showed involvement in neurogenic trigeminovascular dural vasodilatation, which may imply involvement in trigeminovascular nociception (Akerman et al., 2003).

Glyceryl trinitrate

Glyceryl trinitrate, a nitric oxide (NO) donor, can trigger a short-lived headache in healthy control individuals and in migraineurs, but in these patients it often also induces a delayed migraine attack (Olesen et al., 1994). The initial headache is thought to originate from direct action of the NO-cGMP pathway, while the delayed migraine is likely to result from trigeminovascular activation (Akerman et al., 2002). Increasing scientific evidence sug-

gests that NO plays an important role in primary headaches (Thomsen and Olesen, 2001). It has multiple physiological actions such as endothelium-dependent vasodilatation and neurogenic vasodilatation both of which may be mediated via perivascular nerves (Toda and Okamura, 1991, Toda et al., 1997), and may release relevant neurotransmitters such as calcitonin gene-related peptide (CGRP) from trigeminal fibers (Wei et al., 1992, Akerman et al., 2002, Strecker et al., 2002). Moreover, NO plays a role in neurotransmission in the central nervous system (CNS), which is of importance in pain perception (hyperalgesia) (Woolf and Thompson, 1991).

Endothelin 1

In migraine patients, endothelin 1, a potent vasoconstictive peptide, has shown increased plasma levels ictally (Farkkila et al., 1992, Gallai et al., 1994, Kallela et al., 1998) and interictally (Gallai et al., 1994). The biological effects of endothelin 1 are mediated by endothelin type A and endothelin type B receptors. The former are expressed in vascular smooth muscle cells, and the receptor activation leads to vasoconstriction by endothelin 1 (Arai et al., 1990). The endothelial system is complex, however, with a preceding phase of vasodilatation induced by the endothelin type B receptor activation before the endothelin type A receptor-mediated vasoconstriction takes place. Endothelin type A receptors mediate an inhibitory action on NO synthesis in vascular smooth muscle cells by endothelin 1 stimulation (Ikeda et al., 1997).

2.5. Functional studies and insights into the pathophysiology of FHM and EA2

2.5.1. In vitro studies

FHM1

In vitro, some of the FHM1 mutations have produced gain-of-function, others loss-of-function effects, or functional instability of human P/Q-type calcium channels (Hans et al., 1999, Kraus et al., 1998, Kraus et al., 2000). In migraine with aura, single-fiber electromyography (SFEMG) shows mild abnormalities in neuromuscular transmission (Ambrosini et al., 2001). After acetazolamide treatment for several weeks, the SFEMG shows abnormalities in migraine patients as being normalized (Ambrosini et al., 2003), which suggests that genetically modified $Ca_v2.1$ channels may be present in typical migraine as well. This conclusion has caused debate, because the possibility that $Ca_v2.1$ calcium channel dysfunction underlies typical migraine leads to ongoing controversy (van den Maagdenberg and Plomp, 2003).

FHM2

Mutations in the *ATP1A2* gene may result in a loss of function of the *Na^+,K^+-ATPase* pump, which may depolarize neurons. On the other hand, *Na,K-ATPase* may be kinetically altered but fully functional, and cause FHM2 (Segall et al., 2004). The neurons depolarize by impaired maintenance of Na^+ and K^+ gradients across the cell membrane (De Fusco et

al., 2003). Furthermore, the *Na^+,K^+-ATPase* pump alpha2 subunit is localized coincidentally with the Na^+,Ca^{2+} exchanger, which may eventually lead to an increase in intracellular Ca^{2+}. Four isoforms of the catalytic alpha subunit have been identified to have a tissue- and developmental-specific distribution (Lingrel et al., 2003). Their functional roles are as yet unknown, for the most part. Astrocytes that highly express *ATP1A2* may impair K^+ clearance (Juhaszova and Blaustein, 1997). Consequently this, by increased extracellular K^+, may facilitate CSD (Van Den Maagdenberg et al., 2004). Impaired *Na^+,K^+-ATPase* pumps may also cause an electrochemical Na^+ gradient insufficient for astrocytes to remove synaptic glutamate, which in part may modulate susceptibility and sustained migraine attacks (Moskowitz et al., 2004) (Figure 3).

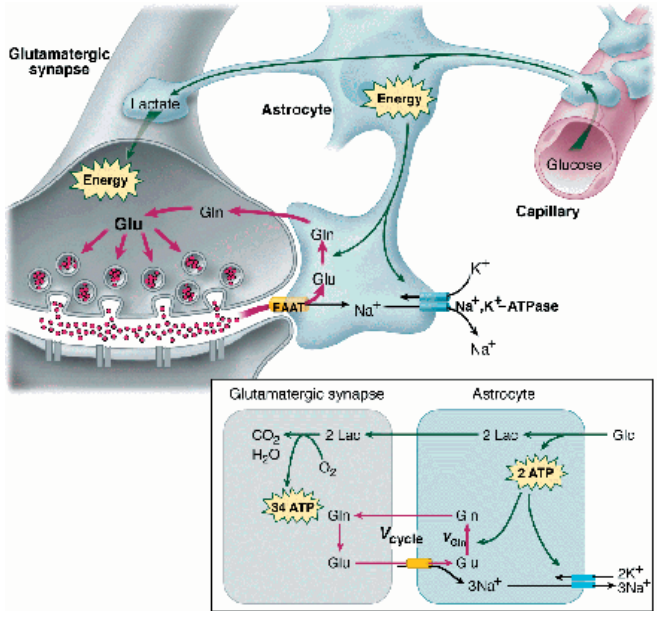


Figure 3. Synaptic activity is coupled to glucose usage (upper panel). After depolarization, glutamate is released into the synaptic cleft regulated by $Ca_v2.1$ calcium channels that gate calcium influx. Glutamate is taken up by astrocytes via specific transporters that use the electrochemical gradient of Na+ as a driving force. The Na+ gradient is maintained by the activity of Na+, K+-ATPase that removes sodium from inside cells. Glutamate (Glu) is converted into glutamine (Gln) and subsequently released by astrocytes and taken up by neuronal terminals, where it is enzymatically reconverted to Glu (lower panel). (Reprinted with permission from Magistretti PJ, Pellerin L, Douglas L, et al.. Energy on demand. Science; Vol 283 (5401):496-497,1999. Copyright 1999 AAAS)

V_{cycle}, flux from glutamate to astrocytic glutamine; V_{gln},rate of glutamine cycle.

EA2

In EA2, missense and nonsense mutations in *CACNA1A* produce mutant channels with diminished whole-cell calcium channel activity in vitro due to loss of function (Jen et al., 2001, Wappl et al., 2002), with some of the mutations producing partially functional channels (Wappl et al., 2002). These altered biophysical properties may contribute to in vivo abnormal neuromuscular transmission, manifesting as weakness in the attack profile. Indeed, SFEMG shows jitter and blocking in EA2 patients (Jen et al., 2001), with signs of presynaptic failure of neurotransmission (Maselli et al., 2003). Neuromuscular transmission in SFEMG in SCA6 patients is normal, suggesting that the nature of the SCA6 mutations produces a distinctive phenotype at the level of neuromuscular transmission (Schelhaas et al., 2004).

2.5.2. Mouse models

In order to investigate mechanisms of ataxia, dystonia, epilepsy, and migraine, at least four mutant mice models with spontaneous *Cacna1a* mutations have been developed: *tottering*, *leaner*, *rolling*, and *rocker* mice. Their phenotypes differ significantly in respect to ataxia severity, absence epilepsy and paroxysmal dyskinesias, cerebellar cell death, and ectopic dendritic spines on Purkinje cells (Zwingman et al., 2001). Ataxia is milder in *tottering* and *rolling* mice than in *leaner* mice (Mori et al., 2000). Significant cerebellar cell loss occurs in *leaner* and *rolling* mice, in contrast to *rocker* and *tottering* mice. The null mutant mice lack expression of the alpha1A subunit and thus have a complete loss of P-type channel function. These mice display severe progressive ataxia with premature death (Jun et al., 1999). *Leaner* and *tottering* mice have absence epilepsy (Noebels, 1984, Fletcher et al., 1996), whereas *rocker* mice have the first allele to present absence seizures without paroxysmal dyskinesias (Zwingman et al., 2001).

Recently, a *Cacna1a* knockin transgenic mouse model was generated with the human R192Q mutation that causes pure FHM1 (Van Den Maagdenberg et al., 2004). The investigators found multiple gain-of-function effects with increased $Ca_v2.1$ current density in cerebellar neurons, enhanced neurotransmission at the neuromuscular junction, and a reduced threshold as well as increased velocity of CSD. They concluded that hyperexcitability possibly causes the increased susceptibility to CSD and aura in migraine.

P/Q type calcium channels that are located on presynaptic terminals as well as on somatodendritic membranes regulate neurotransmitter release, postsynaptic calcium fluxes, and excitability. The functional consequences of FHM1 mutations for the membrane properties of $Ca_v2.1$ are complex. Overall, all FHM1 mutations analyzed thus far display enhanced Ca^{2+} influx through single $Ca_v2.1$ channels for voltages lower than –10mV (single channel gain-of-function phenotype). On the other hand, a loss of function was found in tranfected neurons and HEK293 cells due to a proposed reduced density of functional $Ca_v2.1$ channels in the membrane (Tottene et al., 2002). Van den Maagdenberg et al. (Van Den Maagdenberg et al., 2004) later criticized this conclusion: Their data on the knockin mouse model suggest that transfected cells (either neurons or HEK293 cells) can reliably be used to study the effect of mutations on single channel function, but are unreliable in studying consequences of the ion channel mutation on the whole-cell current density.

Synaptic release of glutamate from cortical neurons depends primarily on the opening of Ca_v channels, whereas these channels are less significant in the regulation of gamma-amino butyric acid, GABA, release (Moskowitz et al., 2004). Accordingly, based on cortical microdialysis, both *tottering* mice and the more severely affected allele *leaner* mice, have shown a decrease in depolarization-induced release of glutamate with almost no change in GABA. *Tottering* and *leaner* mice also showed a 10-fold resistance to CSD and, for *leaner* mice, also a slower velocity of CSD propagation (Ayata et al., 2000). These mice show an opposite electrophysiological phenotype to that of the R192Q KI mutants. The lowered threshold for CSD in the homozygous R192Q KI mice may be due to cortical hyperexcitability resulting from increased glutamate release (Van Den Maagdenberg et al., 2004).

Null mutant *Atp1a2* mice die just after birth (Moseley et al., 2003, Ikeda et al., 2003, Lingrel et al., 2003). In these homozygous mice, Moseley et al. (Moseley et al., 2003) reports a primary neuronal defect in the brainstem. Heterozygous *Atp1a2* mutant mice show hypercontractile hearts but, when compared to the wild type, no gross abnormalities (James et al., 1999).

