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Childhood Absence Epilepsy

An epidemiological, neuropsychological and molecular genetic study

ACADEMIC DISSERTATION
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TO TUIKE, SOLMU AND VILPPU
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1. **List of original publications**


This thesis is based on the four substudies listed above. In addition, some unpublished data are presented.
2. Abbreviations

AED antiepileptic drug  
*CA10* carbonic anhydrase X  
*CACNA1A* voltage-dependent P/Q-type calcium channel, alpha 1A subunit  
*CACNA1G* voltage-dependent T-type calcium channel, alpha 1G subunit  
*CACNA1H* voltage-dependent T-type calcium channel, alpha 1H subunit  
*CACNA1I* voltage-dependent T-type calcium channel, alpha 1I subunit  
*CACNB1* voltage-dependent L-type calcium channel, beta 1 subunit  
*CACNG1* voltage-dependent L-type calcium channel, gamma 1 subunit  
*CACNG3* voltage-dependent calcium channel, gamma 3 subunit  
*CACNG4* voltage-dependent calcium channel, gamma 4 subunit  
*CACNG5* voltage-dependent calcium channel, gamma 5 subunit  
CAE childhood absence epilepsy  
*Cav3.2* voltage-dependent T-type calcium channel, alpha 1H subunit  
*CBZ* carbamazepine  
*CEPH* Centre d'Etude du Polymorphisme Humain  
Cl- chloride ion  
*CLCN2* chloride channel 2  
cM centimorgan  
*CNP* copy number polymorphism  
*CNV* copy number variation  
*CT* computer tomography  
*DNA* deoxyribonucleic acid  
*ECA1* epilepsy childhood absence locus 1  
*EEG* electroencephalography  
*EMA* eyelid myoclonic absence epilepsy  
*ETM* etosuximide  
*FS* febrile seizure  
*FS+* febrile seizure plus
GABRA1 GABA_A receptor, alpha 1 subunit
GABRB3 GABA_A receptor, beta 3 subunit
GABRG2 GABA_A receptor, gamma 2 subunit
GEFS+ generalised epilepsy febrile seizure plus
GLUT1 glucose transporter 1
GTCS generalised tonic-clonic seizure
GWAS genome wide association study
GWS genome wide scan
HOXB2 homeobox-B2
Hz Hertz
IGE idiopathic generalised epilepsy
ILAE International League Against Epilepsy
IBD identical-by-decent
IMPA2 myo-inositol monophosphatase-2
IQ intellectual quotient
JAE juvenile absence epilepsy
JME juvenile myoclonic epilepsy
JRK jerky
JH8 jerky homolog of human on chromosome 8
KCNH4 voltage-gated potassium channel, subfamily H (eag-related), member 4
KCNH6 voltage-gated potassium channel, subfamily H (eag-related), member 6
KCNJ2 inwardly rectifying potassium channel, subfamily J, member 2
KCNJ16 inwardly rectifying potassium channel, subfamily J, member 16
LEV levetiracetam
LOD lod score
LTG lamotrigine
MMD macrophage differentiation associated protein
MKS1 Meckel syndrome, type 1
MRI magnetic resonance imaging
mRNA messenger ribonucleic acid
NPL non-parametric linkage
OMIM Online Mendelian Inheritance in Man
OXC oxcarbazepine
PCR polymerase chain reaction
PHOSPHO1 phosphatase, orphan 1
PHT phenytoin
PS Panayiotopoulos syndrome
PSD95 post-synaptic density protein 95
RAP2A member of RAS oncogene family
RCT randomised controlled trial
RNA ribonucleid acid
RPIP8 Rap2-interacting protein 8
SCN1A sodium channel, alpha 1 subunit
SCN1B sodium channel, beta 1 subunit
SLC2A1 solute carrier family 2, member 1
SLC25 solute carrier family 25
SLC25A39 solute carrier family 25, member 39
SMEI severe myoclonic epilepsy of infancy
SNP single nucleotid polymorphism
SPSS Statistical Package for the Social Sciences
STIM Library of Sensory, Cognitive, and Neuropsychologocal Tasks
TBC1D24 TBC domain family, member 24
TPM topiramate
VPA valproate
WISC-R Wechsler Intelligence Scale for Children, Revised
WPPSI-R Wechsler Preschool and Primary Scale of Intelligence - Revised
3. Abstract

Childhood absence epilepsy (CAE) is a well known syndrome belonging to the group of idiopathic generalised epilepsies (IGE). CAE is considered as a benign epilepsy syndrome with respect to seizure remission and cognitive functions. The genetic etiology of CAE has been under research without conclusive answers. This study was carried out to evaluate the short-term neurocognitive outcome and short- and long-term clinical outcome in patients with CAE and to identify the clinical features and molecular genetic background in two families with CAE and febrile seizure (FS) as main phenotypes.

In the short-term clinical and neurocognitive outcome study (Study 1), eleven patients with typical absence seizures were studied at the time of the diagnosis and after ten months’ antiepileptic drug (AED) treatment by video-electroencephalography (video-EEG) recordings, general intelligence (IQ) measurements and computerized neurocognitive assessments focusing on the fine-motor fluency, sustained attention and visual and spatial memory. Eleven age and gender matched control individuals were studied accordingly. The long-term clinical outcome of patients with CAE is based on the data of Studies 1, 2 and 4. The patients and/or their parents were interviewed and the medical files were reviewed to collect data on the course of the disease, medication, side-effects of AED, remission and family history. In Studies 3 and 4, members of two families (family 98 and family 5) with a proband diagnosed with CAE and other family members with epilepsy or FS participated in the clinical and molecular genetic studies. The following epilepsy syndromes or seizure types were presented in these families: CAE (4), CAE and FS (1), FS only (9), benign temporal lobe epilepsy (2), Panayiotopoulos syndrome (PS) (1), probable PS and FS (1) and FS and secondary generalised epilepsy (1). In both families a genome wide scan (GWS) with fine-mapping of the candidate loci and sequencing of the candidate genes were performed.
In the short-term outcome study (Study 1) the patient group improved in fine-motor fluency, attention and visual memory whereas the control group improved in fine-motor fluency and attention. The impairment in visual memory in the patient group correlated with the duration of the generalised discharges in their EEG recordings and improved significantly as a result to seizure cessation. In the long-term outcome study (Study 2) the mean follow-up time was 10 years (range 2-22 years). Ninety-four percent of the patients had been seizure free for more than 2 years. Thirty-six percent of the patients with CAE had relatives with epilepsy or FS.

All together sixty-one family members of families 98 and 5 participated in the clinical and molecular genetic study in Studies 3 and 4. In family 98 an autosomal dominant inheritance of seizure susceptibility with a contribution of three chromosomal loci, including a probable new locus on chromosome 17q12-24 and two modifier loci on chromosomes 5q11.2 and on 18p11-q11 were identified. Ten brain-expressed ion-channel genes were sequenced. No disease-associated alteration was found. In family 5, the inheritance pattern was suggestive of a polygenic complex inheritance. Two loci on chromosomes 16p12.3-p13.3 and 22q13.1-q13.31 were fine-mapped. After fine-mapping using multipoint non-parametric analysis, a p-value of 0.0014 was obtained between the disease and marker D16S3072 at chromosome 16p13.3. Sequence analysis of voltage-dependent T-type calcium channel alpha 1H subunit (CACNA1H) on chromosome 16p13.3 revealed a novel sequence alteration resulting in the p.Pro686Leu amino acid change. This variant partially segregated with the affection status in the family.

This study demonstrates that most patients with CAE reached seizure freedom and especially visual memory improves after seizure cessation. About a third of the CAE patients had relatives with epilepsy or FS. Clinical and molecular genetic results in two families with heterogenous phenotypes including CAE complied with the complex genetics model of common epilepsies.
4. Introduction

Epilepsies can be caused by both genetic and acquired factors. The cumulative incidence of epilepsy up to age of 74 years is 3% (Hauser et al. 1993). Those epilepsies that are believed to be largely genetic are designated ‘idiopathic’ epilepsies and comprise ~30% of all cases (Hauser et al. 1993, Eriksson and Koivikko 1997, Sillanpää et al. 1999, Jallon et al. 2001). CAE belongs to the IGEs presenting about 25-50% of them. Of pediatric epilepsies CAE is the most common form and it accounts for ~12% of all cases of childhood-onset epilepsy (Berg et al 2000). Juvenile absence epilepsy (JAE) and juvenile myoclonic epilepsy (JME) phenotypically partially resembling syndromes belong as well into the IGEs, but are more rare (Eriksson and Koivikko1997, Sillanpää et al. 1999, Waaler et al 2000, Berg et al. 2000, Jallon et al. 2001).

CAE is an epilepsy beginning after age of 4 years. The child has a normal development and is in most cases the only member with epilepsy in his/her family. The rareness of CAE leads to an obvious parental question: why our child? What is the etiology of CAE? How to treat the condition? Is it necessary to treat the child with AED? The short absence seizures consist of very subtle clinical features. Occasionally the child may have had seizures for several months or longer before a diagnosis is made. The effect of seizures on the cognitive performance is not very obvious. Commonly the child is able to continue whatever she/he was doing after the short absence seizure is over. These phenomena show the benign nature of CAE (Berg et al 2010).

The classification of epilepsies has been under re-evaluation by the International League Against Epilepsy (ILAE). The first published proposal for International Classification of the Epilepsies is from 1970 (Merlis 1970). The following ones were published in 1981, 1985 and the fourth revised Proposal for the Classification of Epilepsies and Epileptic Syndromes was published in 1989 (ILAE 1981, 1985 and 1989). Due to increasing knowledge in genetics, neuroimaging and therapeutics in pediatric and adult epileptology an update of the classification has been of interest.
to ILAE. The goal has been to develop a methodologically and conceptually sound and clinically meaningful revision to the classification of the epilepsies and seizures. According to the last Report of the ILAE Commission on Classification and Terminology the main seizures types, generalised and focal seizures do persist but their content is redefined (Berg et al. 2010). The concepts: idiopathic, symptomatic and cryptogenic are replaced by modified concepts: genetic, structural–metabolic and unknown. The organisation of forms of epilepsy is following: electroclinical syndromes, non-syndromic epilepsies with structural–metabolic causes, and epilepsies of unknown cause.

CAE as an apparently simple epileptic syndrome has also been under re-evaluation. Recently published studies have shown that using strict diagnostic criteria according to Loiseau and Panayiotopoulos (http://www.ilae-epilepsy.org/Visitors/Centre/ctf/childhood_absence.cfm) an even more favourable outcome than considered earlier can be reached in these patients (Grosso et al. 2005, Valentin et al. 2007). The diagnostic criteria of the proposals of years 1985 and 1989 state that the child with CAE has normal neurocognitive development (ILAE 1985, 1989). Cognitive function may be defined as the capacity of the brain to process information accurately and to program adaptive behaviour. It involves e.g. the ability to solve problems, communicate, memorize information and focus attention (Aldenkamp et al. 2004). A study using simultaneous video-EEG monitoring and computerised neurocognitive tests has shown that time estimation ability in children with absence seizures is affected by the characteristic generalised 3 Hz spike-wave discharges of CAE (van Luijtelaar et al. 1991). It has also been shown that short-term spatial and verbal memory is impaired during generalised discharges in half of the patients with various epilepsy types (Binnie et al. 1987). Cognitive functioning can be indirectly measured by evaluating academic achievements although it is clear that other factors also affect the success at school (Sturniolo and Galletti 1994, Oostrom et al. 2003, McNelis et al. 2005). Patients with absence epilepsy, including CAE and JAE performed significantly worse than the control group at primary and secondary school and were less likely to graduate from high school or to attend higher education (Wirrell et al. 1997).

It is difficult to answer definitively to the parents’ question: what is the reason for CAE in my child? Since the time of Hippocrates, epilepsy has been recognised to have a familial component. William Lennox presented the first concordant twin pair
Constance and Kathryn McN. with the diagnosis of petit absence (Figure 1) (Lennox 1951, Vadlamudi et al. 2004). Later family and twin studies have confirmed the genetic etiology of CAE (Italian League Against Epilepsy Genetic Collaborative Group 1993, Berkovic et al. 1998, Marini et al. 2004). The first genetic locus on chromosome 8q24 was mapped in a large Indian family with CAE and generalised tonic-clonic seizures (GTCS) (Fong et al. 1998). Since then other chromosomal loci and genes have been linked to CAE (reviewed in Helbig et al. 2008). CAE, while belonging to the IGEs, is designated to present complex genetics with a polygenic heterogeneity model (Dibbens et al. 2007).

This study was undertaken to study prospectively the short-term neurocognitive outcome in patients with newly diagnosed typical absence seizures, which has not so far been evaluated in Finland and to study retrospectively the long-term outcome in patients with CAE. Moreover, clinical seizure phenotyping and molecular genetic studies were performed in two families with a proband diagnosed with CAE and other family members with heterogeneous seizure phenotypes. These families were among the first Finnish families of their kind to be studied and as such valuable in the complex puzzle aiming at unravelling the complex genetics in CAE.

Figure 1. Photograph and EEG of Constance and Kathryn, monozygous twins concordant for childhood absence epilepsy, both with seizure onset at age 6 years, Lennox 1951, Vadlamudi et al. 2004. Reprinted with permission.
5. Review of the literature

5.1 Childhood absence epilepsy

Childhood absence epilepsy is a well-characterized syndrome belonging to the IGEs and the proposed diagnostic scheme of ILAE can be followed for describing individual patients (Table 1) (Engel 2001). The other main IGE syndromes are JAE, JME and epilepsy with tonic-clonic seizures only.

The diagnostic scheme consists of five axes: ictal phenomenology, seizure type, syndrome, etiology and impairment. The three first axes facilitate a logical clinical approach to the development of hypotheses necessary to determine the diagnostic studies and therapeutic strategies to be undertaken in an individual patient. For the fourth axis, etiology of CAE, the basis was laid by the seminal twin study of Lennox (1951) and the study on the inheritance of generalised spike-wave discharges of Metrakos and Metrakos (1961). The fifth axis, impairment, has not generally considered a major problem in this patient group, although poor psychosocial outcomes in adulthood have been reported (Wirrell et al. 1997).

Table 1. A Proposed Diagnostic Scheme for Patients with Epileptic Seizures and with Epilepsy

<table>
<thead>
<tr>
<th>Axis</th>
<th>Description</th>
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<tbody>
<tr>
<td>Axis 1</td>
<td>Ictal phenomenology</td>
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<tr>
<td>Axis 2</td>
<td>Seizure type</td>
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<td>Axis 3</td>
<td>Syndrome</td>
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<tr>
<td>Axis 4</td>
<td>Etiology</td>
</tr>
<tr>
<td>Axis 5</td>
<td>Impairment</td>
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</table>
5.1.1 Epidemiology of CAE

The mean annual incidence for CAE varies between 1.2 and 7.1 /100 000 in different populations (Granieri et al. 1983, Olsson 1988, Loiseau et al. 1990, Sidenvall et al. 1993, Wirrell et al. 1996a). Cumulative incidence has been estimated at 98/100 000 in children up to 15 years of age (Olsson 1988). The prevalence of CAE has been found to range from 0.1 to 0.7/1000 persons (Granieri et al. 1983, Sidenvall et al. 1993, Waaler et al. 2000). In Finland, the prevalence of CAE in two population-based studies varied from 30 to 60/1000 in children (< 16 years) with epilepsy (Eriksson and Koivikko 1997, Sillanpää et al. 1999).

In large cohorts of epilepsy patients the frequency of CAE varies between 15 to 120/1000 (Oka et al. 1995, Berg et al. 1999). The variation of prevalence depends largely on the mode and source of case definition. CAE is reported more often in girls than in boys (Waaler et al. 2000).

5.2 Ictal phenomenology and seizure type in CAE

A typical ictus in CAE is an absence seizure described in terms according to the ILAE's glossary of descriptive terminology for ictal semiology as a spontaneous and hypokinetic event associated with cognitive dysfunction in perception, attention, emotion, memory and executive function (Blume et al. 2001).

An epileptic seizure is defined as a transient occurrence of signs and/or symptoms due to abnormal excessive and/or hypersynchronous neuronal activity in the brain. The elements of a definition for an epileptic seizure are mode of onset and termination, clinical manifestations and abnormal enhanced synchrony in EEG (Fisher et al. 2005). All these elements are well known in CAE. The typical absence seizure has a clear onset and end. As a clinical manifestation impaired consciousness may occur as a sole manifestation or in various combinations with mild myoclonic elements of the eyes, eyebrows or eyelids. Automatisms such as lip smacking or swallowing may also occur (Valentin et al. 2007, http://www.ilae-epilepsy.org/Visitors/Centre/ctf/childhood_absence.cfm). The generalised 3-4 Hz synchronous symmetric spike-wave discharges in the EEG represent for the
abnormal enhanced synchrony. A typical absence seizure lasts between 5 and 15 s and those lasting longer than 30 s are rare (Loiseau et al. 2002). The absence seizure in CAE is of the self-limited epileptic seizure type (Engel 2001). Besides CAE typical absences are also seen as the defining seizure type in JAE. About 1/3 of patients with JME have absence seizures but they are rarely seen in epilepsy with generalised tonic-clonic seizures only (Duron et al. 2005).

5.2.1 Basic mechanisms of absence seizure in CAE

The first theories on the basic mechanisms of an absence seizure are results of studies on EEGs of patients with absence seizures, on surface EEG recordings of laboratory animals and thalamic depth electrode recording in one child (Jasper and Kershman 1941, Jasper and Droogleever-Fortuyn 1947, Williams 1953).

When EEGs of patients with typical absence seizures were analysed an abrupt onset and termination in both hemispheres and a high interhemispheric synchronization of spike-wave activity was found (Jasper and Kershman 1941). Because no evidence could be found for a cortical origin Jasper and Kershman proposed that the seizures had a subcortical origin. The landmark experiments demonstrated that electrical stimulation of the midline and intralaminar nuclei of the thalamus in cats at a stimulus frequency of 3 Hz could produce bilaterally synchronous spike-wave discharges on the cortical EEG (Jasper and Droogleever-Fortuyn 1947). The relevance of this finding to humans was demonstrated in 1953 when a depth electrode was utilized to record from the thalamus of a child with absence seizures. Bilaterally synchronous 3 Hz spike-wave discharges were observed to arise from the thalamus (Williams 1953). The five theories on the basic mechanisms of an absence seizure (reviewed in Meeren et al. 2005) are illustrated in Figure 2 and summarised below.