2.6. Acetazolamide – a possible preventive medicine in EA2 and migraine

Acetazolamide is an inhibitor of the carbonic anhydrase enzyme, which catalyzes the following reaction:

$$CO_2 + H_20 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO^-_3$$

The exact preventive mechanism of acetazolamide in ion-channel disorder disease attacks is as yet unknown. However, pH changes under acetazolamide treatment are evident, with a possible stabilizing effect on dysfunctioning ion channels. By inhibiting carbonic anhydrase, acetazolamide causes metabolic acidosis with extracellular pCO_2 increase and extracellular pH decrease. The carbonic anhydrase enzyme occurs in erythrocytes as well as in many other tissues such as the nervous system, kidney, gastric mucosa, and the eyes (Settakis et al., 2003). In the CNS, carbonic anhydrase occurs in the external surface of glial cells (Giacobini, 1962).

Acetazolamide suppresses EA2 attacks effectively (Griggs et al., 1978, Baloh and Winder, 1991), although it seems not to prevent progressive ataxia that may start later in life. There are few reports on acetazolamide responsiveness in FHM (Athwal and Lennox, 1996, Battistini et al., 1999, Kors et al., 2003b), on typical migraine (Vahedi et al., 2002, Haan et al., 2000), or on dominantly inherited migraine with episodic vertigo and essential tremor (Baloh et al., 1996).

In prevention of typical migraine, one acetazolmide trial was prematurely halted because of the high number of withdrawals, primarily due to acetazolamide-induced multiple side-effects (Vahedi et al., 2002). No obvious prophylactic beneficial effect of acetazolamide on migraine attacks had, however, appeared. On contrast to this, migraine aura status patients benefited from acetazolamide, both in treatment of ongoing symptoms and in prevention of further migraine aura status periods (Haan et al., 2000).

3. The peripheral and central vestibular system

The inner ear is located within the petrous portion of the temporal bone inferior to the temporal lobe. The inner ear is made up of a bony labyrinth comprising a vestibular and a cochlear part. The cochlear part is further composed of membranous partitions of scala vestibule, scala media, and scala tympani. The scala media houses the organ of Corti that is responsible for hearing. The vestibular partitions consist of three semicircular canals and two otolith organs. The semicircular canals respond to angular acceleration of the head and the otoliths to linear acceleration.

Moreover, the maintenance of body equilibrium and posture involves multiple other receptor organs and neural centers in addition to the labyrinths. Visual and proprioceptive reflexes must be integrated with vestibular reflexes to ensure postural stability. The functioning of the overall system is integrated in the brain stem and in the cerebellum by means of commissural, inferior olivary, and reverberating circuits.

3.1. The peripheral system

The vestibular system works in conjunction with the visual and proprioceptive systems to achieve gaze stability and postural stability. The peripheral vestibular system comprises the ear and the vestibular labyrinth with two otolith organs (the saccule and utricule) and a set of three semicircular canals (Figure 4). These form a coordinate system with the anterior and posterior canal in vertical positions almost orthogonal to each other, while the horizontal canal makes a 30° angle with the horizontal plane. Each of the canals enlarges at its opening to the utriculus to form an ampulla.
A crest-like septum, the crista, crosses each ampulla and consists of a sensory epithelium of hair cells lying on a mound of connective tissue. The endolymph movement within the canals stimulates these hair cells and thus transmits a biologic signal to an afferent neuron.

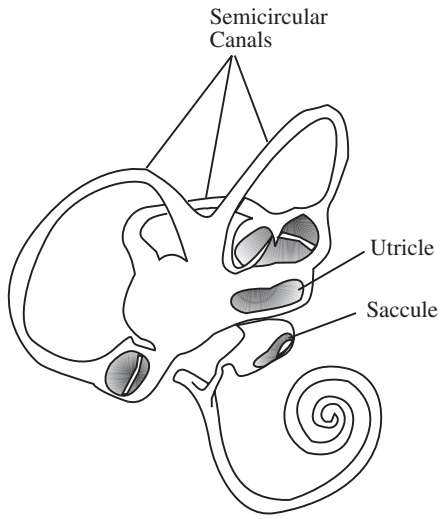


Figure 4. Vestibular labyrinth with two otolith organs (saccule and utricule) and semicircular canals.

The utriculus and the sacculus each have a sensory area with hair cells, the macule, which is covered by the gelatinous otolithic membrane. This membrane contains superficial calcareous deposits, the otoconia. Hair cells transmit signals to afferent neurons through the earth gravitation axis of the otoconia. The saccular and utricular macules sense linear acceleration, while the cristae of the three semicircular canals sense angular acceleration of the head. The labyrinthine blood circulation arises from the basilar artery: the anterior inferior cerebellar artery, labyrithine artery, common cochlear artery, anterior vestibular artery, and posterior vestibular artery, and their branches.

3.2. The central system

The central vestibular system consists of vestibular nuclei in the brain stem, cerebellar-vestibular interaction, vestibulospinal pathways, visual-vestibular interaction, and neck-vestibular interaction (Figure 5). The vestibular nuclei are four: superior, lateral, medial, and inferior. Signals from thousands of primary afferent vestibular nerve fibers bombard the

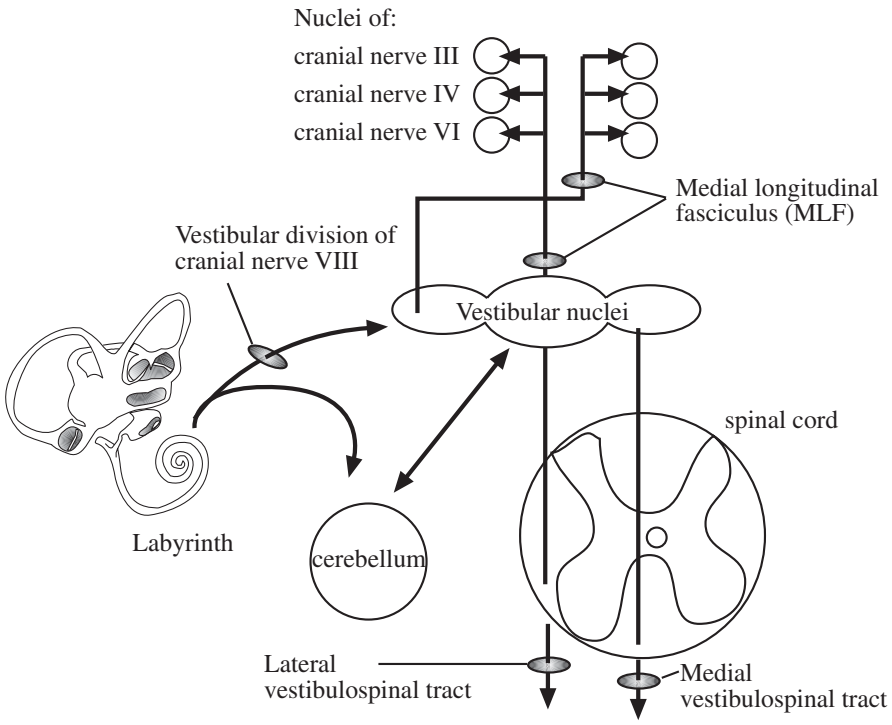


Figure 5. Diagram of the central vestibular system with multiple interactions.

vestibular nuclei in a specific orientation, depending on their organ of origin. After entering the brain stem, they divide into secondary ascending and descending branches each to reach its specific part of the vestibular nuclei. In addition to labyrinthine afferents, afferent fibers from other systems (proprioceptive) and centers (especially the cerebellum) interact with signals from the vestibular organs.

The vestibulo-ocular reflexes — connections between the labyrinthine end-organs and eye muscles — involve the activity of many nuclei within the cerebellum and the brain stem and countless numbers of neurons that form disynaptic and polysynaptic excitatory and inhibitory pathways. The activity of a receptor organ produces activity in a group of motor neurons that coordinate eye-muscle contraction to compensate for a specific head movement and to maintain gaze stability. Alternate pathways also exist, with chains of interneurons forming reverberating circuits. These interact and fine-tune more specific end-organ reflexes. (Baloh, 2001)

3.3. Nystagmus

Nystagmus is a combination of nonvoluntary rhythmic slow and fast eye movements alternating in opposite directions. The direction of the fast component defines the direction of the nystagmus. Lesions of the labyrinth, of the vestibular nerve, of the vestibular nuclei, of

the vestibulo-ocular pathways, and of the cerebellum may cause nystagmus. The magnitude of induced nystagmus depends on the state of arousal and mental alertness as well.

Several types of abnormal nystagmus exist: peripheral spontaneous, central spontaneous, congenital, periodic alternating, gaze-evoked (symmetric, asymmetric, rebound, or dissociated), positional (paroxysmal or persistent), head-shaking, and hyperventilation-induced nystagmus, in addition to other ocular oscillations. Complete darkness often provokes nystagmus. When tested with eyes open in darkness, normal subjects commonly have low-velocity postitional nystagmus (range from 20% to 75%) (McAuley et al., 1996, Bisdorff et al., 2000).

Differentiation between peripheral and central types of nystagmus is essential, but is quite difficult thus far. In spontaneous nystagmus of the central type, the appearance is often pure vertical, horizontal, or torsional; fixation usually has little effect; nystagmus may change direction. In comparison, the peripheral spontaneous nystagmus has a combined torsional or horizontal appearance; fixation inhibits nystagmus and is unidirectional. Position change may alter central positional nystagmus, suggesting that peripheral otolith input can alter the central imbalance.

Gaze-evoked nystagmus

Gaze-evoked nystagmus causes difficulties in maintaining stable conjugate eye deviation away from the primary position. The eyes drift back toward the center with corrective saccades (fast components) constantly resetting the desired gaze position. Gaze-evoked nystagmus is always in the direction of the desired gaze. The causes of abnormality can occur anywhere from the neuromuscular junction to the oculomotor nuclei of the brain stem. Common causes are drugs, metabolic disorders, tumors, infarction, atrophy, multiple sclerosis, or myasthenia gravis. Symmetric gaze-evoked nystagmus (equal amplitude to the left and right) is often caused by drugs (e.g., diazepam, phenytoin), alcohol, myasthenia gravis, multiple sclerosis, or cerebellar atrophy. Asymmetric horizontal gaze-evoked nystagmus, on the other hand, may indicate a structural lesion in the brain stem or the cerebellum. Rebound nystagmus occurs in patients with cerebellar atrophy or a focal cerebellar lesion, and is thus thought to be specific for cerebellar involvement. Rebound nystagmus either disappears or reverses direction when the lateral gaze position is held. When the eyes return to the primary position, another burst of nystagmus occurs in the direction of the return saccade. Dissociated eye movements with gaze-evoked nystagmus are called internuclear ophthalmoplegia: The adducting eye lags behind and develops a low-amplitude nystagmus while the abducting eye overshoots the target and develops large-amplitude nystagmus. Internuclear ophthalmoplegia usually results from lesions of the medial longitudinal fasciculus, MLF, that most commonly result from multiple sclerosis or vascular disease of the brain stem.