In theory 1, the centrencephalic system was held responsible for the generalised seizures with an initial loss of consciousness and a bilateral onset of discharges as seen in the EEG during an absence seizure. This system was thought to be located in the brainstem and diencephalon. In theory 2, the corticoreticular theory both the cortex and the reticular system of thalamus had essential roles in the genesis of
discharges. The crucial factor responsible for the spike-wave discharges was a diffuse increase in excitability of the cortex. Once the oscillation was set in motion, the thalamus and cortex appeared to drive each. The theory 3, the cortical theory was based on clinical observations, which showed that primary generalised epilepsy was the expression of a cortical abnormality, particularly in the frontal lobe, which was rapidly propagated over the cortex through corticocortical pathways. The fourth theory, the thalamic clock theory stated that cortical cells and thalamocortical relay cells fired in synchrony with the EEG spike, whereas reticular thalamic nucleus neurons fired during the slow-wave component. The rhythmic epileptic discharges in the EEG were the result of an abnormal rhythmic oscillation in the intrathalamic network, which imposed its rhythm on the cortex. In the fifth theory, the cortical focus theory a consistent focus was found within the perioral region of the somatosensory cortex. From this focus, seizure activity generalises rapidly over the cortex. The intrathalamic circuitry alone is not sufficient for cortical and thalamic oscillations to occur. During the first cycles of the seizure the cortex drives the thalamus, while thereafter cortex and thalamus drive each other, thus amplifying and maintaining the rhythmic discharge.

Figure 2. Schematic presentation of the five theories on the origin of generalised absence epilepsy, Meeren et al. 2005. Reprinted with permission.
5.3 CAE as a syndrome

A syndrome is a condition that has a clinically recognizable pattern; the clustering of signs and symptoms that form this pattern suggest shared mechanisms. Those who have a particular epilepsy syndrome differ in some fundamental way(s) from those who have other forms of epilepsy. The vast majority of the syndromes recognized in epilepsy are genetic and developmental disorders with onset predominantly in infancy, childhood and adolescence (www.ilae-epilepsy.org/Visitors/Centre/ctf/CTFsyndromes.cfm). The diagnostic criteria used in this study are defined by ILAE in the revised classification for epilepsies and epileptic syndromes published 1989 (ILAE 1989). New criteria for CAE have been proposed by Loiseau and Panayiotopoulos on the ILAE web site (http://www.ilae-epilepsy.org/Visitors/Centre/ctf/childhood_absence.cfm) with the aim of distinguishing a population of children with a better prognosis. Both criteria are presented in Tables 2 and 3.

Typical absence seizures start most often between four and ten years of age, and the peak age at onset varies from five to seven years. The seizures are frequent and daily. In older epilepsy literature the term pyknolepsy was used for typical absence seizures (Adie 1924). The term pyknolepsy is a combination of the Greek word pyknos and epilepsy, the first meaning frequent, closely packed and aggregated. In contrast to frequent seizures in CAE the absences in JAE are infrequent (Panayiotopoulos et al. 1989). CAE responds favourably to treatment, and has commonly a good prognosis. CAE occurs in children with a strong genetic predisposition who are otherwise normal (Loiseau et al. 1995). Approximately 40-50% of patients will develop GTCS (Bouma et al. 1996, Loiseau et al. 2002). An evolvement of CAE to JME has also been recognized (Wirrell et al. 1996a, Martinez-Juarez et al. 2006).
**Table 2. Criteria for CAE defined by ILAE 1989**

1. Age of onset at school age (peak manifestation age 6-7 years)
2. Strong genetic predisposition in otherwise normal children
3. More frequent in girls than in boys
4. Frequent (several to many per day) absences are characteristic
5. EEG reveals bilateral, synchronous symmetrical spike-waves, usually 3 Hz, on a normal background activity
6. During adolescence GTCS often develop
7. Absences may remit or, more rarely, persist as the only seizure type

**Table 3. Inclusion and exclusion criteria of CAE according to ILAE**

Inclusion criteria:

1. Age at onset between 4 and 10 years and a peak at 5 years to 7 years.
2. Normal neurologic state and development.
3. Brief (4 seconds to 20 seconds, exceptionally longer) and frequent (tens per day) absence seizures with abrupt and severe impairment (loss) of consciousness. Automatisms are frequent but have no significance in the diagnosis.
4. EEG ictal discharges of generalized high-amplitude spike and double (maximum occasional three spikes are allowed) spike-and slow-wave complexes. They are rhythmic at around 3 Hz with a gradual and regular slowdown from the initial to the terminal phase of the discharge. Their duration varies from 4 seconds to 20 seconds.

Exclusion criteria:

1. Other than typical absence seizures such as GTCS, or myoclonic jerks prior to or during the active stage of absences.
2. Eyelid myoclonia, perioral myoclonia, rhythmic massive limb jerking, and single or arrhythmic myoclonic jerks of the head, trunk, or limbs. However, mild myoclonic elements of the eyes, eyebrows, and eyelids may be featured, particularly in the first 3 seconds of the absence seizure.
3. Mild or no impairment of consciousness during the 3-Hz to 4-Hz discharges.
4. Brief EEG 3-Hz to 4-Hz spike-wave paroxysms of less than 4 seconds, multiple spikes (more than 3) or ictal discharge fragmentations.
5. Visual (photic) and other sensory precipitation of clinical seizures.
5.3.1 EEG in CAE

The classic EEG pattern associated with absence seizures in CAE consists of generalised, bilaterally synchronous, regular, stereotyped and symmetrical 3 Hz spike-wave complexes that have an abrupt onset and end (ILAE 1989). Hyperventilation is the most effective activator of the classic spike-wave pattern, although photic stimulation may elicit the abnormality in a smaller number of susceptible patients. Single sporadic spikes or spike-wave complexes can appear without obvious clinical accompaniment. The EEG background is normal. Often there is a progressive and regular slowing of the discharge from about 3.5 to 2.5 Hz without discharge fragmentation (Panayiotopoulos et al. 1989).

5.3.2 Procedures at diagnosis

At the time of the diagnosis the interview of the child and the parents consists of the past history of pregnancy, delivery, early development, and learning milestones. Family history of possible FS and epilepsy is evaluated. A routine neurological examination is made along with routine EEG with hyperventilation and photic stimulation.

In clinical practice, a magnetic resonance imagin (MRI) is not necessary in patients who are neurologically normal, have seizures consistent with CAE and a typical EEG. MRI should be performed if the seizures are intractable (Nguyen et al. 2006). However, if quantitative MRI analysis is performed the volumes of cerebral grey and white matter have indicated larger cortical grey matter volumes in IGE patients (mostly adults) than in healthy controls (Woermann et al. 1998). As well, anterior thalamic volumes were larger in patients with absence seizures than in controls (Betting et al 2006). In children with CAE the left orbital frontal gyrus as well as both left and right temporal lobes had significantly smaller grey matter volumes when compared to the age- and gender-matched children without epilepsy (Caplan et al. 2009).
5.3.3 Antiepileptic medication in CAE

In most cases CAE is easy to treat. Ethosuximide (ETM) and valproate (VPA) and are the most commonly used drugs for typical absence seizures. Lamotrigine (LTG) is the third choice treatment (Wheless et al. 2007). A systematic review of three small randomised trials (RCT) from early 1980s comparing efficacy of ETM and VPA concluded that there was no evidence to favour either of the drugs (Posner et al. 2005). Recently, Glauser et al. (2010) provided evidence-based data indicating that ETM is the most appropriate treatment for CAE. Their large RCT on 453 patients with newly diagnosed CAE showed that ETM and VPA are equally effective and more effective than LTG in the treatment of CAE. ETM was associated with fewer adverse attentional effects than VPA (Glauser et al. 2010). The efficacy of ETM for controlling GTCS has been questioned in light of clinical experience (Camfield and Camfield 2005). Schmitt et al. (2007) reported that ETM and VPA have equal efficacy against GTCS.

The SANAD study compared VPA, LTG and topiramate (TPM) in an unblinded RCT in patients with generalised and unclassified epilepsy. In a subanalysis with 450 IGE patients including 230 patients with CAE, JAE or JME TPM was most effective but least tolerated AED. VPA remained the first line treatment for most patients with IGE. LTG was well tolerated but its efficacy was inferior (Marson et al. 2007). In an open label study three out of four patients with CAE became seizure free with levetiracetam (LEV) (Di Bonaventura et al. 2005). Of the newer AEDs, zonisamide has been used in absence seizures (Wilfong and Schultz 2005).Gabapentin is the only drug proven in a published, double blind-trial to be ineffective in CAE (Trudeau et al. 1996).

5.3.4 Discontinuing antiepileptic medication

There are no randomized trials that address the optimal length of treatment. The duration of the medication varies at its shortest from 1-2 years after remission (Camfield and Camfield 2005). The tapering of the AED dose is discussed individually, but a reduction of the dose lasting 3-6 months is commonly used. The risk for recurrence of seizures in CAE has been reported to be as high as 20% (Shinnar et al. 1994).
5.3.5 Outcome of CAE measured by remission rates

In older studies describing small series of patients with absence seizures the remission of seizures occurred spontaneously after persisting 5 - 9 years and the patients remained seizure free for up to 5 - 10 years (Adie 1924). Later, in a larger sample of patients, a tendency for remission of absence seizures has been reported at all ages. A slightly better prognosis was found in those patients whose absence seizures commenced before the age of ten years and who did not have GTCS at all (Gibberd 1966).

A meta-analysis of the outcome of absence epilepsy including 26 publications showed remission rates between 21% and 89% with development of GTCS in about half of the patients included in the studies (Bouma et al. 1996). Different classification criteria and diverse follow-up times have been used and these may explain the variable outcome (Bouma et al. 1996, Trinka et al. 2004). In one population-based study, 65% of patients with an initial CAE diagnosis were seizure free and off therapy, 17% had discontinued AED treatment and had definitive or suspected seizures, and 18% had continued on AED and most of which were seizure free. Fifteen percent of the total population had progressed to JME (Wirrell et al. 1996a). In another population-based study absence seizures persisted beyond the age of 20 years in only 10% of patients. Isolated or rare GTCSs occurred in 26% and were related to the patient’s age at onset of typical absence seizures, being more common (44%) among patients with the onset of typical absences between ages 9 and 10 years than in those for whom typical absences onset was before 9 years (16%) (Loiseau et al.1995).

In two recent studies the criteria developed by Loiseau and Panayiotopoulos (Table 3) were used with the conclusion that remission rates of patients with CAE are greatly influenced by the classification criteria used for selection. Stricter diagnostic criteria allow the definition of a homogeneous group of patients. Factors predicting unfavourable prognosis were GTCS in the active stage of absences, myoclonic jerks, eyelid myoclonia or perioral myoclonia, clinical photosensitivity and EEG features atypical for CAE (Grosso et al. 2005, Valentin et al. 2007). In the first study a high rate of seizure control (95%) and high rate of terminal remission defined by >1 year without AED (82%) were reached (Grosso et al. 2005).
second study complete remission defined by >1 year with or without AED was 65% (Valentin et al. 2007).

5.4 Cognitive impairment and psychosocial aspects in patients with CAE

CAE is often considered a benign form of epilepsy due to mild seizures, which may continue for months before a diagnosis is made. The typical absence seizures commonly cease after AED medication is started. The remission is most often permanent and the patients stay seizure free after drug withdrawal. Some studies have reported the negative impact of absence seizures and/or CAE on cognitive function and psychosocial outcome.

5.4.1 Cognitive impairment

General intelligence, verbal and visual memory, visuospatial perception, verbal learning, word fluency, attention and fine motor fluency have been evaluated in several studies focusing on cognitive functioning in children with different types of seizures (Table 4). Some of the studies have identified lower IQ in children with CAE than in control children or in children with other seizure types (Caplan et al. 2005, 2008, Mandelbaum and Burack 1997, Pavone et al. 2001). Findings of somewhat lower IQ could reflect specific problems in neurocognitive functioning. Difficulties have been found most often in attention and memory. These neurocognitive impairments together with the recently reported smaller frontal and temporal volumes in patients with CAE imply that CAE may not be a benign disorder after all (Caplan et al. 2009). Most of the studies in children with CAE are performed on relatively small patient groups. The children with epilepsy are grouped according to seizure type and often different syndromic entities are grouped together (Fedio and Mirsky 1969, Jambaque et al. 1993, Mandelbaum and Burack 1997, Nolan et al. 2004, Boelen et al. 2005, Henkin et al. 2005, Bhise et al. 2009, Mandelbaum et al. 2009). There is evidence that subclinical generalised spike-wave discharges seen on EEG may have a transient effect on cognition. Reaction times appear to be prolonged when the stimulus is presented during a spike-wave
discharge (Aldenkamp and Arends 2004). It has been questioned if it is warranted to
increase the dose of AED to try to suppress subclinical EEG discharges in a child
with CAE and a clinical cessation of absence seizures. Cognitive side effects of a
higher dose of AED may negate any benefit from eliminating subclinical discharges
(Camfield and Camfield 2005). However, the effect of subclinical EEG discharges
affecting scholastic skills has been shown (Kasteleijn-Nolst Trenite et al. 1988).
There is also evidence that serious accidents may occur as the result of uncontrolled
absence seizures so that the motivation for suppressing all absence seizures is based
in part on physical safety (Wirrell et al. 1996b) in addition to cognitive issues.

5.4.2 Psychosocial aspects

Dieterich et al. (1985) followed up 194 patients in whom the first seizure type
was absence seizure and noted that patients with ongoing seizures, especially
recurrent GTCS, had unfavourable social integration meaning marriage, 
employment or professional training. In the previous study some of the patients
probably had JME, myoclonic absence epilepsy and other absence epilepsies than
CAE. Loiseau et al. (1983) compared the outcome of children with typical absence
epilepsy to that of children with benign rolandic epilepsy and found that the group
with absence epilepsy had poorer social adaptation and higher incidence of
behavioural problems. In a Swedish follow-up study, young adults with a diagnosis
of CAE, JAE or JME in their past were interviewed regarding the social impact of
their epilepsy. Seventy-four per cent considered that schooling, occupation, routines
of daily life, relations to friends or leisure time activities had been affected by their
epilepsy. The result was independent of whether or not they had achieved seizure
control (Olsson and Campenhausen 1993). Additionally, a comprehensive
population-based study from Nova Scotia showed that patients with typical absence
epilepsy also had greater difficulties in the academic-personal lives and in their
behaviour than the control group of patients with juvenile rheumatoid arthritis
(Wirrell et al. 1997). In recent studies comorbidities such as anxiety, externalizing or
disruptive behaviour, ADHD and linguistic deficits have been associated with CAE
Table 4. Results of neuropsychological studies focusing on absence seizures and CAE.

<table>
<thead>
<tr>
<th>Study design / studied cognitive functions</th>
<th>Medication yes / no</th>
<th>Remission yes / no</th>
<th>Identified cognitive problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 centrencephalic epilepsy, TLE / controls</td>
<td>Comparison between groups. IQ, verbal and nonverbal memory, learning and delayed memory, sustained attention.</td>
<td>Yes</td>
<td>Not all</td>
</tr>
<tr>
<td>12 CAE, 3 JAE, 3 IGE, 42 focal epilepsy / 60 healthy controls</td>
<td>Comparison between groups. IQ, verbal and nonverbal memory.</td>
<td>Yes</td>
<td>Not all</td>
</tr>
<tr>
<td>17 generalised non-convulsive, 12 generalised convulsive 11 simple partial, 3 complex partial / no healthy controls</td>
<td>Comparison between groups and at follow-up 6 and 12 mo. IQ, academic functioning, visual motor integration, CBCL (40% drop outs).</td>
<td>No AED at baseline</td>
<td>No data</td>
</tr>
<tr>
<td>16 AS / 16 healthy controls</td>
<td>Comparison between groups. IQ, logical-deductive capacity, visuospatial perception, memory and learning.</td>
<td>Yes</td>
<td>Yes, 13/16</td>
</tr>
<tr>
<td>13 CAE, 32 TLE; 25 FLE / no controls</td>
<td>Comparison between groups. Focus on memory functions only.</td>
<td>Yes</td>
<td>Yes, 7/13</td>
</tr>
<tr>
<td>12 AS, 12 GTCS / 20 healthy controls</td>
<td>Comparison between groups. IQ, attention, verbal learning and memory, word fluency, complex figure recall, fine motor fluency.</td>
<td>Yes</td>
<td>Yes, 6/12</td>
</tr>
<tr>
<td>23 AS, 5 other IGE, 44 cryptogenic focal epilepsy/ 107 healthy controls</td>
<td>Comparison between groups /motor skills, visual and auditory reaction time.</td>
<td>Yes</td>
<td>No data</td>
</tr>
<tr>
<td>62 CAE, 90 focal seizures/ 91 healthy controls</td>
<td>Comparison between groups. IQ, language development psychiatric disorders, CBCL, social skills.</td>
<td>Yes</td>
<td>Not all</td>
</tr>
<tr>
<td>69 CAE /103 healthy controls</td>
<td>Comparison between groups. IQ, language development, psychiatric disorders, CBCL.</td>
<td>Yes, in most</td>
<td>Not all</td>
</tr>
<tr>
<td>18 generalised non-convulsive, 5 generalised convulsive, 34 focal seizures / no healthy controls</td>
<td>Comparison between groups. Learning, memory, attention, motor skill, before AED started at the diagnosis.</td>
<td>No</td>
<td>Not all</td>
</tr>
<tr>
<td>10 generalised non-convulsive, 1 generalised convulsive, 20 focal epilepsy</td>
<td>Baseline 0 mo, follow-up 6 mo and 12 mo. IQ, memory and learning, verbal memory, cognitive processing, attention, eye and hand coordination, perception and motor dexterity.</td>
<td>No AED at baseline</td>
<td>Yes, 9/10</td>
</tr>
</tbody>
</table>

CAE = childhood absence epilepsy, IQ = intellectual quotient, JAE = juvenile absence epilepsy, IGE = idiopathic generalised epilepsy, TLE = temporal lobe epilepsy, FLE = frontal lobe epilepsy, AS = absence seizure, AED = antiepileptic drug, CBCL = Child Behavior Check list, ADHD = attention deficit hyperactivity disorder
5.5 Genetics in idiopathic generalised epilepsy

Twin studies, familial aggregation studies and molecular genetic studies have shown that IGEs have a strong genetic component. Genetic counseling for common IGE syndromes rests on a foundation of twin and family studies conducted over 20 years ago (Blandfort et al. 1987).

5.5.1 Twin studies

Studies of epilepsy in twins have shown an excess of monozygous twins concordant for epilepsy compared with dizygous twins, supporting claims of a genetic component in the common epilepsies (Lennox 1951, Sillanpää et al. 1991, Berkovic et al. 1998, Vadlamudi et al. 2004, Kjeldsen et al. 2005). In an Australian study, in which the classification of seizures and syndromes was based on re-evaluation of original medical files and structured personal interviews, 94% of seizure concordant monozygotic and 71% of dizygotic twins had the same major epilepsy syndrome (Berkovic et al. 1998). The method for data collection affects the concordance rates among twins. If the seizure and epilepsy classification is based on self-reported questionnaires or on hospital discharge registry and medical certificates to enable reimbursement for AEDs the concordance rates remain lower, while still confirming the importance of genetic factors in epilepsy. A proband-wise concordance rate of epilepsy in monozygous twins was 28% compared to 7% concordance rate in dizygotic twins in a study based on self-reported questionnaires (Kjeldsen et al. 2005). In a Finnish population-based study, in which medical certificates to reimbursement for AEDs were used as data source, the proportion of concordant twins for epileptic seizures was 3.4% in monozygotic and 1.4% in dizygotic twins (Sillanpää et al. 1991).

5.5.2 Family studies

Familial aggregation studies on IGE have yielded data on the relationship between different subsyndromes (Italian League Against Epilepsy Genetic
Collaborative Group 1993, Winawer et al. 2003, Marini et al. 2004). Sometimes multiple subsyndromes - CAE, JAE, JME and generalised tonic-clonic seizures only – occur in large families with IGE. In these families a shared genetic influence is assumed to generate the seizure types belonging to the IGES (Ottman 2005). On the other hand, a distinct genetic influence is assumed in CAE and JAE based on familial aggregation studies (Winawer et al. 2003, Marini et al. 2004). Data suggest that CAE and JAE share a close genetic relation, whereas JME is a more distinct entity (Janz et al. 1992, Italian League Against Epilepsy Genetic Collaborative Group 1993, Winawer et al. 2003, Marini et al. 2004). A cumulative incidence of 4.9% of epilepsy has been calculated in first degree relatives of the probands with CAE or JAE (Janz et al. 1992). In some families only one IGE subsyndrome is diagnosed (Italian League Against Epilepsy Genetic Collaborative Group 1993, Cossette et al. 2002, Winawer et al. 2003, Marini et al. 2004, Nabbout et al. 2007). Phenotypic concordance within the CAE proband families was 28%. In JAE proband families 31% of relatives had CAE. But in CAE proband families only 5% had JAE. JME was rare among affected relatives of CAE probands (Marini et al. 2004). The recurrence risk for epilepsy in first degree relatives was three times greater than that of general population (Annegers et al. 1982). Additionally, the decay of risk beyond first degree relatives is known, completing the profile of a complex disease (Heron et al. 2007).

The female preponderance in transmission of seizure liability has also been recognized although not explained. No enhanced risk to the offspring was evident if the father had epilepsy (Annegers et al. 1976, Marini et al. 2004). Moreover, the possibility of bilinear inheritance of IGE may cause a high risk of epilepsy in the offspring. In two families, with both parents diagnosed with IGE the offsprings had more severe seizure phenotypes. Their epilepsy was suggested to be due to the combination of a putative "double dose" as epilepsy genes could be inherited from both parents (Marini et al. 2003a). In another report a benign seizure phenotype was reported with a similar bilinear family history of IGE (Jansen et al. 2004).

Even though the genetic background in CAE is evident familial cases are still rare. Most commonly CAE occurs in a single sporadic patient in a family. The genetic background in these patients may be a de novo mutation or that of a polygenic, multifactorial e.g. complex disease (Dibbens et al 2007, Helbig et al. 2009).
5.5.3 Molecular genetic etiology of idiopathic epilepsies

Based on the current knowledge, the etiology of IGEs is polygenic, i.e. caused by many predisposing alleles, unknown environmental factors and also by a spectrum of epigenetic factors and processes (Berkovic et al. 2006, Qureshi and Mehler 2010). Some reports of rare monogenic forms exist (Cossette et al 2002, Maljevic et al. 2006, Suls et al. 2009).

There have recently been major advances in understanding the genetic basis of Mendelian epilepsies. The first gene for idiopathic epilepsy was identified in 1995 (Steinlein et al. 1995). In the Mendelian epilepsies, most of the established genes encode for subunits of ion channels, both voltage-dependent and ligand-gated (reviewed in Reid et al. 2009). The discovery of these epilepsy genes was facilitated by the availability of large families with many affected members where a single mutated gene segregated with most affected individuals. The variation in expressivity and penetrance associated with mutations of large effect in monogenic Mendelian epilepsies strongly suggests that variation in additional modifier genes and/or environmental factors act in combination with the major gene in modulating the final phenotype. These modifier genes may act as susceptibility genes underlying epilepsy with complex genetics (Dibbens et al. 2007). The monogenic and complex idiopathic epilepsies merge into a continuum from fully penetrant monogenic epilepsies to those caused by less penetrant alleles of large effect, many alleles of small effect (polygenic mode of inheritance), or finally by environmental factors with no recognizable genetic component (Figure 3).

The broad field of complex genetics includes two non-exclusive models for the genetic architecture of a common disease, such as CAE. The ‘common disease, common variant’ hypothesis posits that common diseases are attributable in part to allelic variants present in more than 1–5% of the population (Reich and Lander 2001). For the idiopathic epilepsies no common variants have been discovered (Kasperaviciute et al. 2010, Scheffer and Berkovic 2010). The second model is the ‘common disease, rare variant’ model, where multiple rare variants combine to cause a disease (Manolio et al. 2009). Based on the current knowledge ‘common disease, rare variant model’ suits best for explaining the genetics of polygenic idiopathic epilepsies such as CAE.
5.5.3.1 Molecular genetic etiology of CAE

The first locus linked to CAE on chromosome 8q24 was identified by studying a large multigenerational Indian family (Fong et al. 1998). To date no mutation in this locus has been identified. Susceptibility loci for IGE, including CAE, JAE and JME, were identified on chromosomes 3q26, 14q23 and 2q36.1 (Sander et al. 2000). The chromosomal regions 3q27-q28 harbouring the chloride channel 2 gene (CLCN2) and 16p12-p13.1 harbouring the voltage-dependent calcium channel γ3 subunit gene...
(CACNG3) may be susceptibility loci in subsets of CAE families (Everett et al. 2007a, Everett et al. 2007b). When using genome wide SNP-based linkage analysis a susceptibility locus was identified on chromosome 3p23-p14 (Chioza et al. 2009).

Data on the genes involved in CAE are currently sparse, but points to neuronal ion channels and GABA receptors (Table 5). Heterogeneous epilepsy phenotypes associated with mutations in GABAA receptor γ 2 subunit gene (GABRG2) vary from FS, FS and CAE to generalised epilepsy febrile seizures plus – syndrome (GEFS+) (Wallace et al. 2001, Kananura et al. 2002, Marini et al. 2003b). With some detected mutations functional studies have been performed and the data shows that Cl- currents mediated by GABAA receptor have been reduced and benzodiazepine action is abolished (Wallace et al. 2001). A mouse model harbouring the R43Q mutation in GABRG2 develops spike-wave discharges in the EEG and behavioral arrest compatible with CAE, with ETM treatment reducing spike-wave discharges (Tan et al. 2007). In GABAA receptor α1 subunit gene (GABRA1) functional studies with the first detected mutation in a boy with CAE showed no detectable GABA-evoked currents for the mutant, truncated receptor, which was not integrated into the surface membrane (Maljevic et al. 2006). Recently, in the GABAA receptor β3 subunit gene (GABRB3) functional studies showed that three evaluated mutations reduced GABA-evoked current density. The mutations in GABRB3 were found both in families and in sporadic patients with remitting CAE (Tanaka et al. 2008).

CAE was the first phenotype associated to CACNA1H in which identified variants are present at low frequencies in the general population and are predicted to have a minor functional effect in the voltage-dependent T-type calcium channel α1H subunit (Cav3.2) (Chen et al. 2003, Heron et al. 2007, Liang et al. 2007). The CACNA1H gene variants have altered activation and in-activation of membrane potentials and increased expression of Cav3.2 in membrane (Khosravani et al. 2004, Vitko et al. 2005, Peloquin et al. 2006, Vitko et al. 2007). Furthermore, in voltage-dependent P/Q-type calcium channel α1A subunit gene (CACNA1A), a point mutation is connected to cerebellar ataxia and CAE in one three generation family (Imbrici et al. 2004). Early onset absence epilepsy that usually presents with movement disorder and in some patients intellectual deficit has been linked to inherited or de novo mutations in SLC2A1 gene coding glucose transporter, GLUT1 (Suls et al. 2009).
<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene (if known)</th>
<th>Method</th>
<th>Study subjects</th>
<th>Phenotypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p35-p31.3</td>
<td>SLC2A1/GLUT1</td>
<td>Candidate gene sequencing</td>
<td>34 patients</td>
<td>Early onset absence epilepsy</td>
<td>Suls et al. 2009</td>
</tr>
<tr>
<td>3p23-p14</td>
<td><strong>GWS (SNP)</strong></td>
<td></td>
<td>41 nuclear pedigrees</td>
<td>CAE</td>
<td>Chioza et al. 2009</td>
</tr>
<tr>
<td>5q31.1-q33.1</td>
<td>GABRG2</td>
<td>GWS</td>
<td>4 - generation family</td>
<td>CAE, GEFS+, FS, FS+, MAE</td>
<td>Wallace et al. 2001</td>
</tr>
<tr>
<td>5q34</td>
<td>GABRA1</td>
<td>Candidate gene sequencing</td>
<td>135 patients</td>
<td>Idiopathic absence epilepsy</td>
<td>Kananura et al. 2002</td>
</tr>
<tr>
<td>8q24</td>
<td><strong>GWS</strong></td>
<td></td>
<td>5 - generation family</td>
<td>CAE, generalised spike-wave</td>
<td>Fong et al. 1998</td>
</tr>
<tr>
<td>15q11.2-q12</td>
<td>GABRB3</td>
<td>Candidate gene sequencing</td>
<td>48 patients</td>
<td>Remitting CAE</td>
<td>Tanaka et al. 2008</td>
</tr>
<tr>
<td>16p13.3</td>
<td>CACNA1fH</td>
<td>Candidate gene sequencing</td>
<td>118 patients</td>
<td>CAE</td>
<td>Chen Y et al. 2003</td>
</tr>
<tr>
<td>16p12-13.1</td>
<td>Regional linkage</td>
<td></td>
<td>100 patients</td>
<td>CAE</td>
<td>Liang J et al. 2006</td>
</tr>
<tr>
<td>16p12-13.1</td>
<td>Regional linkage</td>
<td></td>
<td>192 patients</td>
<td>IGE, TLE, FS, MAE</td>
<td>Heron et al. 2007</td>
</tr>
<tr>
<td>19p13.2-13.1</td>
<td>CACNA1A</td>
<td>Candidate gene sequencing</td>
<td>1 patient</td>
<td>Absence epilepsy, ataxia and GTCS</td>
<td>Jouveneau et al. 2001</td>
</tr>
<tr>
<td>19q13.1</td>
<td>SCN1B</td>
<td>Candidate gene sequencing</td>
<td>1 family</td>
<td>CAE and cerebellar ataxia.</td>
<td>Imbriici et al. 2004</td>
</tr>
</tbody>
</table>

CAE = childhood absence epilepsy, FS = febrile seizure, GEFS+ = generalised epilepsy febrile seizure plus, GTCS = generalised tonic-clonic seizure, GWS = genome wide scan, JME = juvenile absence epilepsy, MAE = myoclonic astatic epilepsy, SNP = single nucleotide polymorphism, TLE = temporal lobe epilepsy
Copy number variations (CNV) (Conrad et al. 2010) have also been identified in CAE. A recurrent microdeletion at 15q13.3 was recently shown to constitute a genetic risk factor for common IGE syndromes and was found in 1% of IGE patients (Helbig et al. 2009). Furthermore, microdeletions were detected in 1.8% of DNA samples of IGE patients in a study focusing on known genomic hot spot regions (1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2) in various neuropsychiatric disorders including autism, intellectual disability and schizophrenia. Significant associations with IGEs were detected for the microdeletions at 15q11.2 and 16p13.11 (de Kovel et al. 2010). Some of the microdeletions have been de novo mutations and some inherited from an affected or an unaffected parent (Helbig et al. 2009, de Kovel et al. 2010).

5.6 Positional gene identification

Positional cloning, first introduced in 1986 (Royer-Pokora et al. 1986), allowed the isolation of disease genes without direct information about the basic biochemical background. The identification of a defective gene is based on its chromosomal map position. In positional cloning the defective gene is first assigned to a chromosomal position using linkage analysis. The candidate interval is narrowed using additional markers and finally the disease gene identified among the genes in the region (Collins 1992).

5.6.1 Linkage analysis

In a linkage study, the segregation of genetic marker alleles is compared to the segregation of the study phenotype in the pedigree.

First, families where the disease of interest is segregating are identified and the phenotype is evaluated carefully followed by sampling of DNA. Families with two or more patients are suitable for linkage analysis, but the size and number of families needed vary depending on the mode of inheritance and analysis method used.
For genotyping, microsatellite markers have been increasingly used since a detailed microsatellite map of the human genome was derived in 1992 (Weissenbach et al. 1992). Microsatellite markers are used to genotype a candidate locus for a disease in question or if there is no known locus, the affected and healthy family members are genotyped with multiple microsatellite markers covering the whole genome. If the family material is large enough, 350-400 informative microsatellite markers covering the genome evenly are enough for detecting linkage. Single nucleotide polymorphisms (SNPs) are nowadays used for GWSs, too. Although SNPs are less informative than microsatellite markers, the higher density of SNPs give finer resolution and array-based SNP genotyping techniques are more high-throughput than genotyping microsatellite markers.

Finally, the likelihood of two (a single disease locus; two-point linkage) or more loci (multipoint linkage) being co-inherited between affected relatives more often than expected by chance can be calculated (Ott 1999).

5.6.1.1 Parametric linkage analysis

In parametric linkage analysis a disease inheritance model is specified *a priori*. This method is best suited to the analysis of single gene disorders exhibiting a Mendelian mode of inheritance, where a major gene makes a large contribution to the phenotype. The disease model parameters include the mode of inheritance, the penetrance and the disease allele frequency (Ott 1999). When analysing epilepsy families, it is important to include a rate for sporadic patients (phenocopies) and incomplete penetrance, in which only a proportion of affected individuals are explained. The fact that many idiopathic epilepsies show a familial pattern of disease consistent with a major gene component does not necessarily imply that there is only one gene that may lead to disease. There is a wide range of methods and software packages for performing linkage analysis (Dudbridge 2003).

5.6.1.2 Non-parametric linkage analysis

Since common diseases do not follow the Mendelian principles of inheritance, non-parametric, also known as model-free, statistical analysis methods have been
developed (e.g. the SimWalk, the Genehunter, and the Merlin programs) (Kruglyak et al. 1996, Sobel and Lange 1996, Abecasis et al. 2002). When multiple genes contribute to disease risk, each gene may act in a dominant, recessive or X-linked fashion, but the mode of familial inheritance is usually unrecognized or uncertain. Non-parametric linkage analysis does not require specification of a disease model. Instead it maps regions showing excess identical-by-descent (IBD) allele sharing among affected relatives compared to the expected sharing that is based only on their familial relationship and random Mendelian allele segregation. This approach can be applied to affected sibling pair studies, affected relative pairs and multiplex families. However, parametric linkage analysis offers a distinct advantage over non-parametric approaches as it can offer increased power when the disease model is specified correctly (Ott 1999).

5.6.1.3 Two-point analysis

Two-point linkage analysis is based on the fact that two closely related loci on the same chromosome are inherited together more often than would be expected by chance. The recombination fraction (theta, $\theta$) indicates the probability that a recombination, that is a crossing-over event during meiosis, is observed between the two loci. Two loci are genetically linked when theta ($\theta$) is less than 0.5 (or 50%). The genetic distance between two loci is measured in centiMorgans (cM). If two loci are 10 cM apart from each other, there is a 10% chance of recombination between these loci as the chromosome is passed to the next generation. The genetic distance is not linear compared to physical distance, but on average 1 cM corresponds to 1 Mb (one million nucleotides). The recombination fractions differ between females and males (Terwilliger and Ott 1994).

Linkage studies are used to localise predisposing candidate loci. Calculation of the LOD score ($Z$; logarithm of odds) represents an efficient statistical test for proving that the result is not simply due to chance (Morton 1955). The LOD score gives a logarithm based likelihood ratio of a pedigree on two alternative hypotheses: is linkage with a recombination fraction of $\theta$ more likely or is it more likely that the two loci are not linked ($\theta = 0.5$) (Terwilliger and Ott 1994).
For a disease with a Mendelian inheritance a LOD score of 3.0 or higher is taken as evidence of linkage with a 5% chance of error (p=0.05) for a single test. A LOD score of 3.3 being equivalent to \( p = 5 \times 10^{-5} \) is regarded as a threshold for a genome wide significance and LOD score threshold of 1.9 as suggestive evidence of linkage (Lander and Schork 1994). When the LOD score is \( \leq -2 \) the linkage is considered to be excluded. LOD scores between -2 and +3 do not give conclusive results (Lander and Kruglyak 1995).

5.6.1.4 **Multipoint analysis**

Multipoint analysis uses a map of genetic markers to reconstruct inheritance along a chromosome. Linkage analysis can be more efficient if data for more than two loci are analysed simultaneously. Multipoint analysis helps to overcome problems caused by the limited informativeness of genetic markers. The highest peak marks the most likely location of the disease gene (Strachan and Read 2004). Large pedigrees, however, create a computational challenge. Simwalk2 (www.genetics.ucla.edu/software/simwalk_doc/) is a statistical genetics computer application that do not subdivide large families (Sobel and Lange 1996). In the location score analysis, the most likely initial genetic-descent graph of a pedigree is examined and its likelihood or probability is computed for the joint transmission of the disease and the marker loci. This likelihood is a function of the location, in map units, of the disease locus (Sobel and Lange 1996). Location scores are used to position a trait locus relative to an existing set of mapped markers (Lange and Sobel 1991). The p-value of 0.01 is considered suggestive for linkage and the p-value of 0.001 as a significant evidence of linkage (personal communication of E. Sobel in Tikka-Kleemola et al.2010).