Positional nystagmus

If a semicircular canal cupula is altered so that its specific gravity no longer equals that of the surrounding endolymph, or if debris inappropriately enters a semicircular canal, the canal becomes sensitive to changes in the direction of gravity and can produce positional

nystagmus. The classifications of positional nystagmus are type I (direction changing), type II (direction fixed), and type III (less clearly defined type). Most investigators agree on two general categories of positional nystagmus: paroxysmal and persistent.

Paroxysmal positional nystagmus results most commonly for benign peripheral reasons but can also arise from lesions of the brain stem and the cerebellum. Benign paroxysmal positional vertigo of the posterior canal (BPPV) with a specific type of nystagmus is induced by a rapid change from erect sitting to the supine head-hanging left or right position (Dix-Hallpike test). It is initially high in frequency but rapidly diminishes. There is a 3- to 10-second latency before onset, and the nystagmus rarely lasts longer than 30 sec. The nystagmus shows combined torsional and vertical components. A burst of nystagmus occurs in the reverse direction when the patient moves back to the sitting position. An essential feature is the severe vertigo that the patient experiences on return to the initial position. With repeated positioning, vertigo and nystagmus rapidly disappear.

The central type of paroxysmal positional nystagmus does not decrease in amplitude or duration with repeated positioning, does not have a clear latency, and usually lasts longer than 30 seconds. The direction is unpredictable and may be different in each position. It is often vertical with a downward fast phase.

Persistent positional nystagmus remains as long as the position is held, although it may fluctuate in amplitude and frequency. Patients with compensated vestibular imbalance due to either peripheral or central lesions may develop a transient nystagmus after vigorous head-shaking. (Baloh, 2001)

3.4. Laboratory examinations of the vestibular system

To examine the vestibular system adequately, one must isolate it from other sensory systems. This is a difficult task at bedside. Here we introduce laboratory examinations used in Studies I, II, and V.

Electronystagmography
Electronystagmography (ENG) acts as an electric dipole that measures the difference in potential between the cornea and retina (corneal-retinal potential). An electrode placed in the vicinity of the eye becomes more positive when the eye rotates toward it and less positive when it rotates in the opposite direction. Recordings are usually made with a three-electrode system; two of the electrodes are placed on each side of the eye, and the reference (ground) electrode is placed somewhere remote from the eye. With ENG, nystagmus and visually controlled eye movements (saccades, smooth pursuit, and optokinetic nystagmus) can be recorded and quantitatively assessed. Often abnormalities not observed during the bedside examination are identified. ENG is relatively inexpensive, easily administered, noninvasive, does not require head restraint, and is reasonably accurate. On the other hand, ENG does not measure torsional eye movements, vertical eye movements are contaminated by eye lid artifacts, and the signal-to-noise ratio is poor.

Computer algorithms have been developed to quantify saccade velocity and accuracy. Saccade accuracy is defined as the ratio of the saccade amplitude divided by the target displacement amplitude times 100. Overshooting of the target rarely occurs in normal subjects. Slowing of saccadic eye movements, both voluntary and involuntary saccades, can occur due to lesions anywhere in the diffuse central pathways involved in generating saccades. The most pronounced slowing results from lesions of the pontine gaze centers, the oculomotor neurons, or the extraocular muscles.

Impaired saccade accuracy is most often seen with cerebellar disorders (Zee et al., 1976). These diseases most commonly display impaired saccade accuracy with saccade overshoot dysmetria. Of the cerebellar syndromes, saccade dysmetria is most prominent with Friedreich's ataxia (Furman et al., 1983). Diffuse degeneration of supranuclear pathways and centers in Parkinson's disease, Huntington's disease, and in progressive supranuclear palsy cause slowing of saccades and hypometria of voluntary saccades.

Video-oculography

Infrared video recording of eye movements (video-oculocraphy, VOG) is a newer, more flexible eye-movement recording system that is gradually replacing ENG. VOG uses an infrared video camera with images stored by a computer for subsequent data analysis. Digital signal-processing algorithms are used to determine horizontal, vertical, and even torsional eye position. It provides a paper- as well as video recording. Disadvantages include inability to wear eyeglasses during the test and difficulty with the digital tracking system in some patients with poor contrast between the pupil and iris. VOG is ideal for identifying the posterior canal variant of benign paroxysmal positional nystagmus that produces mostly vertical and torsional nystagmus. Eye blinks can interfere with both ENG and VOG recording systems (Baloh, 2001), and the current capture rate is insufficient for analysis of saccades.

Caloric testing

The caloric test uses a nonphysiologic stimulus (water or air) to induce endolymphatic flow in the horizontal semicircular canals by creating a temperature gradient in the canal system. Irrigation of the external auditory canal with water or air that is below or above body temperature transfers a temperature gradient from the external auditory canal to the inner ear by conduction. The horizontal semicircular canal develops the largest temperature gradient because it lies closest to the source of temperature change. For performance of caloric testing of the horizontal canal, the patient lies in a supine position with head tilted 30° upwards. With warm caloric stimulus, the endolymph nearest the middle ear rises due to decreased density. This causes the cupula to deviate toward the utricle and produces horizontal nystagmus with the fast component directed toward the stimulated ear. A cold stimulus produces the opposite effect. The caloric test is the most widely used clinical test of the vestibulo-ocular reflex, because it is easy to apply, and each labyrinth can be stimulated individually. Normal subjects show large variability in caloric responses with many methodologic variables in different laboratories. For these reasons, each laboratory should establish its own normal range. Usually vestibular paresis is defined when there is >25% asymmetry between left- and right-sided responses and a directional preponderance with >30% asymmetry.

Posturography

The role of the vestibulospinal system is difficult to assess when isolated from other sensory systems. A force platform, the simplest method to record postural sway, records the position of a subject's center of mass, in fact the center of pressure, in a standing position with the body moving slowly. Some major limitations are evident in static posturography: 1) the nervous system uses a combination of sensory modalities while standing, and 2) static force platforms do not provide controlled stimulus-response measures of vestibulospinal function and thus must rely on spontaneous movements of the body. The measurement of postural sway might prove useful as a screening test for imbalance, but the information it gives is nonspecific and cannot identify vestibular lesions (Baloh et al., 1998).

Dynamic posturography has been designed to overcome these limitations by coupling the platform to the sway of the subject. However, moving-platform studies cannot provide information on the individual suborgans of the vestibular labyrinth, because in the test situation many of these suborgans are simultaneously stimulated. Dynamic posturography is not a diagnostic test, but rather a method to quantify balance dysfunction under differing sensory conditions. (Baloh et al., 1998, Baloh, 2001)

3.5. Neurotologic findings in migraine

Patients with common types of migraine often present with abnormalities in vestibular tests; recent studies report abnormalities in up to 34 to 80% of migraine patients (Toglia et al., 1981, Kayan and Hood, 1984, Cutrer and Baloh, 1992, Cass et al., 1997). Results concerning peripheral labyrinthine dysfunction show variety: abnormalities range from 21 to 80% in migraine patients, with or without vertigo (Toglia et al., 1981, Cutrer and Baloh, 1992, Savundra et al., 1997).

ENG abnormalities in migraine show variable results. Most ENG studies in migraine populations have been carried out in those with ongoing neurotologic symptoms. These studies show a range of ENG abnormalities from 34 to 77.5% of migraine patients (Kayan and Hood, 1984, Cutrer and Baloh, 1992). Migraine patients with vertigo have presented with both central and peripheral vestibular findings (Savundra et al., 1997).

Posturography studies, as well, have shown conflicting results in migraine patients. Dynamic posturography showed abnormalities in 86% of migraine patients with severe, persistent vertigo (Whitney et al., 2000). In contrast, only 19% of patients with migraine-related vestibulopathy showed abnormalities in dynamic posturography (Cass et al., 1997). Migraine-related vestibulopathy included chronic movement-associated disequilibrium, unsteadiness, space and motion discomfort, and occasionally, episodic vertigo as an aura.

In migraine patients, it is not common to find hearing loss. In a study with 100 migraine-related vestibulopathy patients and another study with 91 patients with migraine-related dizziness, hearing loss was a rare finding (Cass et al., 1997, Cutrer and Baloh, 1992). On the other hand, an older study showed that 18% of migraine patients as presenting with sensorineural hearing loss (Kayan and Hood, 1984).

3.6. Varying oculomotor dysfunction in FHM

FHM1 patients have shown a wide variety of ocular motor disturbances, such as downbeating nystagmus (Elliott et al., 1996), horizontal gaze-evoked nystagmus (Joutel et al., 1993, Elliott et al., 1996), and non-specific nystagmus (Joutel et al., 1994, Ducros et al., 1999), as well as no nystagmus (Joutel et al., 1993, Ducros et al., 1999). Only the study of Elliot et al. used quantitative eye-movement measurements (ENG). In other studies, a neurologist had clinically evaluated possible pathological nystagmus.

To our knowledge, no previous studies cover neurotology of FHM2. Neurological examinations of FHM2 patients have been unremarkable, except in one family, where patients showed cerebellar signs (Spadaro et al., 2004).

3.7. Definite and subclinical neurotologic findings in EA2

In EA2, interictal nystagmus is a common finding together with other signs of cerebellar dysfunction. Oculomotor studies show interictal gaze-evoked, rebound, and downbeat nystagmus (Baloh et al., 1986, Baloh et al., 1997, Jen et al., 2004) with features similar to those of SCA6 patients (Ying et al., 2001). The findings localize in the oculomotor region of the posterior vermis and the fastigial nuclei, namely in the vestibulocerebellum (Ohtsuka and Noda, 1995, Ying et al., 2001). In a recent study, subclinical vestibulocerebellar dysfunction was reported in an EA2 family (Subramony et al., 2003). Regardless of age or interictal clinical status, all affected subjects demonstrated abnormal saccades, ocular fixation, and postural stability. Interestingly, in ocular motor studies, three patients with normal neurological status showed several oculomotor abnormalities such as square-wave jerks with lateral gaze, hypometric saccades, and both horizontal and vertical components during diagonal saccades. Saccadic peak velocity was reduced significantly in these patients, which led the authors to suggest that changes in saccade peak velocity might be the earliest manifestation of abnormal oculomotor function in EA2 (Subramony et al., 2003).

4. Proton (^{1}H) Magnetic Resonance Spectroscopy (MRS) in migraine and EA2

Magnetic resonance spectroscopy (MRS) is an emerging method to study brain metabolism noninvasively. Proton MRS is available in many clinical MR imagers.