5.6.2 **Mutation detection**

The genes in the candidate region are evaluated and prioritized with respect to their known or predicted function and tissue expression profiles before picking candidate genes for sequencing. The genome browsers make the sequence data easy to access. There are three widely used genome browsers available on-line: the
The final step in identifying a disease gene is to detect disease-associated mutations in patient samples. Sequencing is nowadays used as a primary method because of its lower costs. However, sequencing of only the coding regions of a gene is not an optimal method if the mutation lies in an intronic region. Alternative methods, such as quantitative polymerase chain reaction (PCR), Southern blotting or array-based comparative genomic hybridization are required to detect larger chromosomal aberrations e.g. deletions, insertions or duplications.

Distinguishing between a mutation that changes the phenotype and a harmless polymorphism can be a difficult task. After detecting a genomic variant in a patient, the segregation of the change in the family is studied. If the change is a mutation, it should co-segregate with the disease in the family. Different types of mutations are possible (Strachan and Read 2004). Nonsense mutations causing a premature stop codon, frameshift or deletion of the whole gene almost certainly impact on gene function. The effect of a missense variation has to be analysed based on the type of the replaced amino acid (conserved versus nonconserved). Substitutions changing conserved splice site sequences lead to exon skipping and altered splicing, which can have an impact on the expression or function of the gene. A mutation should not be detected in a panel of healthy control individuals or, if detected, it should not exceed the estimated carrier frequency in the population.
6. Aims of the study

The aims of the study were:

• To study prospectively the neurocognitive functioning in newly diagnosed CAE patients and evaluate the short-term outcome after effective AED treatment (Study 1)

• To characterize mainly retrospectively the clinical features and evaluate the long-term outcome in patients with CAE measured with seizure remission (Studies 1, 2 and 4)

• To characterize the clinical features and molecular genetic background in two families with heterogeneous seizure phenotypes and a proband with CAE (Studies 3 and 4).
7. Material and methods

7.1 Ethical aspects

The study protocols were approved by the Ethics Committee of Pirkanmaa Hospital District, Tampere, Finland and for genetic studies also by the Ethics Committee of the Hospital for Children and Adolescents, University Central Hospital, Helsinki, Finland.

All patients, control individuals (Study 1), parents and relatives (Studies 3 and 4) provided written consent prior to attending the study.

7.2 Patients with CAE in short-term clinical and neuropsychological outcome evaluation (Study 1)

Between June 1997 and March 2002 twelve newly diagnosed patients with typical absence seizures were recruited to participate in the study at the Department of Pediatric Neurology of Tampere University Hospital. The inclusion criteria were the diagnostic criteria for CAE according to the 1989 revised classification (Table 2) (ILAE 1989). Seizure freedom, as an additional inclusion criterion was elicited at follow-up evaluation. The exclusion criteria were: other than possible genetic etiology, mental retardation based on clinical evaluation, the continuation of clinical absence seizures at the follow-up assessment. One patient was excluded from the analysis due to data collection error. For a control group gender and age matched children were recruited from the outpatient clinic for asthma at the Department of Pediatrics of Tampere University Hospital.

7.3 Patients with CAE in long-term outcome evaluation (Studies 1, 2, 4)

All 52 patients with CAE evaluated for the long-term outcome were identified through following sources.
1. Eleven patients with CAE participating in Study 1.
2. Thirty seven patients with CAE identified for Study 2
3. Four patients with CAE participating in Study 4

The inclusion and exclusion criteria were as described in 7.2 complemented with compulsory participation in the follow-up interview if no up-dated medical records were available.

7.4 Multiplex families with a CAE patient as proband (Studies 3 and 4)

Six families with three or more additional individuals with IGE or FS were identified in Study 2 through a CAE proband. One multiplex family was diagnosed at the Department of Pediatrics in Southern Ostrobothnia Central Hospital, Seinäjoki, Finland. The parents and other relatives of five CAE probands agreed to participate in the clinical and genetic study of FS and epilepsy. Two of these families were studied in detail (Studies 3 and 4). The seizure types and syndromes were classified according the ILAE (ILAE 1989) and Panayiotopoulos syndrome according to the consensus view of an international consortium (Ferrie et al. 2006). FS were classified as simple or complex (Shinnar and Glauser 2002).

For genetic linkage analysis individuals were considered affected if they had a diagnosis of epilepsy, if they had a medical record of FS or if at least one close relative confirmed that they had had FS. Family members who spontaneously described repeated episodes of symptoms of focal seizures were also considered to be affected. The cornerstone of epilepsy diagnosis of these family members rested entirely on anamnestic data (Aicardi 2008).

7.4.1 Seizure and epilepsy phenotypes in family 98

Family 98 was identified through a female patient with CAE. Seven other individuals had a history of seizures. Altogether, twenty-seven family members were interviewed and venous blood samples were drawn from 25 individuals (Figure 4). None of the spouses had a history of seizures. We performed sleep deprivation EEGs with hyperventilation and intermittent photic stimulation on nine
individuals. These recordings were normal. The clinical characteristics of the patients are summarised in Study 3 (please see Table 2 in Study 3).

Figure 4. Pedigree of Family 98.
? = unknown affection status.

7.4.2 Seizure and epilepsy phenotypes in family 5

Family 5 was recognized through a sibship with three affected individuals (5-21, 5-22 and 5-23) with three different seizure phenotypes (Figure 5). The descendants of 5-1, 5-2, 5-3, 5-4 5-34 and 5-51 are later in this study called the core family. During the field trip to interview and collect the blood samples of the core family two additional affected individuals (5-18 and 5-33) connected to the core family through an unaffected spouse (5-17) were recognized. Later in this study, individuals 5-18, 5-33 and their parents are called the extended family. All patients except 5-18 showed a benign seizure phenotype. Altogether 36 family members were interviewed and venous blood samples were collected for DNA extraction. Eleven individuals had a history of seizures. The clinical characteristics of the patients are presented in Study 4 (Table 1 in Study 4).
• The yellow bar marks one of the haplotypes segregating on chromosome 16p12.3-p13.3 containing the SNP P686L in CACNA1H.
• The red bar marks the other haplotype segregating on chromosome 16p12.3-p13.3.
• The blue bar marks the haplotype segregating on chromosome 22q13.1-q13.31.
• DNA of individuals marked with an asterisk was used in the GWS.
• Individuals marked with identifiers in bold face belong to the core family.
• The haplotype on the individual 5-51, in italics is constructed based on the haplotypes of her children and siblings.

Figure 5. Pedigree of Family 5
7.5 Methods

7.5.1 Patient characterisation, clinical methods (Studies 1, 2, 3, 4) and neuropsychological assessment (Study 1)

The following topics were covered when reviewing the medical records and interviewing patients and their family members: development, family history and personal history of FS and afebrile seizures, age at onset of seizures, age at epilepsy diagnosis, AED, remission and relapse. All EEG reports were reviewed. Information of imaging studies (CT and MRI) was collected. In Study 2, remission was defined as seizure freedom >2 years with or without AED. Relapse was defined as a seizure occurrence of any kind. Intractability was defined as continued seizures in a patient in spite of adequate AED therapy and proven good compliance.

The baseline and the follow-up computer-based neuropsychological assessments were obtained during the 24-hour video-EEG recording for the newly diagnosed CAE patients. Both assessments included the same tasks. For the CAE group, the mean interval between the two assessments was on average 10 months (7 – 13 months). For the control group, follow-up neuropsychological assessment without simultaneous video-EEG recording was done on average 11.5 months (6 – 18 months) after the first assessment. The difference between the two assessments did not differ significantly between the groups. General cognitive level was assessed in a separate session without video-EEG recording. The shortened version of the standardized Wechsler Intelligence Scale for Children—Revised (WISC-R) was used for 18 children (age range 7.2 - 14.7 years) and the Wisconsin Preschool and Primary Scale of Intelligence—Revised (WPPSI-R) for four children (age range 5.5 – 5.8 years) (Wechsler 1984, Wechsler 1995).

Neuropsychological assessment during video-EEG recording included STIM™ tasks (a Library of Sensory, Cognitive and Neuropsychological Tasks, Neuro Scan, Inc. Charlotte, USA) involving fine-motor fluency, sustained attention, and visual and spatial memory. In the fine-motor task, the fluency of finger tapping was measured. The attention task measured the ability to sustain attention in responding correctly to the number stimuli on the screen. In the visual memory task, children
had to remember four sequentially presented pictures. In the spatial memory task, children had to remember the location of blocks presented on a computer screen.

7.6 Genetic analysis

7.6.1 Genealogy (Study 4)

We asked from the oldest sampled generation (generation I in Figure 5) information on the birth-places of their parents and continued further collecting church record information on older generations to identify possible common ancestors. The aim was to detect possible consanguinity between different branches in the family.

7.6.2 Linkage calculation schemes (Studies 3 and 4)

In Study 3, two different calculation schemes were used. Individuals were considered affected in linkage calculation scheme 1 if they had a diagnosis of epilepsy, if they had a medical history of FS or if at least one close relative confirmed that they had had an episode consistent with FS. In linkage calculation scheme 2 family members who described episodes consistent with aura originating from the temporal lobe were also considered affected. Individuals with no history of FS or epilepsy were considered unaffected.

In Study 4, one calculation scheme was used. An affecteds-only strategy was utilised; individuals diagnosed with FS or epilepsy were treated as affected and all other individuals were marked as unknown.
7.6.3 Genotyping (Studies 3 and 4)

DNA extracted from EDTA-blood with phenol-chloroform or Gentra Puregene Blood Kit (QIAGEN, Germany) was used both for genotyping and sequencing. The GWS in Studies 3 and 4 were performed with two slightly different techniques.

In Study 3, the genotyping was based on the Weber set 9A microsatellite marker set (Research Genetics, Inc., Hunstville, AL, USA) of 366 markers, with an average of 10 cM marker distance and performed using the gel electrophoresis on LI-COR model 4200 dual laser automated fluorescent DNA sequencer followed by fragment analysis using Gene ImagIR 4.03 (LI-COR, Inc. Lincoln, NE, USA). DNA samples of all family members were used in the GWS.

In Study 4, the genotyping was based on the LMS-MD10 microsatellite marker set (Applied Biosystems, Foster City, CA, USA) of 382 markers and performed using the ABI 3730xl DNA Analyzer, at the Finnish Genome Center / FIMM Technology Center, University of Helsinki. In the GWS and fine-mapping samples of the core family were used.

After the initial GWS each region with a multipoint parametric location score >1.5 was fine mapped using markers from www.ncbi.nlm.nih.gov/projects/genome/guide/human with AmpliTaq Gold (Perkin Elmer Inc., Waltham, MA, USA) reagents and an automated DNA sequencer (ABI 3730xl DNA Analyzer) and genotyping software (Genemapper 4.0, Applied Biosystems, Foster City, CA, USA). The order of the markers and the intermarker distances were deduced from the NCBI Map Viewer (www.ncbi.nlm.nih.gov/projects/mapview/). The haplotypes in the fine-mapped loci on chromosomes 17q12-q24 and 18p11-q11 in Study 3 and on chromosomes 16p12.3-p13.3 and 22q13.1-q13.3 in Study 4 were constructed by haplotyping option in SimWalk2 v.2.91 (Sobel and Lange 1996). The haplotypes on chromosome 5p13.1-q13 in study 3 were constructed manually.

7.6.4 Sequencing and mutation analysis (Studies 3 and 4)

The sequencing was performed with standard methods presented in detail in Studies 3 and 4. The sequenced genes are listed in Table 6.

In Study 3, for all sequenced genes samples of at least two patients of family 98 and one unrelated Finnish control were used. Nine positional and functional brain-
expressed ion channel genes as primary candidate genes from the chromosome 17q12-q24 locus and myo-inositol monophosphatase-2 (IMPA2), a previously suggested candidate gene for FS locating on the chromosome 18p11-q11 locus were sequenced (Nakayama et al. 2004). In addition, rap2-interacting protein 8 (RPIP8) and solute carrier family 25, member 39 (SLC25A39), identified as candidate genes from the microarray-based expression analysis (see 7.6.5) and residing on chromosome 17q12-q24 were sequenced (Janoueix-Lerosey et al. 1998, Haitina et al. 2006). In Study 3, all family members were also screened for the triplet repeat variation near the transcription start point of the carbonic anhydrase X (CA10) gene, expansion of which has previously been implicated as a candidate for neurological disorders (Kleiderlein et al. 1998).

In Study 4, two ion channel genes CACNA1H on the chromosome 16p12.3-p13.3 locus and voltage-dependent T-type calcium channel, α 1I subunit gene (CACNA1I) on the chromosome 22q13.1-q13.3 locus were sequenced as positional and functional candidate genes (Monteil et al. 2000, Chen et al. 2003, Heron et al. 2007). CACNA1H was sequenced using DNA samples of four family members (5-21, 5-8 and 5-30, 5-17, Figure 5). The variant c.2057C>T in CACNA1H was sequenced in all family members in family 5 and in 95 CEPH and 259 Finnish controls. CACNA1I was sequenced using samples of two family members (5-23, 5-30, Figure 5). TBC domain family, member 24 (TBC1D24), a novel candidate gene for epilepsy on chromosome 16p13.3 locus and sodium channel α 1 subunit (SCN1A) as a candidate gene for PS and GEFS+ on chromosome 2q24.3 were sequenced using DNA samples of two trios (5-23, 5-8, 5-9 and 5-30, 5-16, 5-17, Figure 5) and in samples of three patients (5-21, 5-25 and 5-30, Figure 5), respectively (Zara et al. 2000, Mantegazza et al. 2005, Grosso et al. 2007, Livingston et al. 2009, Falace et al. 2010, Corbett et al. 2010).

In Study 3 the effect of coding SNPs was predicted by SNPs3D software (www.snps3d.org) and in Study 4 by PolyPhen program (http://genetics.bwh.harvard.edu/pph). The effect of intronic SNPs was predicted with ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi). Alterations with a probably damaging effect identified only in patient samples were analysed in all family members.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Chromosome</th>
<th>Protein name</th>
<th>Expression and function</th>
<th>OMIM</th>
<th>Accession</th>
<th>Sequenced in family</th>
</tr>
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<tbody>
<tr>
<td>SCN1A</td>
<td>2q24</td>
<td>Sodium channel alpha 1 subunit</td>
<td>Expressed in brain. Essential for the generation and propagation of action potentials.</td>
<td>182389</td>
<td>NM_001165963.1</td>
<td>5</td>
</tr>
<tr>
<td>CACNA1H</td>
<td>16p13.3</td>
<td>Voltage-dependent T-type calcium channel alpha 1H subunit</td>
<td>In brain, expression most abundant in amygdala, caudate nucleus, and putamen. Currents generate short burst firing.</td>
<td>607904</td>
<td>NM_001005407</td>
<td>5</td>
</tr>
<tr>
<td>TBC1D24</td>
<td>16p13.3</td>
<td>TBC domain family, member 24</td>
<td>Brain expressed. Presumed to have a role in regulating cell growth and differentiation.</td>
<td>613577</td>
<td>NM_020705.1</td>
<td>5</td>
</tr>
<tr>
<td>SLC25A39</td>
<td>17q12</td>
<td>Solute carrier family 25, member 39</td>
<td>Mitochondrial carrier protein. Brain expressed SLC25 members are important for neurons in energy production and neuronal signaling. No studies of tissue expression on member 39.</td>
<td>610820</td>
<td>NM_016016</td>
<td>98</td>
</tr>
<tr>
<td>CACNB1</td>
<td>17q21-q22</td>
<td>Voltage-dependent calcium channel, beta 1 subunit</td>
<td>Brain and skeletal muscle isoforms. Able to regulate CACNA1A function.</td>
<td>114207</td>
<td>NM_000723.3, NM_199247.1</td>
<td>98</td>
</tr>
<tr>
<td>KCNH4</td>
<td>17q21.2</td>
<td>Voltage-gated potassium channel, subfamily H, member 4</td>
<td>Expressed only in brain. May be involved in cellular excitability of neurons in the human central nervous system.</td>
<td>604528</td>
<td>NM_012285.1</td>
<td>98</td>
</tr>
<tr>
<td>RPIP8</td>
<td>17q21.3</td>
<td>Rap2-interacting protein</td>
<td>Expressed in brain. Interacting with RAP2A, which is expressed in excitatory synapses.</td>
<td>605448</td>
<td>NM_006695.3</td>
<td>98</td>
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<tr>
<td>CACNA1G</td>
<td>17q22</td>
<td>Voltage-dependent calcium channel, alpha 1G subunit</td>
<td>Expressed dominantly in brain. Modulates generation of GABA&lt;sub&gt;e&lt;/sub&gt; receptor mediated spike and wave discharges in the thalamocortical pathway.</td>
<td>604065</td>
<td>NM_018896.3, NM_198376.1, NM_938406.1</td>
<td>98</td>
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<tr>
<td>KCNH6</td>
<td>17q23.3</td>
<td>Voltage-gated potassium channel, subfamily H,member 6</td>
<td>Expressed in the brain. May differentially control the firing of neurons engaged in several networks.</td>
<td>608168</td>
<td>NM_030779.2, NM_173092.1</td>
<td>98</td>
</tr>
<tr>
<td>Symbol</td>
<td>Chromosome</td>
<td>Protein name</td>
<td>Expression and function</td>
<td>OMIM</td>
<td>Accession</td>
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</tr>
<tr>
<td>CACNG5</td>
<td>17q24</td>
<td>Voltage-dependent calcium channel, gamma 5 subunit</td>
<td>Expression in adult and fetal brain. May regulate function of other voltage dependent calcium channels.</td>
<td>606405</td>
<td>NM_014404.1 NM_145811.1</td>
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<tr>
<td>CACNG4</td>
<td>17q24</td>
<td>Voltage-dependent calcium channel, gamma 4 subunit</td>
<td>Expressed in brain. Mice with mutations in Cacng4 have increased seizure activity.</td>
<td>606404</td>
<td>NM_014405.2</td>
<td></td>
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<tr>
<td>CACNG1</td>
<td>17q24</td>
<td>Voltage-dependent calcium channel, gamma 1 subunit</td>
<td>Expressed in fetal and adult brain. Integral membrane protein.</td>
<td>114209</td>
<td>NM_000727.2</td>
<td></td>
</tr>
<tr>
<td>KCNJ2</td>
<td>17q24.3</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 2</td>
<td>Expressed in brain. Forms a functional brain potassium channel.</td>
<td>600681</td>
<td>NM_000891.2</td>
<td></td>
</tr>
<tr>
<td>KCNJ16</td>
<td>17q24.3</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 16</td>
<td>Expressed in brain. Forms a functional brain potassium channel by interacting with PSD95. Controls negatively KCNJ2 channel activity.</td>
<td>605722</td>
<td>NM_018658.1</td>
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</tr>
<tr>
<td>IMPA2</td>
<td>18p11.2</td>
<td>Myo-inositol monophosphatase-2</td>
<td>Expressed in brain. Possible SNP association with febrile seizures.</td>
<td>605922</td>
<td>NM_014214.1</td>
<td></td>
</tr>
<tr>
<td>CACNA1I</td>
<td>22q13.1</td>
<td>Voltage-dependent T-type calcium channel, alpha 1I subunit</td>
<td>Almost exclusively expressed in brain and activated by small membrane depolarizations, can generate burst firing and pacemaker activity.</td>
<td>608230</td>
<td>ENST00000400164 ENST00000336649 ENST00000407673 ENST00000401624 ENST00000404898</td>
<td></td>
</tr>
</tbody>
</table>
7.6.5 mRNA expression profiling (Study 3)

Microarray-based mRNA expression profiling was performed in three affected (Figure 4: 98-10, 98-12, 98-14) and three unaffected (Figure 4: 98-23, 98-24, 98-25) age- and gender-matched family members in order to identify differently expressed genes on the chromosome 17q12-q24 locus. The microarray experiments were performed as described earlier (Laaksonen et al. 2006) using Sentrix® Human-6 Expression BeadChips (Illumina, San Diego, CA, USA). The differentially expressed genes in each comparison were selected requiring $|\log_2 \text{fold-change}| > 0.5$ (fold-change 1.41) and p-value <0.05.