In MR imaging (MRI), tissue water protons (hydrogen nuclei) are detected, while in MRS, protons of molecules other than water are of interest. Thus, MRS allows detection and measurement of cellular metabolites of various tissues and organs. In MRS, the proton concentration of the metabolites of interest is about 10 000 lower than that of water, so the sensitivity of MRS is much lower than that of MRI. This shows as low signal to noise ratio, low spatial resolution, and long data-acquisition time in metabolic imaging (MRS) compared to traditional water-based imaging (MRI). The MRS experiment is further complicated by the need to saturate the abundant (>80 mol/l) protons of tissue water in order to acquire the small signal from the protons of metabolites that appear in mmol/l concentrations. However, the large but relatively constant tissue water signal can serve as a reference to quantitate the metabolite signals (Ross and Bluml, 2001, deGraaf, 1998).

Important concepts in MR experiments are chemical shift and relaxation times T_1 and T_2. T_1 and T_2 characterize the rate of realignment of protons parallel to the external magnetic field after the radio-frequency pulse. This behavior is strongly affected by the differing chemical and physical environments of the individual protons. Protons of different molecules resonate at different frequencies due to their different environments, and thus appear at different positions on the x-axis of the MR spectrum (chemical shift axis)(deGraaf, 1998).

The main metabolites visible in the proton spectrum of the brain are N-acetylaspartate (NAA), choline-containing compounds (Cho), creatine-phosphocreatine (Cr), lipids, and lactate (Lac). The NAA system appears to reflect a high degree of cellular intergration (Baslow et al., 2003) as well as to be a reversible cerebral osmolyte (Bluml et al., 1997). Loss of NAA may indicate loss of number or viability of neurons. NAA appears at low levels in tumors, in many white-matter diseases (MS plaques, hypoxic encephalopathy), and in atrophic tissue (Bjartmar et al., 2000, Ross and Bluml, 2001). Choline compounds (Cho) take part in the lipid metabolism and are present, for example, in cell membrane and in the synaptic ends of cholinergic neurons. Cho often shows high levels in many disorders because of anabolic or catabolic membrane metabolism. Total creatine (Cr + pCr) represents the sum of creatine and phosphocreatine. Cr probably plays a role in maintaining energy-dependent systems in neurons by serving as a reserve for high-energy phosphates and as a buffer of cellular ATP/ADP (Miller, 1991). Brain trauma or hyperosmolar states may elevate Cr, while hypoxia, stroke, and tumors may reduce it (Ross and Bluml, 2001). Lactate (Lac) is a final product of the anaerobic glycolysis cycle, often increased in ischemic brain tissue and in tumors because of hypoxia.

The brain metabolites show regional differences in the white and gray matter areas. NAA is distributed quite homogeneously throughout the brain. Cho show marked regional variability with the highest concentration in the cerebellum and lowest in the gray matter, with a strong caudally decreasing gradient. Cr shows higher concentrations in the gray than in the white matter, with its highest levels in the cerebellum. This reflects the distribution of creatine kinase and of energy-requiring processes in the brain. Evidence on the metabolic gender differences is conflicting, and so age- and sex-matched control groups in MRS studies are preferable (Tedeschi et al., 1995, Pouwels and Frahm, 1998, Riehemann et al., 1999). Brain aging is characterized by a reduction in NAA, whereas both Cho and Cr increase (Angelie et al., 2001). This suggests reduction in neuronal viability with an accelerated membrane degradation or an increase in glial cell number or both.

4.1. Severe migraine syndromes show decreased brain metabolites

In common types of migraine, ^{1}H MRS and ^{31}P MRS studies show conflicting evidence for brain occipital Lac during and shortly after a migraine attack (Watanabe et al., 1996, Welch et al., 1988). Interictal occipital phosphocreatine (PCr) and cortical magnesium show decreased levels in MA, with the lowest metabolite levels correlating with severity of migraine syndromes, as in hemiplegic migraine (Lodi et al., 2001, Boska et al., 2002, Uncini et al., 1995). Additionally, MA patients with hemisensory, visual, and with motor or speech impairment or both displayed reduced cerebellar Cho (Macri et al., 2003).

4.2. High cerebellar lactate peaks in EA2 patients

Studies using ^{1}H MRS on EA2 are scarce. Bain et al. (1992) and Sappey-Marinier et al. (1999) show an increase in cerebellar lactate, but no decrease in metabolites or changes in ratios. Acetazolamide treatment, on the other hand, causes a pH decrease as well as a decrease in PCr/Pi, and ATP/Pi ratios (Sappey-Marinier et al., 1999). These findings possibly explain the beneficial effects of acetazolamide in EA2.

AIMS

This study was undertaken to better elucidate pathophysiological mechanisms underlying common types of migraine, familial hemiplegic migraine (FHM), and episodic ataxia type 2 (EA2). The aims were to study neurotological findings in migraine and EA2, and to study cerebellar metabolism and genetics in one EA2 family.

More specifically, the aims of this study were to characterize:

- neurotologic findings by means of quantitative measures of vestibular and auditory function in migraine patients with and without aura (I).
- neurotologic findings in familial hemiplegic migraine (II).
- possible vestibulocerebellar involvement and the effect of acetazolamide on these abnormalities in EA2, and by genetic methods to identify a mutation in one EA2 family (III, V).
- cerebellar metabolic abnormalities in EA2 by proton magnetic resonance spectroscopy (IV).

PATIENTS AND METHODS

5. Patients and controls

5.1. Migraine with and without aura

For the neurotologic study (I), we examined 36 migraine patients: 12 with MA and 24 with MO. Each patient group had a strong family history of its type of migraine. MA patients were recruited from six unrelated families, while MO patients were identified from the Finnish Twin Cohort Study (Kaprio et al., 1978), in 14 unrelated families. MA patients had several types of migraine with aura: basilar migraine (two patients), FHM (two), hemiplegic migraine (three), and migraine with typical visual aura (five) (Table 2). Five patients were on beta-blocker medication and eight on anticoagulants, acetosalicylate, or statins.

Controls (I) were 41 healthy nonmigrainous volunteers (Table 3), collected from among the personnel of the Department of Otolaryngology of the Helsinki University Central Hospital. Control groups were test-specific. Static posturography and ENG controls were studied in 1998 and 1995 by the same technique and with the same equipment as in the present studies (I, II, V), and these results have been of clinical use ever since (Isotalo, 1999, Juhola et al., 1989, Starck et al., 1992).

Table 2. Demographic features of patients from Studies I-V, including the extended EA2 family.

	Number of patients	Female	Male	Mean age	SD
MA	12	11	1	44	17
MO	24	19	5	47	8
FHM2	9	7	2	30	14
EA2	18	11	7	37	14

MA = migraine with aura, MO = migraine without aura, FHM2 = familial hemiplegic migraine with aura type 2, EA2 = episodic ataxia type 2, SD = standard deviation

Table 3. Demographic features of controls in Studies I-II, IV-V.

	Number of controls	Female	Male	Mean age	SD
VOG	12	6	6	40	11
ENG	16	9	7	40	9
Static posturography	13	8	5	46	8
^{1}H MRS	9	6	3	36	8

VOG = video-oculography, ENG = electronystagmography, ^{1}H MRS = proton magnetic resonance spectroscopy

5.2. Familial hemiplegic migraine type 2

From one large FHM2 family, 15 subjects underwent examination (Figure 6 A). Nine FHM2 patients underwent neurotologic tests (Study II; Table 2). All were carriers of a T345A mutation of the *ATP1A2* gene (Kaunisto et al., 2004). The patients were on no permanent medication.

5.3. Episodic ataxia type 2

Neurologic examination was performed on 18 patients (9 women) from a large, extended EA2 family (Figure 6 B). Each of these patients, of whom one was asymptomatic, had a novel *CACNA1A* splice site mutation (Study III; Table 2). Neurotologic tests were carried out on 12 patients (6 were women, mean age 36; SD 16 years), 3 of them were examined with and without acetazolamide treatment (V). ^{1}H MRS studies (IV) were carried out on 9 patients (6 women, 3 men), mean age 35, SD 9 years, 7 of these were on acetazolamide 500 mg/day, but those studied had a week of wash-out time before examination.

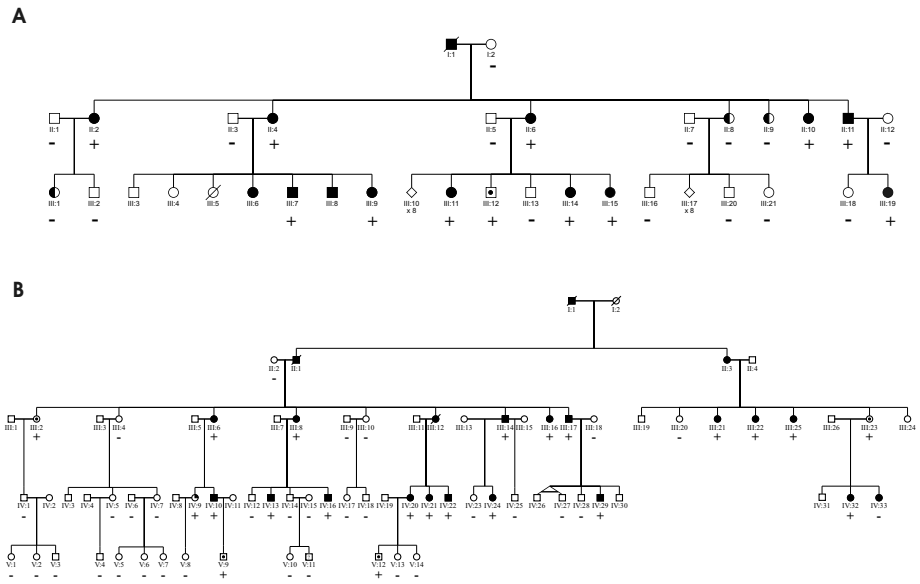


Figure 6. Pedigrees with familial hemiplegic migraine type 2 (A) and with episodic ataxia type 2 (B). Mutation status as + or -. Black symbols denote those clinically affected. Those containing a dark dot denote an asymptomatic mutation carrier. In family A, half-darkened symbols denote patients with migraine without aura. In family B, the quarter-darkened symbol denotes an individual with a specific sensitivity to alcohol.

Controls for the ^{1}H MRS study (IV; Table 3) were 9 healthy volunteers. Two of the controls had migraine with aura, with their last attacks 6 and 11 years previously. Controls used no medication.

All these study protocols were approved by the Ethics Committee of the Helsinki University Central Hospital, and all subjects gave their informed consent.

6. Genetic methods

Linkage analysis
Linkage to the 19p13 *CACNA1A* locus was tested by use of microsatellite markers D19S221, D19S1150, and D19S226. Genotyping was performed with the ALFexpress sequencer (Amersham Biosciences, Uppsala, Sweden) (Virolainen et al., 2000). Asymptomatic EA2 family members under age 20 and an individual (IV:9) with an unusual sensitivity to alcohol as her only symptom were considered as being of unknown status. Equal allele frequencies were used.

Two-point LOD scores were calculated with the MLINK option of the LINKAGE program package under the assumption of an autosomal dominant inheritance with incomplete penetrance of 0.8 and disease gene frequency of 0.0001 (Lathrop and Lalouel, 1984).

Mutation analysis
The proband (IV:13) and his mother (III:18) were analyzed for mutations in *CACNA1A*. Two healthy subjects served as controls. All exons and immediate flanking intronic sequences were amplified by polymerase chain reaction (PCR) and analyzed by cycle sequencing using the BigDye Terminator Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on the ABI Prism 377 automatic sequencer (Applied Biosystems). All PCR products were sequenced in both forward and reverse directions. Nucleotide numbering of *CACNA1A* is according to GenBank sequence X99897.

Within the EA2 family, cycle minisequencing was used to demonstrate the segregation of the specific mutation and to exclude the presence of the mutation in 132 healthy, unrelated controls (Jarvelainen et al., 2001). Exon 37 was amplified by PCR using primers 5´-CCAGTCTCTCCCACTCCTTC-3´ and 5`-TGAAGAACCCCAAGCCACA-3`. We used biotinylated reverse primer 5`biotin-CCTTATAATGAATCCGACCGC-3`and 3H-labeled nucleotides dTTP (wild type) and dCTP (mutated sequence) for the cyclic primer extension.

Controls
A total of 132 healthy, unrelated, voluntary blood donors of Finnish origin served as controls. None was found to have the mutation in question.

7. Neurotologic methods

7.1. Video-oculography

Eye movements were measured during discrete periods of 30 seconds in different eye, head, and body positions. The interval between different test conditions was approximately 1 min. A rubber mask-mounted small video camera (Sensomotoric Instruments, Berlin, Germany) recorded movements of the dominant eye that was in total darkness while the other eye was covered. Patients were instructed not to blink and to keep their eyes open during measurements. There was no fixation target illuminated. Test conditions were 1) body prone, head straight ahead, and gaze forward; 2-3) gaze approximately 30° to the left or right; and 4) after passive head shaking to the right and left 30 times in the yaw plane. After this, positional nystagmus was evaluated by Dix-Hallpike positioning: 1) supine, facing straight ahead, and 2-3) supine, head rotated 45° to the left or right and the neck overextended. Finally, with the subject remaining supine, lateral head turns for 15 seconds were applied six times to study BPPV of the horizontal canal. VOG recordings were stored as eye-position curves in the horizontal and vertical plane, and also on standard VHS videotape. Analysis was done off-line.

The VOG analysis included simultaneous evaluation of eye position curves and videotapes for spontaneous nystagmus, gaze-evoked nystagmus, head-shaking nystagmus, and positional nystagmus. An abnormal nystagmus was defined by calculating maximal slow phase velocity and direction, and was compared to normal values (mean ± 2 SD) derived from healthy controls.

7.2. Electronystagmography and caloric testing

Saccade velocity, accuracy, and latency were determined for pseudorandom saccades of 20° and 60° to the left and right (Juhola et al., 1989). Saccades were classified as abnormal when the mean accuracy of either 20° or 60° saccades was abnormal with regard to the normal limits (mean ± 2 SD). Caloric testing was evaluated for side difference (a 30% difference considered significant) and bilateral hypofunction (maximal slow phase velocities of nystagmus for cold (30°) and warm (44°) caloric stimulus not exeeding 12° per second).

7.3. Static posturography and audiometry

Four test conditions were used in posturography: sway velocities (mm/second) with 1) eyes open and 2) closed, as well as 3-4) with or without bilateral calf-muscle stimulation (vibration perturbation of 30 Hz with calf-attached vibrators weighing 400 grams). Each test condition was recorded for 30 seconds: first with eyes open, then eyes open with 12-second calf-muscle stimulation; after that the same procedure with eyes closed. The interval between the second and third paradigm was 15 seconds (Starck et al., 1992). Audiometry was carried out by the standard procedure in clinical use.

8. Proton (^{1}H) Magnetic Resonance Spectroscopy (MRS)

We used a 1.5 T whole-body MR system (Siemens Magnetom Sonata, Erlagen, Germany) equipped with a standard head coil. Oblique axial, coronal, and sagittal T_1-weighed gradient echo images (FLASH sequence with repetition time (TR) 225 ms, echo time (TE) 4.8 ms, and a 70° flip angle) were used to position the spectroscopic voxels in cerebellar hemispheres, vermis, and left thalamus (Figure 7). Single-voxel ^{1}H MRS measurements were performed using the point-resolved spectroscopy sequence, PRESS, with the chemical shift selective water suppression scheme, and with TE 288 ms and TR 1600 ms. Voxel volumes ranged from 3.38 to 8.00 cm^3. Spectral width was 1000 Hz, number of aquired complex data points was 1024, and 256 acquisitions were recorded. The spectra were processed and analyzed by a MR-spectroscopy package of Mrease-software (Siemens, Erlangen, Germany). The time domain signals were apodized by use of the Hanning function with a 400 ms width at half-maximum and zero filled once prior to Fourier transformation. N-acetylaspartate (NAA, 2.02 ppm), total creatine (3.03 ppm, including signals from creatine, Cr, and phosphocreatine, PCr), and choline-containing compounds (Cho, 3.22 ppm) signal intensities were measured with Gaussian lineshape fitting. Intensities were corrected for differences in coil loading and voxel size.

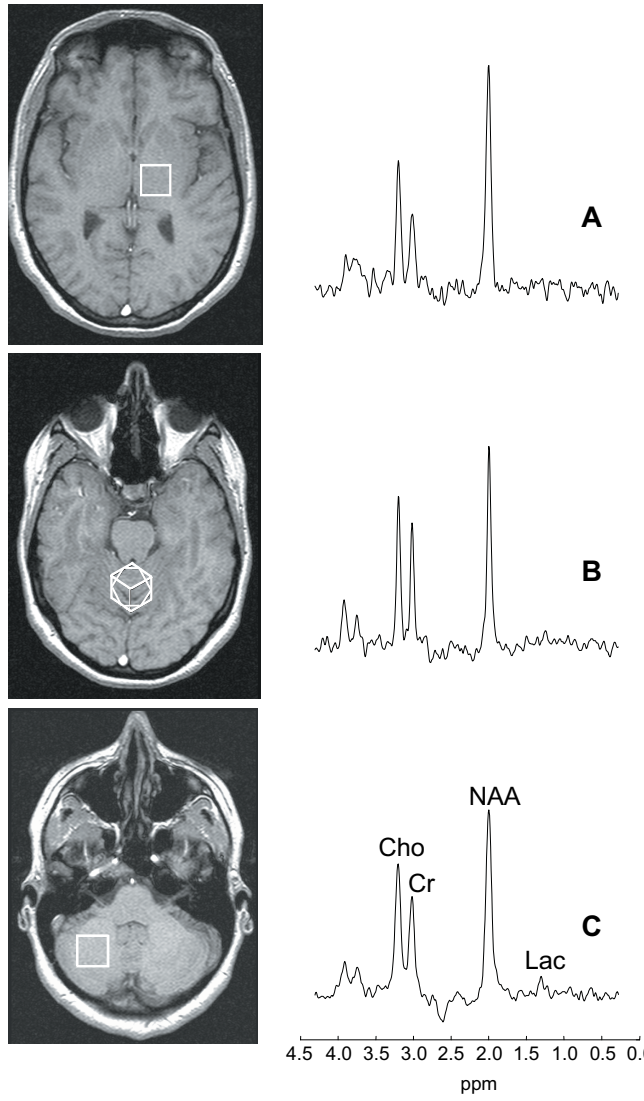


Figure 7. Voxel positions in the left thalamus (A), the vermis (B), and cerebellar hemispheres (C) with patient spectra.
Cho, choline compounds; Cr, creatine: Lac, lactate; NAA, N-acetylaspartate.

9. Statistical methods

Results for neurotologic tests (means ± 95% CI) between the groups were compared by Kruskal-Wallis κ^2 test or Mann-Whitney *U*-test for noncontinuous parameters (SPSS, version 8.0, SPSS Inc., Chicago, IL).

The ^{1}H MRS metabolite signal intensities (in arbitrary units) were analyzed by two-tailed *t*-test assuming unequal variances.The level of significance was set at $p<0.05$.

RESULTS

10. Clinical findings

10.1. Patients with common types of migraine

Of the 36 migraine patients, 12 had MA and 24 MO. Of the 12 MA patients, seven had a severe type of aura (vertigo, hemisensoric symptoms, or mild hemiparesis), while five had only a visual aura. During migraine attacks, two with MO and one with typical MA had nonspecific dizziness (a giddy sensation), while two patients with BM had severe vertigo (an illusion of rotation).

Patients were mostly asymptomatic interictally: Occasional dizziness occurred in two MA patients between attacks, and two with MA had a history of positional vertigo.

Neurologic examination was normal in 34 of 36 migraine patients. Two patients, one with BM and one with FHM, had nystagmus in lateral gaze.

10.2. Variation in clinical features of attacks in FHM2

From a large FHM2 family (Figure 6 A), 11 patients fulfilled criteria for FHM according to the IHS criteria (International Headache, 1988, Headache Classification Subcommittee of the International Headache, 2004). Neurologic examination in all FHM patients was normal. The clinical picture of migraine attacks showed heterogeneity: Most patients had migraine attacks that often started with a gradually spreading hemisensoric aura and thereafter were accompanied by dysarthria, dysphasia, mild to moderate hemiparesis, and, in some individuals, confusion or decreased consciousness. For most of these patients, the hemiplegic aura could last for days or even weeks. In four patients, severe vomiting could last for days. Two patients did not report migraine attacks, but when hospitalized due to mild head trauma that had induced confusion and decreased consciousness, a barely detectable hemiparesis was recorded in the medical history. Mild head trauma triggered migraine attacks in five patients, but three of these had migraine attacks even without specific triggers.

The patients from this FHM2 family considered migraine attacks as placing constraints on their lives and causing social withdrawal. Acetazolamide (250 mg twice a day) and also verapamil (80 mg twice a day) exhibited good efficacy in preventing migraine attacks in two of the patients, thus helping them to lead normal lives.