7.6.6 Array-based comparative genomic hybridization (Study 3)

Array-based comparative genomic hybridization a diagnostic test by GeneDx Inc. (Gaithersburg, USA) was performed on one patient (98-8) using GenomeDx microarray v3.0 containing ~105 000 oligonucleotide probes.

7.7 Statistical analysis

7.7.1 Analysis of the neuropsychological assessments (Study 1)

In Study 1, non-parametric tests were used because the groups were small and data were not normally distributed. Matching of the study and control groups was tested using the Mann–Whitney test (U). The effect of AEDs, cessation of absence seizures, and epileptiform discharges on cognitive functioning within the study group and the effect of the assessment delay within the control group were tested with the Wilcoxon rank test (Z). To investigate the relationships between variables, Spearman’s rho (rs) was used. All calculations were performed with SPSS/PC+ Version 13.0. A p value <0.05 was considered to indicate statistical significance.
7.7.2 Linkage analysis (Studies 3 and 4)

In Study 3, first the parametric multipoint linkage analyses were performed. For the analyses an autosomal dominant model of inheritance with 70% penetrance and a phenocopy rate of 3% were assumed. The disease gene frequency was set at 0.001. The recombination fraction was considered to be equal in males and females. Allele frequencies were extracted from the family data. The parametric multipoint location scores were calculated with SimWalk2 v.2.91. These location scores are directly comparable to multipoint LOD scores (Sobel and Lange 1996). Parametric two-point analyses were performed on the fine-mapped loci using the MLINK program (Lathrop et al. 1984) utilizing the ANALYZE program package (Hiekkalinna et al. 2005). The AUTOGSCAN v1.0.3 software tool was used to automate the genome wide linkage analyses (Hiekkalinna et al. 2005).

In Study 4, in two-point parametric analyses with dominant and recessive models of inheritance, a disease allele frequency of 0.001, a phenocopy rate of 3% and penetrance rates of 70% and 100%, respectively were used. Allele frequencies were calculated from the genotypes of all individuals. Parametric two-point analyses were performed as described in Study 3. Then multipoint NPL analyses on autosomal chromosomes were calculated with SimWalk2, v.2.91 (Sobel and Lange 1996). The X chromosome was analysed with Merlin (Abecasis et al. 2002), since Simwalk2 does not support this analysing option.

To evaluate the expected maximum LOD score and the power of the available pedigree material to achieve a significant LOD score under Mendelian inheritance models simulation was performed with SLINK (Ott 1989). In Study 3, simulation with the model parameters described above reached maximum LOD scores of 3.0 and 3.2, with schemes 1 and 2 respectively. In Study 4, a recessive and a dominant disease model with the parameters mentioned above were used. Simulation with the recessive disease model produced a maximum LOD score of 0.02 (θ=0.0) and with the dominant disease model a maximum LOD score of 3.32 (θ=0.0). The schemes were calculated with 1000 replicates.
8. Results

This study was based on the data of 52 CAE patients of whom 48 were diagnosed at the Department of Pediatric Neurology, Tampere University Hospital between 1974 and 2002 excluding the years 1995 and 1996. Four additional CAE patients belonged to family 5 and were diagnosed at the Department of Pediatrics in Southern Ostrobothnia Central Hospital, Seinäjoki, Finland (5-23), the Division of Child Neurology, Jorvi Unit, in Helsinki University Central Hospital, Finland (5-30, 5-32) and the Department of Pediatrics in Vaasa Central Hospital, Finland (5-33).

The referral district of Tampere University Hospital includes the city of Tampere and the surrounding municipalities. All children with newly diagnosed epilepsy in this area are referred to the hospital’s Department of Pediatric Neurology. Therefore all diagnosed children with CAE from the region were included. The total regional population increased from 370,000 to 470,000 between 1975 and 2000. About 75,000 – 80,000 children (<15y) were living in the study area (www.vaestorekisterikeskus.fi). Every year 0 – 5 new patients with CAE were diagnosed. The incidence of CAE was 2.3/100,000/year.

8.1 Short-term outcome – video-EEG recordings and neurocognitive assessments

Twelve newly diagnosed consecutive patients with typical absence seizures underwent video-EEG recordings and neurocognitive assessments at the time of the diagnosis and after an average follow-up of ten months to monitor short-term outcome in these patients. Ten of the patients had CAE. One of the patients had onset of absences at the age of 14 years and was diagnosed with JAE. The data of one patient was lost due to data collection error. Seven patients had a normal 24-hour video-EEG recording at follow-up. Three patients had mild abnormalities (single spike-wave, slow wave during hyperventilation or short 3 Hz spike-wave
discharge without clinical events) outside the neurocognitive assessments. One patient with infrequent short 3 Hz spike-wave discharges with clinical absences was excluded from the analysis.

The IQ was within normal limits in the study and control group. The verbal IQ improved significantly only in the control group during the nine-month follow-up. Both groups improved significantly in dominant hand fine-motor fluency and in the attention task (Table 2 in Study 1). The visual memory task performance correlated significantly with duration of generalised 3 Hz spike–wave discharges and clinical absence seizures during the first assessment and a significant improved in this task was observed in the study group (Figure 1 in Study 1).

8.2 Long-term outcome of patients with CAE

The clinical characteristics of 52 patients with CAE are given in Table 7. The outcome measured by clinical remission lasting at least two years was 94% during the AED treatment and 87% of patients stayed seizure free after withdrawal of AED. Seventy-three per cent of the patients were female. A family history of epilepsy was recorded in 27% and family history of FS in 17% of the patients. Eight of the patients continuing AED treatment had either subtle absence seizures detected only in video-EEG (two patients), or developed GTCS (five patients) or had only recently reached two years of seizure freedom with AED (one patient). None of the patients had GTCS during the active stage of absences. Patients with GTCS often had a family history of FS or epilepsy. Seven patients with a mean follow-up of 7 years (6 – 15 years) had a history of an onset of absence seizures before four years of age. Five of them had an excellent outcome with remission without AED and two patients remained on AEDs and were seizure free.

Information of the first AED and the AED with which the remission was achieved is presented in Table 8. Sixty-three per cent of the patients achieved remission with the first AED. ETM was most commonly used as the first drug of choice. Monotherapy was effective in 83%. Fifty-two per cent of the patients achieved remission with ETM and 31% of them achieved remission with VPA.
Reported side-effects during the AED treatment were generally mild and mostly disappeared during the initial phase of the treatment (Table 9). Only a few patients had to change the AED due to the side-effects.

Table 7. Clinical characteristics of CAE patients

<p>| | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>52</td>
</tr>
<tr>
<td>Females (n/%)</td>
<td>38/73</td>
</tr>
<tr>
<td>Family history of FS(^a) (n / %)</td>
<td>14/27</td>
</tr>
<tr>
<td>History of FS (n%)</td>
<td>5/10</td>
</tr>
<tr>
<td>Family history of FS(^a) (n / %)</td>
<td>9/17</td>
</tr>
<tr>
<td>Duration of clinical follow-up, mean (range, yr)</td>
<td>10.4 (2-22)</td>
</tr>
<tr>
<td>Age at the onset of AS(^b), mean (range, yr)</td>
<td>5.8 (2 - 8.5)</td>
</tr>
<tr>
<td>No of patients with age at onset between 2 - 4 years (^c) (n / %)</td>
<td>7/13</td>
</tr>
<tr>
<td>Age at diagnosis (range, y)</td>
<td>6.6 (2-11.0)</td>
</tr>
<tr>
<td>No of patients with GTCS (n / %)</td>
<td>8/15</td>
</tr>
<tr>
<td>Age at onset of GTCS, mean (range, yr)</td>
<td>10.5 (6.5-18)</td>
</tr>
<tr>
<td>Family history of FS or epilepsy in patients with GTCS (n/%)</td>
<td>6/75</td>
</tr>
<tr>
<td>Duration of treatment mean (yr, range)</td>
<td>4.2 (1.6-8.8)</td>
</tr>
<tr>
<td>Patients in remission(^d) (n / %)</td>
<td>49/94</td>
</tr>
<tr>
<td>Patients with onset of AS between 2-4 years of age in remission (n/%)</td>
<td>6/86</td>
</tr>
<tr>
<td>Patients in remission(^d) without AED(^e) (n/%)</td>
<td>42/87</td>
</tr>
<tr>
<td>Difficult to treat (n / %)</td>
<td>3/6</td>
</tr>
</tbody>
</table>

\(^a\) 2 pairs of siblings, 1 child and aunt, 2nd cousins in Family 5 counted only once
\(^b\) data not available in 2 patients
\(^c\) data not available in 1 patient
\(^d\) in remission >2 years
\(^e\) data not available in 3 patients

Abbreviations: n = number, FS = febrile seizure, AS = absence seizure, AED = antiepileptic drug, GTCS = generalised tonic-clonic seizure.
Table 8. The first AED and the AED or combination with which remission (n=49) was reached.

<table>
<thead>
<tr>
<th>First AED</th>
<th>No</th>
<th>Clinical remission reached with</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPA</td>
<td>12</td>
<td>VPA</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA+CLB</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA+ETM</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETM</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA+CBZ</td>
<td>1</td>
</tr>
<tr>
<td>ETM</td>
<td>39</td>
<td>ETM</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA+CLB</td>
<td>2</td>
</tr>
</tbody>
</table>

AED = antiepileptic drug, VPA = valproate, CLB = clobazam, ETM = ethosuximide, CBZ = carbamazepine

Table 9. Side-effects of AED reported in 20 patients.

<table>
<thead>
<tr>
<th>AED</th>
<th>ESM</th>
<th>VPA</th>
<th>CBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic reaction</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressivity</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth retardation</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporary increase of amylase</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AED = antiepileptic drug, VPA = valproate, ESM = ethosuximide, CBZ = carbamazepine
8.3 Heredity of CAE

The pedigrees of 19 families with at least one additional individual with FS or epilepsy are shown in Figures 4, 5 and 6. Based on the pedigree structure a familial IGE diagnosis can be proposed in five families where at least two members had IGE (families 4, 6, 7, 9, 13). FS and CAE were diagnosed in six families (1, 2, 3, 97, 98 and 99). In seven families at least one additional individual had an unclassified epilepsy (families 8, 10, 11, 12, 14, 15 and 16). The families 2, 97, 98 and 99 were contacted and the family members were interviewed during field trips. An additional family (family 5) with several affected individuals was identified at the Department Pediatrics in Southern Ostrobothnia Central Hospital, Seinäjoki, Finland.

Of families 98 and 5 detailed seizure phenotypes are presented in Studies 3 and 4 (Table 2 in Study 3, Table 1 in Study 4). In family 98 a Mendelian dominant inheritance pattern with reduced penetrance was assumed. In this family only the proband had CAE. Five other patients had FS and two patients had benign temporal lobe epilepsy. In family 5 no definitive inheritance pattern was observed. In family 5 three patients had CAE, one patient had CAE and FS, four patients had FS only, one patient had PS and one patient FS and probable PS. Only one patient had intractable epilepsy with a history of FS followed by secondarily generalised frontal lobe seizures since adolescence. GWS was performed in these two multiplex families in order to assign genetic loci for the epilepsy phenotypes found in the families.
8.3.1 Genealogy in family F5

In family 5, by searching the church registers it was found that the several parents of generation I (Figure 5) originated from Southern Ostrobothnia. Most of the ancestors of the core family originated from the villages of Kortesjärvi and Lappajärvi. A link was established between the family members 5-1 and 5-2 four generations earlier at around 1790 in Kortesjärvi. The ancestors of married-in
spouses 5-3 and 5-52 originated from the Karelia region and Central Finland. The ancestors of the extended family originated from the southern part of Ostrobothnia, the locality of Teuva. This line of ancestors was searched until the 1850’s and no link to the core family was established. In Figure 7 the birthplaces of the ancestors originating in Southern Ostrobothnia are marked.

Figure 7. Birthplaces (1-3) of ancestors originating from Southern Ostrobothnia. Common ancestors were found to originate at Kortesjärvi (Birthplace 1). The ancestors of individual 5-1 and the ancestors of the mother of individuals 5-2, 5-4 and 5-51 were born in Birthplace 1. Birthplace 2 indicates the origin of the ancestors of the father of the individuals 5-2, 5-4 and 5-51. Birthplace 3 indicates the origin of the ancestors of the extended family.
8.3.2 Identified genetic loci

In family 98, after the GWS parametric multipoint linkage analyses were carried out in each autosome using Simwalk2 v.2.91. The multipoint location score value (corresponding to the multipoint LOD score value) of >1.5 as a limit was used to identify candidate loci for further fine-mapping. Four loci on chromosomes 5q11.2, 17q12-q24, 18p11-q11 and 18q21 were fine-mapped with 12, 16, 10 and 4 additional microsatellite markers. Suggestive linkage evidence was obtained on chromosome 17q12-q24 with the highest two-point LOD scores of 2.6 and 2.8 at marker *D17S1606* (at recombination fraction, theta 0.0) with calculation schemes 1 and 2 respectively. The parametric multipoint linkage analysis with Simwalk2 produced location score values of 2.5 and 2.7 (using schemes 1 and 2 respectively) between seizures (epilepsy and FS) and 17 markers covering *D17S1814* to *D17S1786* (Figure 1 in Study 3). The segregating haplotypes on chromosome 17q12-q24 are presented in study 3 (Figure 1 in Study 3). The two-point LOD scores on chromosome 5q11.2 were 2.0 and 2.1 at markers *D5S628* and *D5S2076* (theta 0.0) with calculation schemes 1 and 2 respectively. On chromosome 18p11-q11 the highest two-point LOD scores calculated with schemes 1 and 2 were 1.3 and 1.5 respectively at *D18S1153* (theta 0.0). The fourth locus on chromosome 18q21 was excluded by fine-mapping with partly negative multipoint location scores.

In family 5, after GWS in the two-point parametric linkage analysis using the dominant model none of the markers reached the level of suggestive linkage (data not shown). Genome wide multipoint NPL analysis indicated location scores >1.5 (p-value ≤ 0.032) between the disease phenotype (epilepsy and FS) and markers *D16S423* and *D22S274* (p-values of 0.017 and 0.028, respectively).

Six microsatellite markers were used for fine-mapping the chromosome 16p12.3-p13.3 locus. Multipoint NPL analysis showed suggestive evidence for linkage with a p-value of 0.0014 between the disease phenotype and marker *D16S3072*. Seven microsatellite markers were used for fine-mapping the chromosome 22q13.1-q13.31 locus. Multipoint NPL analysis showed the highest p-value of 0.037 between the disease and the marker *D22S274*, remaining, however, below the level for a suggestive linkage. No evidence for linkage to any of the X chromosomal markers was identified (data not shown).
Haplotype analysis in family 98 showed that all affected individuals carried the disease-associated haplotypes on all three loci showing positive linkage evidence. Four of the nine unaffected individuals had only one or two of the segregating haplotypes. Two obligate carriers (98-1, 98-2) who were labelled unknown for the linkage analysis did carry all three segregating disease-associated haplotypes (Figure 2 in Study 3).

Haplotype analysis in family 5 showed two different haplotypes segregating on the chromosome 16p12.3-p13.3 locus (Figure 1 in Study 4). The haplotype on the chromosome 22q13.1-q13.31 locus co-segregates in six of the eight affected individuals in the core family. The segregating haplotypes of the loci on 16p12.3-p13.3 and 22q13.1-q13.31 are presented in Figure 5.

8.3.3 Candidate gene sequencing

Altogether eleven ion channel genes as positional and functional candidate genes were sequenced. Two genes, *SLC25A39* and *RPIP8*, were identified by mRNA expression profiling, and two genes, *IMPA2* and *TBC1D24*, were positional, functional candidate genes. Additionally, *SCN1A* was sequenced as a candidate gene for PS and the GEFS+-like phenotype. One hundred and six polymorphisms were identified. Of these polymorphisms 80 matched sequences in the reference SNP database. Fifty one polymorphisms located in the coding regions. Of these variants 23 were silent and did not cause amino acid change (synonymous change) 13 variants changed amino acid (missense SNP). Additionally, two variants located in the 5’UTR (untranslated region) and 13 in the 3’UTR regions. Fifty-four variants located in the intronic regions of the sequenced genes. One variant located downstream of the first exon.

None of the variants were confirmed to be disease associated (data not shown). In *CACNA1H* gene the polymorphism in exon 9, (c.2057C>T, p.Pro686Leu) segregated with one of the haplotypes in the core family (Figure 5). Pro686 is located in the segment between domains 1 and 2 of *CACNA1H* encoded Cav3.2 channel (Figure 8) and is an evolutionally conserved amino acid (Figure 2 in study 4). PolyPhen program estimates this amino acid to be probably damaging. The c.2057C>T alteration was found in none of the genotyped 92 general Finnish
controls and in one of the 167 controls originating from the Ostrobothnia region. One of the 95 controls of the European Centre d'Etude du Polymorphisme Humain (CEPH) had this alteration.

8.3.4 mRNA expression profiling and array-based comparative genomic hybridization

On the mRNA expression profiling, five genes RPIP8, SLC25A39, Meckel syndrome type 1 (MKS1), phosphatase orphan 1 (PHOSPHO1) and monocyte to macrophage differentiation associated protein (MMD) on the chromosome 17q12-q24 locus were significantly down-regulated and one gene, homeobox-B2 (HOXB2) was significantly up-regulated (Table 4 in Study 3). RPIP8, SLC25A39, MKS1 and
HOXB2 are expressed in the brain. *RPIP8* and *SLC25A39* were further studied by sequencing (see 8.3.3), but no disease associated variant was identified. For the *MKS1* three family members (98-10, 98-12, 98-14) were tested and found to be negative for the Finnish *MKS1* founder mutation (data not shown).

In array-based comparative genomic hybridization no clinically relevant CNV was identified in the genome and no abnormalities in the fine-mapped regions of interest (average probe resolution in the analysis 37 kb).
9. Discussion

CAE is an epilepsy syndrome with an etiology which has so far not been fully revealed. In this study Finnish CAE patients were studied to better understand the clinical features, short- and long-term outcome, neuropsychological aspects as well as the molecular mechanisms of this complex disease. The annual incidence (2.3/100 000) of CAE in the study population is in line with previous epidemiological studies with reported annual incidences of 1.2-7.1 /100 000 in different European populations (Granieri et al. 1983, Olsson 1988, Loiseau et al. 1990, Sidenvall et al. 1993). The major clinical observation in this study is the good long-term outcome achieved in the majority of patients with monotherapy or with a combination drug treatment. Furthermore, the neurocognitive functioning of CAE patients seems to be comparable with that of the age-matched healthy children and AED treatment does not adversely affect the neurocognitive functioning of these patients during the short-term follow-up. The molecular genetic novelty of this study was the identification of suggestive linkages on chromosome 17q12-q24 in family 98 with CAE, FS and benign TLE and on chromosome 16p12.3-p13.3 in family 5 with CAE, FS and PS. A novel partially co-segregating SNP was identified in CACNA1H gene, which is a previously known susceptibility gene for CAE and other epilepsies.

9.1 Short-term outcome – video-EEG recordings and neurocognitive assessments

The advantage of the present study over earlier studies is the careful control of seizure freedom during follow-up testing. There are two other follow-up studies including patients with absence seizures among other seizure types but without information on how seizure freedom was controlled (Mandelbaum and Burack 1997, Mandelbaum et al. 2009). One of the major limitation in the present study and
indeed of many others on neurocognitive functioning is the small sample size. A comforting observation in this study was that the newly diagnosed CAE patients did not differ from the control group in the general IQ test at the time of diagnosis. However, this result could be different with a larger study group. Others have evaluated similar size or larger study groups with CAE patients and found mean IQ to be lower in CAE patients than in healthy controls (Pavone et al. 2001, Caplan et al. 2005, 2008). The patients in these studies were evaluated only when receiving AED treatment. However, in these three studies not all patients were seizure free as were the patients at follow-up in Study 1 (Pavone et al. 2001, Caplan et al. 2005, 2008). During the ten-month follow-up the IQ did not change in the study group. In the control group a significantly improved verbal IQ was recorded. It is possible that this improvement was due to a practice effect and not to an actual maturation/development of skills. In our longer follow-up study on this topic focusing on 15 CAE patients with a mean 5.1 years of seizure freedom normal but significantly lower general IQ was measured in the patient group than in healthy controls indicating for the need to follow-up these children during the school years (Poutanen 2009).

In the computerized test measuring attention both groups improved similarly during follow-up. A wider range of performance in the study group than in the control group was found at baseline and at follow-up, suggesting that some of the children with typical absence seizures might have problems sustaining attention. A positive improvement in sustained attention has also been reported in an uncontrolled follow-up study with an equal sized study group (10 patients with absence seizures) (Mandelbaum et al. 2009). Attention problems evaluated by questionnaires such as the Child Behaviour Check List by Achenbach completed by the parents (Achenbach 1991) or measured by specific tests focusing on attention have been reported in other studies (Fedio and Mirsky 1969, Henkin et al. 2005, Caplan et al. 2008, Bhise et al. 2009, Poutanen 2009).

In earlier studies visual memory has been reported to be weak in patients with absence seizures when compared with other epilepsy patients or healthy controls (Fedio and Mirsky 1969, Jambaque et al. 1993, Pavone et al. 2001, Nolan et al. 2004). In this study visual memory improved significantly in patients after they had reached remission, which supports importance of the active use of AEDs to control seizures. In the longer follow-up study with a similar test for visual memory
the group with absence seizures did not differ from the controls, but had a wide variation in performance (Poutanen 2009). Although the performance in this short-term outcome study was good physicians should be attentive not only to the child’s seizure outcome but also to the school performance and behavioural problems and parents’ upset as a result of the epilepsy, as show by others (Oostrom et al. 2003).

9.2 Long-term outcome measured by remission rates

In the present study most of the CAE patients were identified retrospectively, thereby preventing a detailed analysis of the EEGs and associated clinical features. Therefore a comparison of the ILAE 1989 diagnostic criteria used in this study with the new proposal was not possible (ILAE 1989, http://www.ilae-epilepsy.org/Visitors/Centre/ctf/childhood_absence.cfm). However, it is likely that the population studied presents a narrow definition similar to the new criteria of CAE as the remission rate in this study was comparable to that of a study following the recently published diagnostic criteria for CAE (Grosso et al. 2005).

Most of the patients achieved remission easily. The first AED was effective in 63% and 83% of patients achieved remission with monotherapy. These results are similar to those reported in other studies (Covanis et al. 1992, Wirrell et al. 2001, Callenbach et al. 2009). Furthermore, the prognosis in patients in whom the initial treatment failed was also good, because nearly 90% of them achieved remission later on. Interestingly, there seems to be a group of patients who do not respond to ETM, but do have a good response to VPA monotherapy. Many of these patients also had a family history of FS or epilepsy. This kind of endophenotype of AED specific responsiveness could result from distinct genetic influences (Winawer 2006).

9.3 Genetics of CAE

Twenty-nine of the patients with CAE in this study were the only individuals with epilepsy in their families. They were sporadic patients and their epilepsy was believed to be due to complex inheritance. Finding definitive answers for the
molecular genetic background of CAE was challenging due to the genetic heterogeneity and the contributing environmental factors, yet fully unknown (Berkovic et al. 2006). Large-scale international collaboration is needed to collect large enough sample size for genome wide association study (GWAS) to allow identification of common variants underlying seizure susceptibility in CAE. This method has been successfully applied in a number of complex human phenotypes, as exemplified in the recent report on several novel loci associated with plasma lipid levels that were also associated as risk factors for coronary artery disease (Teslovich et al. 2010).

In nineteen families there were at least two family members with epilepsy or FS and six of them had a first-degree relative with epilepsy, which concours with previous results (Janz et al. 1992, Italian League Against Epilepsy Genetic Collaborative Group 1993, Marini et al. 2004). Eight of the patients with CAE had at least one family member with FS. It is possible that the CAE and FS patients do not have a common genetic background, because FS is the most common seizure type in childhood, affecting 2-4% of children of Caucasian origin (Shinnar and Glauser 2002). On the other hand, FS has been reported to be associated with CAE in multiplex families, which implicates a shared genetic susceptibility (Italian League Against Epilepsy Genetic Collaborative Group 1993, Marini et al. 2004). The heterogeneity of the seizure phenotypes in families 5 and 98 resemble large GEFS+ families in which a single mutation contributes to the disease outcome (Wallace et al. 1998, Baulac e.t al 1999). Thus, these kind of families highlight the complexity of genotype-phenotype correlation in epilepsies and demonstrate that factors other than “epilepsy genes”- be they acquired factors or modifier genes - in part determine the phenotype (Scheffer and Berkovic 1997).

In five of the identified families (families 4, 6, 7, 9 and 13) at least one other family member was diagnosed with CAE or IGE. In these families a specific genetic mechanism was assumed to produce similar phenotypes, a hypothesis called distinct genetic influence according to Ottman (2005). These five families are individually too small to be studied genetically, but they are good candidates for international collaborative studies aiming at dissecting the complex genetic background of IGE. On the other hand, in the rest of the families (13) a shared genetic influence, the same genes influencing risk for different epilepsy syndromes was assumed (Ottman et al. 1989, 1998).
Additionally, in two multiplex families (5 and 98) a shared genetic influence e.g. the same genes influencing on the risk for different epilepsy syndromes and FS was assumed and molecular genetic studies were performed (Ottman 2005, Winawer 2006).

### 9.3.1 Genetic loci identified

We performed GWS in two families with diverse phenotypes aiming to identify shared genetic influence within both families. Large families suitable for GWS with CAE as the only phenotype are rare. An Indian family with CAE inherited as a dominant Mendelian disease has been described and linked to chromosome 8q24 (ECA1 locus, epilepsy childhood absence) using microsatellite markers, but no disease causing gene has yet been identified (Fong et al. 1998, Sugimoto et al. 2000). This locus was later excluded by linkage analysis using families with at least two affected individuals with CAE affirming a genetic heterogeneity of CAE (Robinson et al. 2002). The second ECA locus on 5q31.1-q33.1 was mapped using a multiplex family with individuals with FS, FS+ and CAE (Wallace et al. 2001).

Of the five loci showing positive linkage evidence in this study two have previously been linked to CAE, while three are novel. The locus on chromosome 18p11-q11 identified in Study 3 is a recognised locus for familial febrile seizure (FEB6) and has been suggested to contain a modifier gene in a family with CAE and FS (Nakayama et al. 2004, Nabbout et al. 2007). The chromosome 16p12.3-p13.3 locus has earlier been linked to CAE by demonstrating susceptibility variants in a candidate gene, CACNA1H (Chen et al. 2003, Heron et al. 2004, Heron et al. 2007, Liang et al. 2007). Statistically the evidence for the locus on chromosome 17q12-q24 was strong with the highest LOD score of 2.8. It is therefore most likely a novel locus for epilepsy. On the other two loci on chromosomes 5q11.2 and 22q13.1-q13.31 the linkage evidence was weaker, and therefore these loci could be modifier loci containing modifying genes The CACNA1I gene on chromosome 22q13.1-q13.31 was sequenced as a functional candidate gene, but no disease associated variants were identified.

The segregating, disease-associated haplotypes (Figure 5 and Figure 2 in Study 3) are a way to present the complex inheritance in these families. In family 98 all
affected individuals did carry all three disease-associated haplotypes inherited in a Mendelian manner. It is therefore possible that a gene of each segregating locus participates in determining the general seizure susceptibility in this family. However, other genes or environmental factors are needed to explain the different seizure phenotypes as suggested in a family with FS, CAE and TLE by Nabbout et al. (2007). In family 5, the haplotype on the chromosome 22q13.1-q13.31 locus is inherited in a dominant Mendelian manner and may contain an epilepsy-associated gene with a low penetrance. The chromosome16p12.3-p13.3 locus may act as a modifier in the seizure phenotype. In family 5, other loci/genes and possibly environmental factors are also to be identified before the seizure phenotypes can be fully understood. Both families exemplify the complex genetic basis of idiopathic epilepsies with an unknown number of susceptibility genes and intrafamilial heterogeneity (Dibbens et al. 2007).

9.3.2 Candidate gene sequencing; focus on CACNA1H

Microsatellite markers with an average distance of 10 cM were used in the GWSs in families 98 and 5 to identify candidate loci for fine-mapping. Even after fine-mapping the identified loci were still large, containing several hundred positional candidate genes. Therefore sequencing them all was an unfeasible task. Thus the candidate gene hunt was performed by targeted sequencing of genes where there was existing relevant knowledge of their function and association with epilepsy. Primary selection criteria were the existing expression data in the brain shown for all sequenced ion channel genes, and for RPIP8 and IMPA2 (Goldin et al. 1986, Pragnell et al. 1991, Yoshikawa et al. 1997, Janoueix-Lerosey et al. 1998, Miyake et al. 1999, Williams et al. 1999, Montiel et al. 2000a, 2000b, Burgess et al. 2001, Derst et al. 2001, Papa et al. 2003, Letts et al. 2005, Singh et al. 2007). For SLC25A39 gene no expression data were available, however brain-expressed members of the solute carrier family 25 (SLC25) are known to be important for neuronal energy production and signalling (Haitina et al. 2006). In four other sequenced genes mutations had previously been reported in patients with epilepsy. SCN1A, a well-known candidate gene for severe myoclonic epilepsy of infancy (SMEI) and GEFS+ has also been linked to myoclonic–astatic epilepsy, Lennox-
Gastaut syndrome and infantile spasms (Gambardella and Marini 2009) and recently also to PS (Grosso et al. 2007, Livingston et al. 2009). In the CACNA1G gene segregating and non-segregating alterations were reported in small families with IGE (Singh et al. 2007). In the TBC1D24 gene compound heterozygote mutations were identified in a family with autosomal recessive myoclonic epilepsy in infancy (Zara et al. 2000, Falace et al. 2010). Finally, the CACNA1H gene that was initially identified as a susceptibility gene for CAE in Chinese Han population has later also been reported to harbour susceptibility alleles in Caucasian patients with different IGE syndromes and focal epilepsies (Chen et al. 2003, Heron et al. 2007, Liang et al. 2007).

In this study altogether 16 genes were sequenced. In one of the candidate genes sequenced, namely in CACNA1H, a probable mutation that segregated with CAE, FS and PS phenotypes was identified in one branch of family 5. The first two phenotypes, CAE and FS have been associated with variants in CACNA1H gene before (Chen et al. 2003, Heron et al. 2007, Liang et al. 2007). PS is a new epilepsy phenotype associated with CACNA1H. The identified variant, c.2057C>T in CACNA1H is a novel one. It is a potential susceptibility variant for epilepsy based on in silico analysis, which showed that the affected amino acid located in intracellular loop linking domains 1 and 2 is highly conserved, and that the change of proline to leucine at this position is probably damaging. The intracellular loop connecting domains 1 and 2 has been found to regulate the membrane expression of CACNA1H. Its disruption by either single amino acid changes or by large sequence removal significantly enhances membrane expression of Cav3.2 channel from intracellular compartments to the plasma membrane (Vitko et al. 2007). The first 62 amino acids of the loop linking domains 1 and 2 are involved in regulating the voltage-dependence of channel gating and inactivation. The last 15 amino acids of the same loop are involved in channel inactivation. The central region (containing the Pro618Leu variant) of this loop regulates surface expression, with no significant effect on voltage-dependence or channel kinetics (Vitko et al. 2007). Functional and/or expression studies are needed to prove the significance of c.2057C>T in CACNA1H. Variants in CACNA1H are considered to contribute to an individual’s susceptibility to epilepsy but are not sufficient on their own to cause epilepsy (Heron et al. 2007). The difficulty in demonstrating that a channel variant is the root cause of epilepsy has been emphasized because IGE is a polygenic disorder and by
definition requires the co-inheritance of variants in other genes, as well as possibly contributions from the environment (Berkovic et al. 2006, Zamponi et al. 2010). The finding in the Study 4 also supports this concept. Hence, other genetic factors are needed to produce variable phenotypes present in family 5.

9.3.3 mRNA expression profiling

Systematic evaluation of comparability of gene expression in blood and brain has shown that whole-blood gene expression profile has significant similarities with that of multiple central nervous system regions (Sullivan et al. 2006), therefore it may provide a suitable surrogate for gene expression in central nervous system diseases (Sharp et al. 2006). The blood gene expression in children who became seizure free on VPA was different from that of children who continued to have seizures (Tang et al. 2004). In this study, gene expression profiling was used to identify additional genes for sequencing in the candidate locus on chromosome 17q12-q24 locus. Six genes were differentially expressed, and two of these (RPIP8, SLC25A39) were chosen for sequencing based on their expression and function, as described in 9.3.2. The chip used contained 1500 different chromosome 17 transcripts of which more than one third, including the nine sequenced ion-channel genes, had a fairly low expression level in blood. Neuronal ion-channel genes related to epilepsy in humans may have much higher expressions in brain tissue and thus, differences in expression levels cannot be detected using blood cell mRNA as a surrogate marker (Helbig et al. 2008).

9.3.4 Array-based comparative genomic hybridization

Copy number variants are a new class among the disorders of the genome architecture (reviewed in Kumar 2008). One area in which this form of genetic susceptibility has been shown to be crucially important is intellectual disability, where large CNVs explain the underlying etiology in 15% of individuals (Mefford and Eichler 2009). On the other hand, these submicroscopic rearrangements often have no phenotypic expression, but may act as rare variants predisposing to complex disease (Scheffer and Berkovic 2010). In 1% of patients with IGE 15q13.3
microdeletion has been reported (Helbig et al. 2009) and family studies have revealed that the 15q13.3 microdeletion behaves as would be expected for a susceptibility variant (Dibbens et al. 2009). In family 98, evaluation of the CNV was used, but no abnormality was identified. However, the disease-associated common copy number polymorphisms (CNPs) were much smaller (20–45 kb) than the resolution in the analysis carried out in family 98 (Manolio et al. 2009). Therefore the possibility of CNVs contributing to seizure susceptibility in family 98 cannot be totally excluded.
10. Conclusions and future prospects

Childhood absence epilepsy is a benign epilepsy syndrome, although some patients are difficult to treat. Video-EEG is not routinely used to assess the seizure status after clinical remission in CAE. However, it should actively be used in situations in which the abolishment of absence seizures is not a certainty. Newer AEDs such as LTG, TPM and LEV should be introduced if the first line drugs ETM and VPA do not produce remission (Curatolo et al. 2009). Zonisamide might also offer remission in some patients with CAE (Marinas et al. 2009). Additionally, nervus vagus stimulation might also be an option for drug resistant CAE patients (Kostov et al. 2007).

In the future it would be important to assess the long-term outcome in a large cohort of seizure free CAE patients by the general IQ test, specific neurocognitive assessments, analysis of behaviour, emotion, social skills and self-image estimation. There are preliminary results for these factors in a small Finnish patient group (Poutanen 2009). Due to the low incidence of CAE, a multicentre follow-up study focusing on these aspects would be needed to better understand the importance of good seizure control, identification and treatment of comorbidities and the effect of AEDs.

CAE is most often a sporadic epilepsy syndrome with a genetic background of a cocktail of several susceptibility genes and possibly one or more rare variants. As found in this study, about 10% of CAE probands have at least one additional family member with IGE. Including these small families in a large collaborative project would be useful to go further in solving the genetic basis of CAE. Rarely identified, multiplex families with heterogeneous IGE phenotypes could be used in linkage analysis to identify a major gene behind the seizure susceptibility by linkage analysis and for the detection of rare and low-frequency variants with new methods (see below) (Manolio et al. 2009). For clinicians, the most important task is to identify these families by obtaining a detailed family history and interviewing the extended family.
The two families studied with molecular genetic methods were too small to detect a major susceptibility gene. In family 98, a major susceptibility variant was located in the chromosome 17q12-q24 region. Additional modifier variants may be located on the other two loci on chromosomes 5 and 18. In family 5, a locus on chromosome 16p12.3-13.3 with a nearly significant linkage signal was identified. The variant identified in CACNA1H is a likely candidate for a susceptibility variant. In both families common variants in different genes and additional low-frequency and rare variants are also likely to contribute significantly to the genetic architecture and heterogeneous seizure phenotypes (1000 Genomes Project Consortium 2010).