10.3. Typical phenotype in EA2

EA2 attacks were primarily of similar type among family members, Figure 6 B. Two of the subjects were asymptomatic (III:23 and IV:9) with a particular sensitivity to alcohol. During attacks the most common symptoms were vertigo, ataxia, dysarthria, nausea, vomiting, blurred vision, and fatigue. Some patients presented with double vision or generalized weakness, one patient presented with migrainous headache during ataxia episodes that fulfilled basilar migraine criteria (Headache Classification Subcommittee of the International Headache, 2004). Attacks commonly started in childhood (mean age 12, range 4-35) and lasted typically about 2 hours, but in one patient (IV:24) could last for days. Triggering factors were often emotional stress, physical exercise, heat, alcohol, sometimes coffee or fasting. Rest, often a short nap, relieved symptoms in all patients.

Seven patients were on acetazolamide to prevent EA2 attacks. Most of these patients responded extremely well to acetazolamide (250 mg twice a day) with a dramatically reduced attack frequency. Two individuals (IV:13 and IV:16) had attacks about once a month despite medication, but the severity, duration, and frequency decreased overall. We increased the acetazolamide dose to 1000 mg per day to better reduce attacks in these individuals, but due to side-effects (urethrolithiasis and metabolic acidosis) the dose had to be reduced to 500 mg per day.

For most patients, neurological interictal examination was either normal (5/18) or with only minor clinical findings (8/18). These included mild limb ataxia on knee-heel testing, difficulties in tandem gait, or nystagmus at lateral gaze. However, four individuals had cerebellar ataxia ranging from mild (3/18) to severe (1/18), accompanied by dysarthria and pronounced gaze-evoked nystagmus. These findings were consistent with cerebellar atrophy in MRI.

Migraine headaches appeared in three patients together with ataxic spells, but six patients had MO attacks independent of EA2 attacks.

11. Genetics of EA2

Linkage analysis
The maximum two-point LOD scores of 4.48 (θ=0), 3.81 (θ=0), and 3.49 (θ=0) revealed linkage to 19p13 in our EA2 family with 13 clinically affected subjects with the markers D19S226, D19S1150, and D19S221.

Mutation analysis
Sequencing of *CACNA1A* showed, in the proband and his mother, a heterozygous single nucleotide change from A to G (IVS36-2A>G substitution) at the 3' acceptor splice site of intron 36. A minisequencing method was used to detect the mutation in the rest of the family. A total of 17 family members carried the mutation, 13 of them clinically affected (II). In further study, the family was extended (V), and the mutation was confirmed from four

additional clinically affected family members (III:21, III:22, III:25, IV:32) in addition to one asymptomatic subject with a particular sensitivity to alcohol (III:23).

12. Neurotologic tests

We examined the 36 patients with common types of migraine, the family with episodic ataxia type 2 (N=12), and the family with familial hemiplegic migraine type 2 (N=9) by a neurotologic test pattern (see Table 4). The results were compared to those of healthy controls.

Table 4. Number of abnormal neurotologic findings in each patient group.

	Number of patients	VOG	ENG	Caloric responses	Static posturography	Audiometry
MA	12	6	4	0	7	3
MO	24	8	7	4	10	2
FHM2	9	4/7 tested	2	1	3	0
EA2	12	8	7	0	7/10 tested	0

VOG = video-oculography, ENG = electronystagmography, MA = migraine with aura, MO = migraine without aura, FHM2 = familial hemiplegic migraine type 2, EA2 = episodic ataxia type 2.

12.1. Subclinical vestibulocerebellar dysfunction in the common types of migraine

In neurotologic tests, 30 of 36 patients (83%) had abnormalities (Table 4). Of the 36 patients, 16 (44%) had abnormalities in one, while 14 patients (39%) had abnormalities in more than one test. Only 17% had all test-results within normal limits: four patients with MO and two with MA (one with typical MA and one with FHM).

Video-oculography
Abnormal nystagmus occurred in 14 of 36 (39%) of the migraine patients (p=0.02). Nystagmus, suggestive for central type, was evident in 10 of 14 subjects, 2 of 14 had BPPV, and 2 had nystagmus with possible peripheral or central etiology. Pathologic findings tended to be more severe in MA (p=0.003) than in MO (p=0.03), although this difference was nonsignificant (p=0.23).

Central pathologic nystagmus was defined as follows: 1) direction-changing gaze nystagmus, which was asymmetric, occurred in a vertical plane despite horizontal gaze, or occurred together with head-shaking nystagmus or spontaneous nystagmus; 2) positional nystamus, which was two-dimensional (occurring simultaneously in both vertical and horizontal planes). Peripheral nystagmus showed, during positioning, typical features of torsional and vertical nystagmus of BPPV of the posterior semicircular canal.

ENG and caloric testing
Saccadic inaccuracy showed a significant difference in 4 of 12 of the MA (33%) and 7 of 24 of the MO patients (29%) when compared to controls (p=0.001). MA and MO did not differ significantly (Study I, Figure 1, p.1750). Saccades were hypermetric in six patients and hypometric in five. Saccadic latencies and peak velocities were within normal limits. Caloric responses in 4 of 24 of the MO subjects were asymmetric; one of these also had bouts of vertigo during a migraine attack. None of the patients with asymmetric caloric responses had any history of vestibular abnormality. There was no bilateral caloric hypofunction.

Static posturography
Static posturography showed increased body sway in 7 of 12 (58%) of the MA patients, and in 10 of 24 (42%) of the MO patients, this difference being significant (p=0.001) compared to those of the controls when the test was performed with eyes open (Study I, Figure 2, p.1750). MA patients tended to have higher body sway velocities than did MO patients.

Audiometry
Hearing level was abnormal for 5 of 36 (14%). One migraineur had a slight conductive unilateral hearing loss, and the other showed a slight unilateral sensorineural hearing loss. Three had symmetric bilateral sensorineural hearing loss: one apparently due to noise exposure and age (71 years), one most likely due to presbyacusis, and one due to unknown etiology.

12.2. Abnormal oculomotor and postural function in FHM2

For neurotologic tests, at least one of the tests was abnormal for 7 of 9 patients. Two patients (II:6, II:10 in Figure 6A) were normal in all tests.

Video-oculography
In video-oculography, 4 of 7 patients had abnormal results with the central type of positional nystagmus and in one patient (III:7) also gaze-evoked nystagmus. VOG results differed significantly from those of the controls (p=0.02).

Saccades and caloric responses
Two patients (II:4, III:9) had hypometria for 60° saccades. Mean saccadic accuracy was decreased for 60° saccades (p=0.02) when compared to controls. Caloric responses were normal, except for one patient (III:14) with unilateral weakness.

Static posturography and audiometry
Body sway velocity was increased in 3 of 9 of the patients (III:9, II:11, III:11). In test conditions with eyes open, mean body sway velocity was significantly increased when compared to that of controls (p=0.03). Audiometry in all patients was normal.

12.3. Definite and subclinical vestibulocerebellar dysfunction in EA2

Of the 12 EA2 patients examined, 11 had abnormalities in at least one test (in part, unpublished data). Only one patient (IV:24) was normal on all tests. Patients with permanent cerebellar atrophy and ataxia had the most severe findings. Abnormal findings seemed to correlate neither with age nor with gender. In particular, we examined the effect of acetazolamide on abnormal neurotologic findings in three patients (Study III).

Video-oculography

VOG showed abnormal nystagmus in 8 of 12 of these patients (p=0.001). Abnormal nystagmus types were spontaneous (4/12), gaze-evoked or rebound (4/12), and central positional (8/12) nystagmus. Acetazolamide improved the VOG findings (Study III, Figure 2, p. 233) in two patients (IV:16, IV:22). Those with cerebellar ataxia had the most severe nystagmus findings (Figure 8).

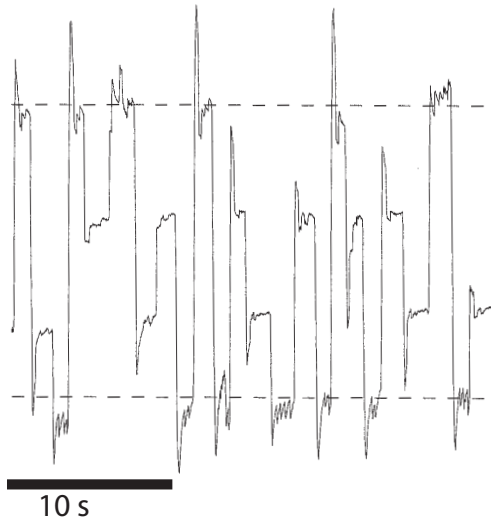


Figure 8. A 40-second sample of an eye-position signal from an EA2 patient during saccade testing. Time scale below. Note the hypermetric eye movements after most eye-position changes. Broken lines indicate ± 30° range of maximal target position.

ENG and caloric responses

Saccadic accuracy both at 60° and 20° saccades was significantly decreased in patients compared to that of controls (p<0.001). Saccadic dysmetria was found in 7 of 12 of the patients. Three subjects had hypermetric saccades, but four had both hypermetric and hypometric saccades. Saccadic velocities and latencies fell within normal limits. Acetazolamide improved hypermetric saccades in one patient (IV:13). None of the patients showed caloric weakness.

Static posturography and audiometry

In static posturography, 7 of 10 of the patients showed increased body sway velocity, especially in eyes-open test conditions, when compared to that of controls (p=0.002). Two patients (III:8, III:14) were not tested because of severely impaired balance. Acetazolamide improved abnormal posturography in one patient (IV:22).

12.4. Comparison of neurotologic findings in common types of migraine, in FHM2, and in EA2

We compared the neurotologic findings in FHM2, MA, and MO (Study II), and on the other hand in EA2 (in part, unpublished data). The accuracy in 20° saccades was significantly decreased in MO patients when compared to that of FHM2 patients (p=0.02). Furthermore, 20° saccadic accuracy in EA2 patients was significantly decreased when compared to that of FHM2 patients (p=0.01) (Figure 9). EA2 patients showed significantly more abnormal nystagmus in the VOG than MO patients did (p=0.03). No significant differences appeared between the groups in static posturography (Figure 10).