The GWAS based on examining the genomes of thousands of individuals for correlations between the presence of genomic variants and the traits of interest have been successfully used in a growing number of diseases displaying a complex genetic basis (Ku 2010) and such studies will hopefully reveal common variants in CAE in the future. Yet, the genomic variants detected through GWAS explain only a small proportion of genetic susceptibility in individual diseases (Manolio et al. 2009). Human geneticists call this problem 'missing heritability'. It has been postulated that most of this missing heritability is due to the effect of rare variants not identifiable through GWAS. The possibility of applying next generation sequencing methods, currently exome sequencing (the coding regions of the genes) and, ultimately, whole genome sequencing should fill in the gap of missing heritability and thus provide the ultimate answer on the genetic basis of complex disease, including CAE.

Future research will also focus on understanding the epigenetic mechanisms associated to epilepsy. Epigenetic mechanisms are suggested to be partially responsible for the phenotypes acquired during developmentally critical periods and for complementary changes that occur throughout life. Epigenetic regulation is said to be important for mediating the interplay between cell intrinsic processes and complex spatiotemporal patterns of local and long distance environmental cues in the brain and can show similar inheritance patterns as Mendelian disorders (Qureshi and Mehler 2010).

Successes in mapping of complex disease loci in population isolates have depended on large pedigrees with either proven or predicted genealogical links between affected individuals (reviewed in Varilo and Peltonen 2004). The reduced genetic diversity in the isolates will simplify the genetic background of any trait. If
not fewer genes, at least fewer alleles and recognizable haplotype signatures among affecteds is an expectation especially in young isolates (Varilo and Peltonen 2004). Therefore, efforts in identifying and recruiting for participation rare large families with several affecteds, more common small families with some affecteds and even sporadic patients with IGE spectrum epilepsies in Finland is emphasized.

Characterization of the genetic determinants of disease provides remarkable opportunities for clinical medicine through an improved understanding of pathogenesis, diagnosis and therapeutic options (Kumar 2008). For example, at the time of diagnosis of CAE it would be of value to test for genetic variants that predict drug response or development of GTCS (Pal et al 2010). The ultimate goal is to understand the genetic basis of CAE and to be able to provide personalised medicine for each CAE patient.
11. Acknowledgements

I wish to express my gratitude to all those who have been involved in this study. The study was carried out at the Department of Pediatric Neurology, Tampere University Hospital, Finland, Centre de Recherché, Centre Hospitalier de l’Université de Montréal, Canada, Laboratory of Experimental Neurology, Department of Neurology, Erasme Hospital, Free University of Brussels, Belgium, Folkhälso Institute of Genetics and Neuroscience Center, University of Helsinki, Finland. The former and current heads of the Departments from 1995 to 2011 are warmly thanked for providing the excellent working facilities at my disposal and for their positive attitudes to my studies.

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12. References


Italian League Against Epilepsy Genetic Collaborative Group (1993): Concordance of clinical forms of epilepsy in families with several affected members. Italian League Against Epilepsy Genetic Collaborative Group. Epilepsia 34:819-826.


13. Web sources

The following web sites were used:
http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi
http://www.ensembl.org
http://www.genenames.org/
http://www.genetics.ucla.edu/software/simwalk_doc
http://genetics.bwh.harvard.edu/ppy
http://www.genome.ucsc.edu/cgi/hgGateway
http://www.hgvs.org/mutnomen/
http://www.ileae-epilepsy.org/Visitors/Centre/ctf/childhood_absence.cfm
http://www.ileae-epilepsy.org/Visitors/Centre/ctf/CTFs syndromes.cfm
http://www.snps3d.org
http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi
Beneficial effects of antiepileptic medication on absence seizures and cognitive functioning in children

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Abstract

In this prospective clinical study, the effects on cognitive functioning of absence seizures, epileptiform EEG discharges, and their abolishment by antiepileptic medication were evaluated in patients newly diagnosed with childhood absence epilepsy or juvenile absence epilepsy. Eleven children in the study group and ten age- and gender-matched controls with mild asthma underwent combined video/EEG and neurocognitive assessment (IQ, fine-motor fluency, attention, visual and spatial memory). The neuropsychological assessment was repeated after the introduction of antiepileptic medication. Ten children with absence epilepsy became clinically seizure free. The study group improved in attention, fine-motor fluency, and visual memory. The controls improved only in fine-motor and attention skills. Duration of generalized 3-Hz spike–wave discharges and clinical absence seizures was negatively correlated with performance on the visual memory task. Cessation of seizures induced by antiepileptic medication may support neurocognitive functioning in children.

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Keywords: Typical absence seizure; Cognitive functioning; Visual memory; Fine-motor fluency; Antiepileptic medication; Remission of seizures

1. Introduction

Typical absence seizures are the main symptom in two idiopathic generalized absence epilepsy syndromes: childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE). Three components are essential in both syndromes: impairment of consciousness coinciding with generalized 3-Hz spike and slow wave discharges, normal development, and normal intelligence. Generalized tonic–clonic seizures (GTCS) rarely affect children with CAE and more often those with JAE. In CAE, the absence seizures tend to occur as often as 10 to 100 times a day. The onset of seizures is usually between the ages of 4 and 10. Children with JAE have fewer absence seizures than those with CAE, and onset is after 10 years of age. The absence seizures are often very short, about 2 to 10 seconds. Thus, they are difficult to detect, and in many cases, it may take months or even years before medical treatment is considered for children with absence seizures [1]. In clinical practice, in these patients, the parents and the children themselves rarely complain of poor performance at school, which could reflect subtle difficulties in cognitive functioning that are hard to recognize albeit they may influence learning. Cognitive impairments and the need for special education are frequently reported in children with epilepsy [2,3]. In studies of cognitive performance, children with idiopathic generalized epilepsy or/and with typical absence seizures are found to have specific problems in attention [4,5], visual memory [4,6–8], and fine-motor fluency [5] despite their normal intelligence level. Findings for verbal memory and other verbal functions have been within the normal range in most studies [4–8]. It is obvious that several factors, such as etiology, type of seizure, epilepsy syndrome, age at onset, and
medication, in combination are related to the cognitive problems in epilepsy [9]. Also, parents’ ability to continue their normal parenting after the diagnosis of epilepsy is important for the prognosis of the child’s cognition and behavior [10]. The absence seizures in CAE and JAE are clinically similar, and these syndromes share a common genetic etiology [11]. Therefore, patients newly diagnosed with the syndromes CAE and JAE are an appropriate population in which to assess neurocognitive functioning before initiation of and after time on treatment with antiepileptic drugs (AEDs).

In patients newly diagnosed with epilepsy who are not yet on medication, the cognitive effects of AEDs can be studied prospectively. There are currently no studies in which solely patients with CAE and JAE have been assessed before introduction of AEDs and later after cessation of seizures (or at the medication discontinuation phase). In a drug withdrawal study (Multicenter Holmfrid Study), a homogeneous subgroup of patients with epilepsy with typical absence seizures was found to be slower in fine-motor fluency and visual searching both during treatment and after drug withdrawal, compared with other patient groups with epilepsy studied [12].

The aim of the present study was to investigate neurocognitive functioning in children with absence seizures and matched control children. Neurocognitive functions were measured before and 10 months (7–13 months) after the introduction of antiepileptic medication to study the influence of cessation of seizures and medication on cognitive processes. General intelligence was included in the assessment procedure to establish whether there was any evidence of general developmental delay either at the time of diagnosis or after the follow-up period. The neuropsychological assessment was done during video/EEG monitoring (24-hour EEG recording under video supervision in long-term monitoring unit) to determine whether the children had clinical or subclinical seizures with epileptiform EEG discharges during the cognitive assessment. The assessment comprised tasks of visual and spatial memory, sustained attention, fine-motor fluency, and auditory and visual reaction times. These subdomains of cognition were selected in line with the findings of previous studies of neurocognitive functioning in epilepsy. These functions were also relatively easy to assess using computer-based tasks.

### 2. Methods

#### 2.1. Participants

The participants were 11 (6 girls and 5 boys) children newly diagnosed with typical absence seizures and generalized 3-Hz spike–wave discharges on the EEG. They were diagnosed between June 1997 and March 2002. They had either CAE (10) or JAE (1) according to the ILAE classification [13]. All participants experienced absence seizures; none had had GTCS. The mean age of the participants in the study group was 8 years 2 months (4 years–14 years 9 months). The control group consisted of 11 gender-

<table>
<thead>
<tr>
<th>Number</th>
<th>Study group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, F/M</td>
<td>10</td>
<td>10</td>
<td>5/5 5/5</td>
</tr>
<tr>
<td>Age at seizure onset</td>
<td>3.0–11.8</td>
<td>3.0–11.8</td>
<td>5.5–14.5</td>
</tr>
<tr>
<td>Age at first assessment</td>
<td>4.0–14.9</td>
<td>4.0–14.9</td>
<td>5.5–14.5</td>
</tr>
<tr>
<td>Median Verbal IQ</td>
<td>99.5</td>
<td>94.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Median Performance IQ</td>
<td>96.0</td>
<td>96.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Medication</td>
<td>Sodium valproate (VPA)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Ethosuximide (ETM)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>VPA + ETM</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>


and age-matched children from the outpatient clinic for asthmatic children. One child from the control group was eliminated from further analysis because she did not participate in the second assessment session. The mean age of the 10 remaining control children was 8 years 10 months (5 years 6 months–14 years 6 months). There was no significant difference between the study and control groups with respect to chronological age. One child in each group was left-handed. The educational levels of the mothers were matched. One child in the study group continued to have infrequent clinical absence seizures despite the use of combination therapy and good compliance, and she was therefore excluded from further analysis. Descriptive information for the two groups is summarized in Table 1. This study was approved by the ethical committee of Pirkanmaa Hospital District. The children and their parents gave informed consent before the study.

#### 2.2. Video/EEG recordings and neuropsychological assessment

Children were asked to participate in the study at the time when the diagnosis of idiopathic generalized absence epilepsy with generalized 3-Hz spike–wave discharges was initially confirmed by electroencephalography. The first computer-based neuropsychological assessments were done during the 24-hour video/EEG recording. General intelligence level and auditory and visual reaction times were assessed in a separate session without video/EEG recording. To confirm that all the children in the control group had a normal EEG, the first neuropsychological assessment of the control group was also done during video/EEG recording, which, however, lasted only an hour.

The follow-up neuropsychological assessment included the same tasks as in the first assessment. For the study group, the mean interval between the two assessments was 10 months (7–13 months). For the control group, follow-up neuropsychological assessments without simultaneous video/EEG recording were done 11.5 months (6–18 months) after the first assessment. The delay between the two assessments did not differ significantly between the groups.

Antiepileptic medication was introduced after the first neuropsychological assessment. Standard AEDs for typical absence seizures, ethosuximide (ETM) and sodium valproate (VPA), were introduced. Four children were treated with VPA, and five with ETM; two children required a combination of VPA and ETM to achieve clinical cessation of seizures. All children in the study group had had typical generalized 3-Hz spike–wave discharges and absence seizures during the first 24-hour video/EEG recording. The video/EEG recordings were analyzed by a clinical neurophysiologist. Analysis included the total duration of generalized 3-Hz spike–wave discharges, clinical absence seizures, and subclinical interictal discharges during the neuropsychological assessment.

Neuropsychological assessment during video/EEG recording included STIM tasks (a Library of Sensory, Cognitive and Neuropsych Tasks, Neuro Scan, Inc.) involving fine-motor fluency, sustained attention, and visual and spatial memory. Based on a pilot study with children, the tasks were modified by reducing the number of trials and task demands. The assessment lasted approximately 45–60 minutes.
In the fine-motor task, the fluency of finger tapping was measured. Children were asked to tap the computer mouse as fast as possible with the index finger of both hands. The tapping time was 10 seconds and was repeated five times with both hands. The mean dominant and non-dominant hand tapping frequencies were measured. The difference between tapping frequencies of the dominant and non-dominant hands was also calculated.

The attention task measured the ability to sustain attention. Numbers from 0 to 9 were flashed (for 500 ms) behind a white square in the middle of a screen. Children had to press the right button of the computer mouse when the number behind the square was 0 and the left button of the mouse when any other number (from 1 to 9) was flashed. The task comprised 100 trials with an interstimulus interval of 2000 ms. The overall percentage of correct responses was measured.

In the visual memory task, children had to remember four sequentially presented pictures. After presentation of the four pictures, a fifth picture was shown (with 3000-ms delay), and by pressing the appropriate button, children indicated whether the fifth picture was among the previously shown pictures. There were 20 trials in the task, and the overall percentage of correct responses was measured.

In the spatial memory task, children had to remember the location of blocks presented on a computer screen. The screen was divided into nine squares, which were the nine different spatial locations to remember. At first, the three blocks appeared at different squares on the screen. After disappearance of the blocks, children had to click the locations of the target blocks using the pointer of the mouse. The first four trials consisted of three blocks, in the next four trials there were four blocks, and in the last four trials there were five blocks. The overall percentage of correctly pointed locations was measured.

The standardized Wechsler Intelligence Scale for Children—Revised (WISC-R) was used for 18 children and the Wisconsin Preschool and Primary Scale of Intelligence—Revised (WPPSI-R) for 4 children [14,15]. The intelligence quotients, that is, Verbal (VIQ) and Performance IQ (PIQ), were estimated from the subtests on information, similarities, arithmetic, picture completion, block design, coding (WISC-R), and geometric design (WPPSI-R). To prevent double estimations of the intelligence level, the full intelligence quotients were not calculated (given the estimated versions of VIQs and PIQs).

Visual and auditory reaction times were measured with computer-based tasks (FePsy) [16]. The children had to react to a sound (800-Hz tone) or to a visual signal (white square) by pressing the space bar as quickly as possible. The reaction times in milliseconds were converted to Z scores separately for the dominant and nondominant hands. Visual and auditory reaction times were assessed only in the study group.

2.3. Statistical analysis

Nonparametric tests were used because the groups were small and data were not normally distributed. Matching of the study and control groups was tested using the Mann–Whitney test (U). The effect of AEDs, cessation of absence seizures, and epileptiform discharges on cognitive functioning within the study group and the effect of the assessment delay within the control group were tested with the Wilcoxon rank test (Z).

To investigate the relationships between variables, Spearman’s rho \((r_s)\) was used. Visual and auditory reaction times were analyzed with Student’s t test because there is no equivalent nonparametric test to measure the difference between sample Z scores and Z scores of the normative average. A \(P\) value \(\leq 0.05\) was considered to indicate statistical significance. All calculations were performed with SPSS/PC+ Version 13.0.

3. Results

3.1. Video/EEG recordings

All children in the study group had typical 3-Hz spike–wave discharges with absences during the 24-hour video/EEG recording. Nine patients had generalized 3-Hz spike–wave discharges on their EEGs during the first computer-based neuropsychological assessment. In total, 104 epileptic discharges (mean, 11.5; range, 2–17) were registered during the neuropsychological assessment. The mean duration of a discharge during the first assessment was 8 seconds (1–34 seconds). Only one participant, an 8-year-old girl, did not have any EEG abnormalities during the first neuropsychological assessment. Another participant had only occasional spikes during the neuropsychological assessment. Unfortunately, a video recording was not available and the data on clinical absence seizures were missing for one patient, a 14-year-old boy with JAE. He had 15 discharges lasting 5–34 seconds. Analysis of the follow-up video/EEG recordings revealed that under AED treatment, 10 of 11 children became clinically seizure free. Seven children had a normal EEG during the second 24-hour video/EEG recording. One child had a few spike–wave complexes during sleep, and another child had a slow wave discharge with spikes provoked by hyperventilation. One child had a few typical 3-Hz spike–wave discharges lasting 3 seconds during the follow-up video/EEG recording. One child continued to have infrequent clinical absence seizures despite the use of combination therapy and good compliance, and she was therefore excluded from further analysis. No EEG abnormalities were recorded during the follow-up neuropsychological assessment of the 10 seizure-free children in the study group.

3.2. Neuropsychological assessment

The neuropsychological tasks were completed by all participants with the following exceptions. In the study group, one patient did not perform the visual memory task and two patients did not complete the attention task. In both groups, the median percentages of performance on all tasks were relatively high (Table 2).

In the fine-motor fluency (tapping) task, the study group performed significantly better with both hands during the second assessment than during the first assessment \((Z = -1.99, P = 0.047 \text{ for the dominant hand}; Z = -2.29, P = 0.022 \text{ for the nondominant hand})\). The control group improved in tapping only with the dominant hand \((Z = -2.43, P = 0.015)\); no change was measured with the nondominant hand \((Z = -0.53, P = 0.594)\). Also, there was a significant increase in the difference between dominant and nondominant fine-motor fluency in the control group \((Z = -2.19, P = 0.028)\), but not in the study group \((Z = -0.969, P = 0.333)\).

Performance on the attention task improved significantly in both groups \((Z = -2.1, P = 0.036 \text{ in study group}; Z = -2.49, P = 0.012 \text{ in control group})\). On the visual memory task, there was a significant improvement (6.25%) only in the study group \((Z = -2.67, P = 0.008)\). On the spatial memory task, there was a decrease in...
performance (−3.2%, Z = −1.79, P = 0.074) that only approached our significance level, in the study group. On the other hand, in the control group, performance on the spatial memory task improved 3.3% (Z = 1.86, P = 0.063), but yet again this only approached the significance level set.

Duration of generalized 3-Hz spike–wave discharges and clinical absence seizures during the first assessment session correlated significantly with performance on the visual memory task (r_s = 0.69, P = 0.041 and r_s = 0.79, P = 0.021, respectively) (Fig. 1). There were no significant correlations between performance on any of the other neuropsychological tasks and duration of epilepsy before treatment, duration of generalized 3-Hz spike–wave discharges, clinical absence seizures, or interictal discharges during the assessment.

General intelligence (IQ) was within the normal range in both groups, and no significant difference was recorded between groups. In the study group, VIQ ranged from 84 to 128 (median, 99.5) and PIQ ranged from 67 to 118 (median, 96). Improvement in VIQ (Z = −2.14, P = 0.033) was observed in the control group during the follow-up assessment, whereas PIQ did not change significantly (Table 2).