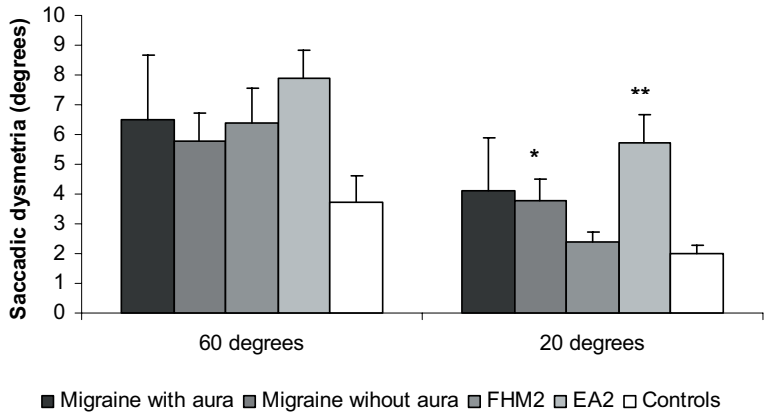


*Figure 9. Patient groups were compared to each other, although controls are shown. In 20° saccadic accuracy, EA2 patients had significantly decreased values compared to those of FHM2 patients. Moreover, MO patients showed decreased saccadic accuracy compared to that of FHM2 patients.*p=0.02, ** p=0.01*
EA2 = episodic ataxia type 2, FHM2 = familial hemiplegic migraine type 2, MO = migraine without aura

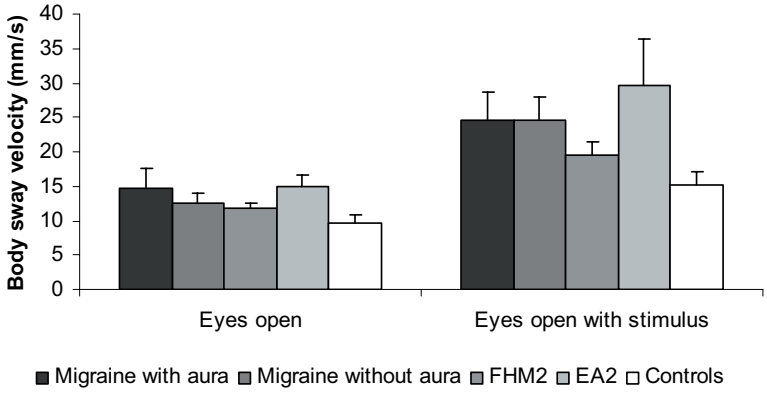


Figure 10. Patient groups showed no significant differences in posturography (controls not taken into account, although shown).

12.5. Neurotologic findings in controls

In video-oculography, nystagmus was quite frequent among control subjects. However, the mean slow-phase velocity for nystagmus was very low. Controls displayed no pathological nystagmus, such as gaze-evoked nystagmus (Levo et al., 2004). In ENG, controls had neither hypermetric nor hypometric saccades. The mean saccadic accuracy (±CI) for controls was 3.7° (±0.9) for 60° saccades and 2.0° (±0.3) for 20° saccades. In static posturography, the controls did not show as much body sway as did the patients, but the individual variance in sway velocity between control subjects was large.

13. Proton magnetic resonance spectroscopy showing decreased cerebellar total creatine in EA2

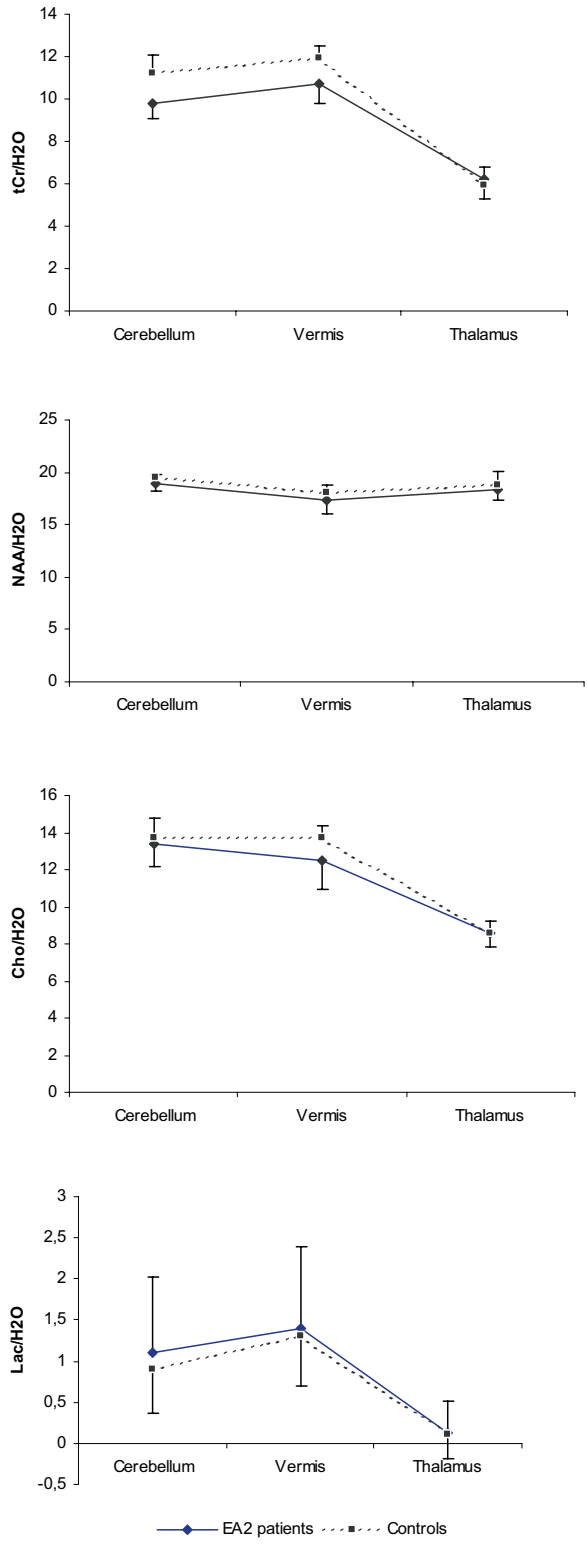
We examined nine EA2 patients by ^{1}H MRS and measured ratios of Cr/H_2O, Cho/H_2O, NAA/H_2O, and Lac/H_2O in both cerebellar hemispheres, in the vermis, and in the left thalamus (Figure 7). Among the nine EA2 patients, one showed marked vermian atrophy in brain MRI.

In the cerebellar hemispheres, tCr/H_2O signal intensities were 12% lower in patients than in controls ($p=0.005$), but no difference appeared in NAA. Neither cerebellar Cho/H_2O nor Lac/H_2O showed a statistical difference when compared to controls (Figure 11).

In the vermis, tCr/H_2O was 16% lower in patients than in controls ($p=0.007$). NAA, Cho, and Lac ratios showed no significant difference from those of controls.

In the left thalamus, no significant differences appeared in NAA/H_2O, tCr/H_2O, Cho/H_2O, or Lac/H_2O between patients and controls.

Figure 11. EA2 patients showed a significant decrease in total creatine/H2O ratio in the cerebellum (p=0.005) and the vermis (p=0.007) when compared to controls. Other metabolites showed no significant difference. Confidence intervals indicated by whiskers.



DISCUSSION

By neurotologic testing (VOG, ENG, caloric responses, static posturography, and audiometry), we studied vestibulocerebellar changes in 36 patients with migraine with or without aura, 9 patients with FHM2, and 12 patients with EA2. Three patients with EA2 were examined with and without acetazolamide treatment. Healthy, nonmigrainous controls were test-specific: 12 controls for VOG, 16 for ENG, and 13 for static posturography. These studies suggest subclinical oculomotor and postural changes in the common types of migraine as well as in FHM2 and EA2. Proton MRS was carried out for nine EA2 patients and nine healthy controls with special reference to the cerebellum. Cerebellar total creatine was lower than in the controls, which may have been an early sign of calcium channel dysfunction in mostly nonataxic EA2 patients. A novel *CACNA1A* mutation was identified in an EA2 family.

14. Migraine with and without aura, FHM2, and EA2 displaying subclinical vestibulocerebellar dysfunction

Neurotologic findings in our study on MA and MO patients (I) suggest subclinical vestibulocerebellar dysfunction in both types of common migraine. These patients were mostly asymptomatic in respect to neurotologic symptoms and had a strong family history of either MA or MO. We thus put our emphasis on migraine as the study focus. Interestingly, for the common types of migraine, a three-dimensional analysis of reaching movements has also shown subclinical cerebellar impairment (Sandor et al., 2001).

Most studies on neurotology of the common types of migraine have been retrospective (Harker and Rassekh, 1988, Cutrer and Baloh, 1992, Savundra et al., 1997, Whitney et al., 2000), have included few patients (Harker and Rassekh, 1988), or have emphasized either episodic or chronic vertigo (Dieterich and Brandt, 1999, Neuhauser et al., 2001, Cass et al., 1997). This complicates the comparison of our data with that of previous studies. Nevertheless, our data with an 83% proportion of neurotologic abnormality in migraine patients agree with data from several other studies (range, 34%-80%) (Toglia et al., 1981, Kayan and Hood, 1984, Cutrer and Baloh, 1992, Cass et al., 1997).

Moreover, neurotologic studies on migraine show somewhat conflicting results. Several show a variety in the proportion of migraine patients with ENG abnormalities (range, 34%-77.5%) (Kayan and Hood, 1984, Cutrer and Baloh, 1992, Dieterich and Brandt, 1999). The diversity is even clearer in the proportion of migraine patients having labyrinthine dysfunction (range, 8%-80%) (Toglia et al., 1981, Cutrer and Baloh, 1992, Dieterich and Brandt, 1999). In our study (I), ENG abnormalities occurred in 29% of MO and 33% of MA patients, while only 17% of the MO patients and none of the MA patients showed unilateral caloric weakness. Others' results differ depending on whether patients have ongoing neurotologic symptoms. Moreover, MA patients that present with vertigo commonly have shown both central and peripheral vestibular dysfunction (Savundra et al., 1997). We, as well, found signs indicative for peripheral vestibular dysfunction: 22% of our patients showed BPPV, unilateral caloric hyporeactivity, or the suggestive peripheral type of nys-

tagmus in the VOG. In short, we detected signs of peripheral and central vestibular nystagmus in migraine, but the abnormalities were mostly of vestibulocerebellar origin.

In Study I, MA and MO showed a similar type of neurotologic findings, with features of central etiology in VOG. The central type of positional nystagmus findings (in 28% of the patients) were composed mostly of horizontal and vertical components with no known peripheral pathology or vertigo symptoms during positional testing. Some of these patients also had simultaneous gaze-evoked nystagmus, which occurred without positional nystagmus in 25% of them. Moreover, our patients showed saccadic inaccuracy with hypo- and hypermetric saccades as well as increased body sway in static posturography, which support — together with the VOG findings — our conclusion of subclinical vestibulocerebellar dysfunction in migraine patients.

FHM2 is such a new entity that, to our knowledge, no previous neurotological studies on FHM2 have appeared. In Study II, neurological examination in all FHM2 patients was normal, but neurotologic tests showed subclinical changes consistent with those seen in more common types of migraine. FHM2 patients showed heterogeneity in oculomotor and postural dysfunction: Most of the patients examined had the abnormal central type of positional nystagmus in VOG, while some had abnormalities only in postural control. This may suggest phenotype variation within specific *ATP1A2* mutation carriers.

Cerebellar signs are present in about 20% of FHM1 families, while the phenotype of FHM2 has not displayed cerebellar signs (Ducros et al., 1997, 2001,Gardner et al., 1997, Marconi et al., 2003), except for one Italian family (Spadaro et al., 2004). The normal neurological examinations in our patients support the theory that the major FHM2 phenotype is pure, i.e., without cerebellar signs. We had heterogeneous, subclinical oculomotor and postural findings that were not specifically localizing. On the other hand, one patient showed gaze-evoked nystagmus which was suggestive of vestibulocerebellar origin. The cerebellar involvement in FHM2 is as yet unclear, but defective ion channel mechanisms in the cerebellum offer interesting insights into their disease pathophysiology. The Na^+,K^+-ATPase pump in astrocytes is impaired in FHM2 due to *ATP1A2* gene mutation. This causes impaired maintenance of an electrochemical Na^+ and K^+ gradient, which may affect neurons and astrocytes in various ways: It may depolarize neurons, facilitate CSD, impair astrocytes in their glutamate removal, and activate the Na^+,Ca^{2+} exchanger (Juhaszova and Blaustein, 1997, Van Den Maagdenberg et al., 2004, Moskowitz et al., 2004). The latter may eventually cause an increase in intracellular Ca^{2+} and thus lead to similar type of changes in neurons in FHM2 as in FHM1 (De Fusco et al., 2003).

A genetic basis and chronic cerebellar ataxia in part unite FHM1 and EA2, with considerable overlap also in their clinical phenotype. Despite these overlapping phenotypes, an increasing number of identified mutations in the *CACNA1A* have become feasible for diagnosing both FHM1 and EA2 patients. The causative mechanisms associated with the development of ataxia are as yet unknown. In one FHM1 family, abnormal eye movements that suggest vestibulocerebellar dysfunction have been documented, possibly indicating early manifestations of cerebellar atrophy (Elliott et al., 1996).

Abnormal interictal oculomotor function has been documented in EA2 with typical features of gaze-evoked, rebound, or downbeat nystagmus (Baloh et al., 1997, Jen et al., 2004). In a recent study of EA2 patients, half the patients had normal interictal status, similar features in oculomotor dysfunction, and abnormal dynamic posturography (Subramony et al., 2003). Our findings with abnormal neurotologic test results in 11 of 12 patients from an EA2 family agree with those results. Vestibulocerebellar dysfunction did not seem to depend on neurological status. These findings may indicate functional changes in the cerebellum before the atrophy and death of Purkinje cells (Subramony et al., 2003).

Acetazolamide prevents, most often extremely well, ataxic episodes, but has not previously improved baseline interictal ocular motor signs (Baloh and Winder, 1991). In Study III, however, three patients from our EA2 family showed improvement in baseline neurotologic tests while on acetazolamide. It possibly stabilizes dysfunctioning $Ca_v2.1$ channels in Purkinje cells where the most abundant neuronal expression of *CACNA1A* gene occurs. Improvement in baseline oculomotor and postural abnormalities, in contrast to previous findings, may also reflect the diversity of dysfunctioning $Ca_v2.1$ channels. With increasing data on genotype-phenotype correlations for EA2, it has become evident that one cannot predict baseline ataxia, interictal nystagmus, or acetazolamide responsiveness by type of mutation (Jen et al., 2004). With documented mutations, clinical features vary between and within families (Van Den Maagdenberg et al., 2002, Jen et al., 2004).

Overall, EA2 patients, although mostly with normal interictal neurological status, showed the most abnormal results in neurotologic tests when compared to MA, MO, or FHM2 patients. These four patient groups showed significant abnormalities in neurotologic tests when compared to controls' tests, with suggestive vestibulocerebellar dysfunction as a common feature. This may reflect intrinsic ion channel dysfunction in cerebellar and brain stem neurons, with the most evident abnormal findings appearing in EA2 patients. FHM2 causing Na^+,K^+ ATPase pump dysfunction may lead to Ca^{2+} overload in neurons (De Fusco et al., 2003), which may explain the features of subclinical oculomotor dysfunction in the present FHM2 family. However, in neurotologic findings, patients with the common types of migraine, with no genetically defined background, showed similar features. This suggests that the cerebellum and most probably the brain stem both play a role in the pathophysiology of various types of migraine. It has been debated whether calcium channel regulating genes occur in the common types of migraine as well, but results are conflicting (Terwindt et al., 2001, Noble-Topham et al., 2002). Interestingly, neurophysiological and imaging studies on common types of migraine have, on the other hand, pointed to subclinical cerebellar dysfunction (Sandor et al., 2001, Kruit et al., 2004) or to possible impaired neuromuscular transmission (Ambrosini et al., 2001). These features may reflect *CACNA1A* dysfunction, but the study populations have not been genetically defined.

EA2 patients showed the most severe saccadic inaccuracy, with a signicant difference from that of FHM2 patients. Interestingly, MO patients showed a significant difference in saccadic inaccuracy from that of FHM2 patients, as well. These comparisons suggest that migraineurs in general may exhibit dysmetria in fast voluntary eye movements, whether

in common type or hemiplegic migraine, but with more severe findings in patients with an actual *CACNA1A* mutation.

15. Complex phenotype-genotype correlation in EA2

Remarkable variation exists in the clinical expression of the EA2 phenotype — symptoms may range from migraine to progressive ataxia (Denier et al., 1999, Jen et al., 2001, Jen et al., 1999, Jouvenceau et al., 2001, Van Den Maagdenberg et al., 2002, Subramony et al., 2003). Our EA2 family was consistent with this observation: Age of onset and duration of attacks, as well as neurological examinations showed variation. Most likely, in addition to disease-causing *CACNA1A* mutation, either modifying genetic or environmental factors, or both, play a role in the clinical expression of EA2 (Van Den Maagdenberg et al., 2002).

In Study IV, we suggest that decreased cerebellar total creatine (tCr) may, in addition to decreased cerebellar PCr/Pi or ATP/Pi, reflect neurochemical changes as a consequence of dysfunctioning neuronal calcium channels. The exact mechanism how an impaired $Ca_v2.1$ channel causes the episodic ataxic symptoms and eventual cerebellar atrophy is unknown. Recent studies on functional consequences of *CACNA1A* mutations associated with EA2 show loss-of-function or partially functional $Ca_v2.1$ calcium channels (Jen et al., 2001). Lowered Ca^{2+} influx is predicted during single depolarizations as well as during repetitive neuronal activity. $Ca_v2.1$ channels are tightly coupled to neurotransmitter release in many neurons and mediate the depolarization-induced calcium current in Purkinje cells. Reduced depolarizations in these neurons may possibly underlie processes that later lead to atrophy.

A brain alkalosis-driven change shifting the creatine kinase reaction towards PCr production (PCr + ADP $\Leftrightarrow$ Cr + ATP) may be one explanation for decreased cerebellar tCr. EA2 patients may present with cerebellar alkalosis that normalizes under acetazolamine treatment. Acetazolamide also reduces PCr/Pi and ATP/Pi ratios (Sappey-Marinier et al., 1999). On the other hand, the T_2-relaxation difference between PCr and Cr may shift the reaction equilibrium and thus lead to a decreased tCr-signal in the long-TE (288ms) spectrum (Ke et al., 2002). This, together with a possible decrease in concentration of Cr or PCr may cause the tCr/H_2O decrease observed. As a whole, decreased cerebellar tCr supports the hypothesis of an alkalosis-driven creatine kinase-reaction shift and may reflect neurochemical changes in dysfunctioning $Ca_v2.1$ channels before a patient develops cerebellar atrophy and NAA decrease.

16. Future prospects

Over the last decade, genetic and phenotypic knowledge of EA2 and FHM has shown huge improvements. The mutations coding for dysfunctioning ion channels and their functional research has helped in understanding some of the pathogenesis of migraine and ataxia. Recently, evidence has emerged on overlapping mechanisms and the etiology of EA2, FHM, and on the other hand, epilepsy (Jen et al., 2004, Vanmolkot et al., 2003).

Vertigo and dizziness in migraine patients will most probably receive more emphasis in the future, because the definition of migraine vertigo is becoming better clarified (Neuhauser et al., 2001, Neuhauser and Lempert, 2004). Neurotologic studies have brought insight into the pathophysiology of migraine and confirmed the subclinical lesions in the cerebellum (Kruit et al., 2004), which may broaden our view of the interconnections in migraine mechanisms.

Many EA2 and FHM patients still lack a proper diagnosis in which a genetic test or a biomarker may serve as a helpful tool. However, clinical diagnosis has a value in itself, even with no genetic confirmation of the disease. Most likely, more *CACNA1A* mutations for EA2 are yet to be expected. Possibly chromosomal loci other than 19p may be found, since not all EA2 families have been linked to this region (Jen et al., 2004). New chromosomal loci will probably be found in FHM as well, because some FHM families do not link to chromosomes 19p13 or 1q23 (Ducros et al., 1997).

Clinical research will be needed on the phenotype of migraine, FHM, and EA2. Different non-invasive clinical and neuroimaging techniques, in vivo physiology, and pharmacological studies integrated with molecular genetics will have implications for the understanding of migraine and EA2 pathogenesis (Goadsby, 2004, Cohen and Goadsby, 2004). Functional studies on the consequences of disease-causing mutations will probably be of great importance. Studies on the rare types of migraine will most probably aid in the understanding of the more common types of migraine when one will see how the functional defects converge as common pathophysiological mechanisms of migraine. These efforts will eventually lead to better diagnostics and treatments for these disorders.

CONCLUSIONS

The common types of migraine, familial hemiplegic migraine, and episodic ataxia type 2 show an interesting unity, with partially shared genetic backgrounds. New scientific data on the pathophysiology and genetics of these diseases is rapidly accumulating. This study was centered on the neurotologic abnormalities of migraine syndromes and on the other hand on the pathophysiology of EA2 with respect to neurotologic and metabolic changes. The main results and conclusions are as follows:

- The common types of migraine seemed to share similar types of neurotologic abnormalities that suggested mainly vestibulocerebellar dysfunction in oculomotor and postural control.

- In FHM2, neurotologic changes were subclinical, consistent with changes seen in the common types of migraine.

- A novel mutation of the *CACNA1A* was identified in a family with EA2.

- EA2 patients showed improved baseline oculomotor and postural function while on acetazolamide treatment, which is a novel finding. These results may reflect the diversity of dysfunctional calcium channels and confirm the action of acetazolamide treatment for EA2.

- EA2 patients, most of whom were nonataxic, showed decreased cerebellar total creatine in ^{1}H MRS. This most probably is the first sign reflecting P/Q-type calcium channel dysfunction in Purkinje cells.

In conclusion, vestibulocerebellar changes in oculomotor and postural function were subclinical in the common types of migraine and partly in EA2 patients as well. ^{1}H MRS for FHM2 patients could bring novel understanding to FHM2 pathophysiology, especially targeted at the cerebellum.

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