Visual and auditory reaction times of six children in the study group were measured. Reaction times were transformed to z scores according to the age-related means of the normative data for typically developing children [17]. The mean z scores of the reaction times revealed that the study group was somewhat below the normative average (mean = 0, SD = 1) with respect to visual and auditory reaction times. This was observed for both hands and during both assessments (Table 3). A one-sample t test, however, revealed that none of the measures were significantly different from the normative average. There were no significant differences between the first and second assessments.

Table 2
Results of cognitive assessments at the time of diagnosis and follow-up

<table>
<thead>
<tr>
<th>Measure</th>
<th>First assessment</th>
<th>Second assessment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Study group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>84.0–128.0</td>
<td>99.5</td>
<td>86.0–136.0</td>
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<tr>
<td>Performance IQ</td>
<td>67.0–118.0</td>
<td>96.0</td>
<td>77.0–131.0</td>
</tr>
<tr>
<td>Fine-motor fluency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant hand</td>
<td>13.2–46.4</td>
<td>39.8</td>
<td>36.0–64.4</td>
</tr>
<tr>
<td>Nondominant hand</td>
<td>13.2–40.4</td>
<td>34.3</td>
<td>30.0–55.0</td>
</tr>
<tr>
<td>Dominant/nondominant hand difference</td>
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<td>3.6</td>
<td>0.4–15.2</td>
</tr>
<tr>
<td>Attention</td>
<td>14.0–96.0</td>
<td>81.3b</td>
<td>55.0–98.0</td>
</tr>
<tr>
<td>Visual memory</td>
<td>46.7–94.7</td>
<td>80.0c</td>
<td>52.6–100</td>
</tr>
<tr>
<td>Spatial memory</td>
<td>59.2–100.0</td>
<td>96.7</td>
<td>89.6–100</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VIQ</td>
<td>74.0–102.0</td>
<td>94.0</td>
<td>74.0–106.0</td>
</tr>
<tr>
<td>PIQ</td>
<td>81.0–106.0</td>
<td>96.5</td>
<td>81.0–111.0</td>
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<tr>
<td>Fine-motor fluency</td>
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<td></td>
</tr>
<tr>
<td>Dominant hand</td>
<td>26.0–54.4</td>
<td>42.3</td>
<td>30.2–60.8</td>
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<tr>
<td>Nondominant hand</td>
<td>23.6–49.6</td>
<td>35.5</td>
<td>23.6–54.8</td>
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<tr>
<td>Dominant/nondominant hand difference</td>
<td>0.8–11.0</td>
<td>5.9</td>
<td>3.0–14.2</td>
</tr>
<tr>
<td>Attention</td>
<td>37.0–98.0</td>
<td>80.5</td>
<td>84.0–99.0</td>
</tr>
<tr>
<td>Visual memory</td>
<td>47.4–100.0</td>
<td>84.5</td>
<td>73.7–90.0</td>
</tr>
<tr>
<td>Spatial memory</td>
<td>75.0–100.0</td>
<td>93.8</td>
<td>77.1–100.0</td>
</tr>
</tbody>
</table>

† Statistically significant.
b Eight participants.
c Nine participants.

Fig. 1. Scatter gap of the correlation between visual memory and total duration of epileptiform discharges during video/EEG assessment. The correlation coefficient, r_s, was −0.686. Scores on the visual memory task are given as overall percentages of correct answers.
Auditory and visual reaction times of the study group (n = 6) converted to z scores

<table>
<thead>
<tr>
<th></th>
<th>Mean z score (SEM)</th>
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<td></td>
<td>First assessment</td>
</tr>
<tr>
<td>Auditory reaction time</td>
<td></td>
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<tr>
<td>Dominant hand</td>
<td>−1.15 (0.66)</td>
</tr>
<tr>
<td>Nondominant hand</td>
<td>−2.35 (1.47)</td>
</tr>
<tr>
<td>Visual reaction time</td>
<td></td>
</tr>
<tr>
<td>Dominant hand</td>
<td>−2.19 (1.25)</td>
</tr>
<tr>
<td>Nondominant hand</td>
<td>−0.29 (0.65)</td>
</tr>
</tbody>
</table>

4. Discussion

Neurocognitive functioning of children with absence seizures and their matched controls was studied before and after introduction of antiepileptic medication. The results indicated improvement in neurocognitive functioning with efficient AED treatment. In the present study, the improvement was evident only with respect to visual memory and fine-motor fluency of the children with absence epilepsy, but not in the normally developing children. The two groups of children did not differ significantly with respect to change in performance on attention and spatial memory tasks. Absence seizures per se seemed not to affect patients’ general intelligence, as at the time of initial diagnosis the intelligence level was average. However, the study group’s reaction times tended to be somewhat slow.

Earlier studies in this particular patient group described diverse cognitive deficits in a variety of subdomains of neurocognitive functioning [4–8,18]. Instead of a wide-scale neuropsychological assessment, we decided to use assessments based on the computer-generated neuropsychological tasks in the STIM system, which we modified to be suitable for children. We observed that visual memory improved when absence seizures were abolished. Deficits in visual memory in this patient group have also been reported by others [4,6–8]. In these previous studies, many patients still had seizures when compared with healthy controls or with patients with other types of epilepsy. In the present study, all patients analyzed attained remission, as confirmed by the 24-hour video/EEG recording, which may be related to the significant enhancement of visual memory observed.

We found that both groups of children improved significantly in performance on the attention task, which measured the ability to sustain attention. It is known that the ability to sustain attention is still developing rapidly between 6 and 15 years of age [19], which is close to the age range of the children in our sample. The improvement in attention-sustaining skills might occur even with the short delay (around 10 months) between the first and the second assessments in our study. This developmental tendency could explain the findings for both groups. It should, however, be noted that there was a wider range of performance in the study group than in the control group. This suggests that some of the children with typical absence seizures may have problems sustaining attention. Fedio and Mirsky observed poor ability to sustain attention in a similar pediatric patient population when compared with patients with temporal lobe epilepsy and healthy controls [4]. In their study, the patients were on AEDs and still experienced seizures. In another study, in which the Coding and Digit Span subtests of the Wechsler Intelligence Scale for Children were used as attention measures, impairment in attention was demonstrated in a patient group similar to that in the present study. Again, in the previous study, half of the patients continued to have seizures [5]. Thus, it is possible that remission is required to support the beneficial development observed in the present study.

We did not observe significant improvement in performance on the spatial memory task in either group. There was only a tendency toward improvement in the control group, and in fact, performance of the study group tended to decline. Some previous studies that measured visuospatial construction and memory skills (e.g., Rey–Osterreith Complex Figure test) in patients with absence epilepsy have shown impairment of performance [4,7,8]. There have, however, been contradictory findings as well [5]. We do not want to draw too strong conclusions from the findings on the spatial memory task in the present study. First, the change in performance between the first and second assessments was only of borderline significance in both groups. Second, task performance in general was very high (around 95%), and it may be that the task was too easy for the children and therefore lacked the sensitivity to measure very subtle changes in performance.

Fine-motor fluency with both hands improved significantly in the study group, whereas improvement was observed only with the dominant hand in the control group. Fine-motor fluency is still increasing at this age in normal development [20] and is more evident with the dominant hand than the nondominant hand [21]. We assume that the significant improvement with both hands in the children with absence epilepsy relates to the general recovery of motor fluency resulting from the cessation of absence seizures and not just to the improvement in fluency of the dominant hand, which is typical of normal development. However, no differences in fine-motor fluency were observed in a large study in which children with newly diagnosed, uncomplicated idiopathic generalized or partial epilepsy were compared with nonepileptic controls [22]. According to this previous study, we do not know whether an improvement in performance would be measurable if the patients had been re-evaluated after attaining remission. Also, Henkin et al. [5] showed that children with typical absence seizures, in particular, performed poorly on fine-motor fluency task with the dominant hand when compared with control children or with children with GTCS. All their patients were being treated with VPA, and half of them were still experiencing absence seizures at the time of assessment. Thus, both of these previous studies seem to support the idea that absence seizures are harmful to
fine-motor fluency, and our observation of improvement in fine-motor fluency immediately after remission provides further proof of this phenomenon.

In addition, Henkin et al. [5], found that the difference in motor fluency between the dominant hand and nondominant hand was statistically lower in children with typical absence seizures than in controls or patients with idiopathic GTCS. In our study, the difference in fine-motor fluency between the dominant hand and nondominant hand increased significantly between the two assessments only in the control group. It should be emphasized here that the purpose of the present study was not to compare the groups but to investigate the improvement in neurocognitive functioning after treatment of children with absence epilepsy. Thus, this finding further supports the fact that especially the fluency of the dominant hand improved in the normally developing children and this was not observed in the study group.

We did not observe significant slowing in visual and the auditory reaction times in the children with absence epilepsy, although their times were somewhat below the normative average. This could be due to a lack of statistical power, as only data for six children with absence epilepsy were available. Slowing of reaction times and motor speed was evident in an earlier study that studied children newly diagnosed with uncomplicated epilepsy (cryptogenic partial and idiopathic generalized epilepsy). At the time of measurement, most of the patients had only just started taking AEDs [22]. Boelen et al. could not confirm any correlation between the prescribed dose of AEDs and decrease in reaction time and motor speed [22]. This finding is in line with our study showing no difference in reaction times after treatment. Slow auditory and visual reaction times in children have been measured during subclinical epileptiform EEG discharges [23]. We did not conduct video/EEG recording simultaneously with the assessment of visual and the auditory reaction times and, therefore, cannot conclude whether slow reaction times would have been more prominent during the discharges than in their absence. Because our sample size for the reaction time data was small, these findings should be considered with caution.

In previous studies with similar patient populations (idiopathic generalized epilepsy with absence seizures), it has been shown that there are no differences in general intelligence (IQ) between patients with typical absence seizures and patients with partial seizures, patients with other types of seizures, or healthy controls [4,6,8]. This was our result as well: the study group and the control group did not differ with respect to VIQ or PIQ at the first assessment. However, in two other studies, the general cognitive level of children with typical absence seizures was reported to be within the normal range, although lower than the general cognitive level of patients with other types of seizures [18] or diabetic controls [7]. In the first study, the difference was measured prior to the introduction of AEDs, but was no longer evident at the 12-month follow-up [18]. In the second study, the children had been under treatment with the regular AED and almost all were seizure free [7]. These studies indicate that continuing absence seizures disturb normal cognitive development. An interesting finding in the present study was that in the follow-up assessment, there was a significant improvement in VIQ only in the control group. The delay between the first and second assessments was only 11.5 months, and therefore, it is possible that the improvement in VIQ in the control group was due to a practice effect and not to actual maturation/development of skills. Regardless, we did not observe a similar improvement in the study group. It is tempting to speculate that the absence seizures at the time of the first assessment attenuated the practice or maturation effect, and therefore, the children in the study group were not able to benefit from having performed the same series of tasks a relatively short time before.

The effect of AEDs on cognition has been the focus of epilepsy research and has been reviewed recently [24]. It has been suggested that clinicians should be aware of the differential cognitive effects of antiepileptic drugs and should therefore monitor cognitive function closely when adding or changing therapy. Our patients had no significant changes in general intelligence between the first and second assessments. Similarly, when general intelligence was used as an indicator in children newly diagnosed with epilepsy, no deterioration was detected in two separate annual follow-up studies [18,25]. We demonstrated significant improvement on four of five neurocognitive tasks when the patients were in remission. Two of these changes were not apparent in the control group. These can be viewed as beneficial effects of the remission induced by AEDs.

The major limitation of the present study was the small sample size. This limitation was at least partly compensated for by careful assessments of AED efficacy to ensure that the patients attained remission by the follow-up. In addition, the computer-based assessment of neurocognitive functioning using the tasks of the STIM system was modified to be suitable for children. Performance on all tasks was relatively high (around 88%). Thus, it could be possible that our modification was not sensitive enough for the children, and therefore, their performance reached a “ceiling.” On the other hand, the variation in performance was relatively wide, which supports the assumption that the measurement sensitivity of these tasks was sufficient.

To conclude, antiepileptic medication seemed to improve the cognitive functioning of the children through the cessation of absence seizures. In the present study, visual memory and fine-motor fluency, in particular, improved. Despite the fact that the children with typical absence seizures did not differ in terms of general intelligence (IQ) from the matched controls, they did not seem to benefit from the practice effect that was evident in the follow-up assessment of VIQ in the typically developing children. The findings of this study demonstrate that attainment of remission is important to the normal cognitive development of children with absence seizures.
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References


Idiopathic generalised epilepsies with 3 Hz and faster spike wave discharges: A population-based study with evaluation and long-term follow-up in 71 patients

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KEY WORDS: idiopathic generalised epilepsy, intractable seizures, long-term follow-up

ABSTRACT - For several years we have been following patients with intractable, childhood-onset idiopathic generalised epilepsies with ≥3Hz spike-wave discharges. Our need to find explanations for their intractability was the starting point for this study. We were interested in identifying characteristics, which would predict intractability; evaluating how these patients were treated and whether polytherapy was useful. We identified patients with ≥3 Hz spike-wave discharges by reviewing EEG reports recorded between 1983 and 1992. Data were collected from medical records and through personal interviews. We identified 82 patients with tentative idiopathic generalised epilepsy. Eleven were excluded. Thirty-eight patients had childhood absence epilepsy, 10 had juvenile absence epilepsy, 13 had juvenile myoclonic epilepsy and two had eyelid myoclonia with absences: 89.5, 78, 38 and 0% of the patients in each group, respectively, had been seizure free for more than 2 years. Twenty percent of the patients had intractable seizures. All intractable patients with juvenile absence epilepsy had rhythmic, random eyelid blinking and generalised tonic-clonic seizures. A history of more than ten generalised tonic-clonic seizures was associated with intractability in juvenile myoclonic patients. Monotherapy with ethosuximide or valproate resulted in seizure control in 65% of patients. Seventeen patients (24%) were treated with polytherapy, six achieved remission. These six patients had childhood absence epilepsy and juvenile absence epilepsy. Positive outcome was found in childhood absence epilepsy and juvenile absence epilepsy. Intractable seizures were more frequent among patients with juvenile myoclonic epilepsy. None of them benefited from polytherapy with conventional anti-epileptic drugs.
The revised 1989 Classification of Epilepsies and Epileptic syndromes of the International League Against Epilepsy (ILAE) [1] defines an epileptic syndrome as a disorder characterised by a cluster of signs and symptoms customarily occurring together. In contradistinction to a disease, a syndrome does not necessarily have a common aetiology and prognosis. Telepathic, primary generalised epilepsy syndromes (IGE) such as childhood absence epilepsy (CAE) are a good example of this. Patients with age-related IGE primarily have absences as well as myoclonic or tonic-clonic seizures with generalised spike-wave bursts at ≥3 Hz superimposed on a normal EEG background, without focal signs, mental retardation or an obvious cause other than genetic factors. In symptomatic or cryptogenic absence epilepsies, tonic or atonic seizures, focal signs and cognitive impairment are common. The EEGs are characterised by spike-wave discharges of 2.5 to 3 Hz and often by slow background activity as well [1,2].

The aim of this study is to evaluate the outcome of patients with idiopathic generalised epilepsy syndromes with ≥3 Hz spike-wave discharges, to identify the characteristics which would predict intractability, and to evaluate how these patients have been treated.

**Methods**

The study was conducted among the inhabitants of the referral district of the Tampere University Hospital (TaUH). This includes the city of Tampere (population ~175,000) and 34 surrounding counties (total population ~440,000) in south central Finland. The study was approved by the Ethics Committee of TaUH.

Potential subjects for the study were identified through three sources. 1) We reviewed all reports of EEGs recorded between 1983-1992 in TaUH. Patients who had generalised ≥3 Hz spike-wave or multiple spike-wave discharges recorded in their EEG were considered as potential subjects for the study. 2) The neuropaediatric database, active since 1972, was used to identify potential subjects. 3) Patients who were diagnosed with an idiopathic generalised epilepsy with ≥3 Hz spike-wave discharges during 1993-1994, in the Paediatric Neurology Unit of TaUH.

**Inclusion and exclusion criteria:** patients were classified according to the 1989 revised ILAE classification [1]. Clinical diagnostic criteria for CAE were: (I) a previously normal child most commonly within the age range of 6-7 years at the onset of epilepsy; (II) observed or reliably described absence seizures (AS), which may occur many times daily; (III) generalised tonic-clonic seizures (GTCS) or myoclonic seizures (MS) may occur. Diagnostic criteria for juvenile absence epilepsy (JAE) were: (I) onset of seizures near puberty; (II) observed or reliably described AS; (III) GTCS or MS being present more often than in CAE. Diagnostic criteria for juvenile myoclonic epilepsy (JME) were: (I) onset of MS near puberty; (II) GTCS and AS very often present. Diagnostic criteria for eyelid myoclonic epilepsy (EMA) were: (I) rhythmic and fast jerks of the eyelids; (II) AS occurring with longer eyelid myoclonus; (III) GTCS may occur. Exclusion criteria: (I) only genetic factors being recognised as predisposing factors; (II) mental retardation, if present at the onset of seizures; (III) patient not willing to participate in a follow-up interview and no up-dated medical records available.

**Data collection:** data were collected from the medical records. We interviewed patients and, when possible, also a relative, between November 1996 and February 1997. We collected data on childhood development; family history of seizure disorders; age at onset of seizures; age at diagnosis; history of different seizure types including febrile seizure (FS); anti-epileptic drug (AED) treatment, its efficacy and duration; remission and relapse; long-term prognosis.

**Definitions:** remission, independent if on- or off-medication, was defined as a period of at least 2 years without seizures. A seizure of any kind was considered as a relapse. Patients who continued to have seizures despite serious attempts to control them with AEDs and good compliance, were considered to have intractable epilepsy. FS is defined as recommended in the guidelines by International League Against Epilepsy [3].

**Results**

Eighty-two patients with tentative IGE were identified. Eleven had to be excluded: seven patients could not be located or were not willing to participate and for whom no updated medical record was available; four remaining patients had myoclonic absence epilepsy (MAE), which is classified as cryptogenic epilepsy [1]. Patients with MAE are not reported here.

Of the 71 patients, 54 were interviewed personally, 12 were interviewed by telephone, and five had an updated medical record. Also, a relative of 24 patients was interviewed. The mean follow-up was 14 years (range 2-53 years). Seventy-five percent of patients with IGE were in remission. The characteristics of these patients with CAE, JAE, JME and EMA are summarised in table 1 and figure 2.

**Childhood absence epilepsy**

We identified 38 (53.5%) patients with CAE. They had been followed for a mean period of 10.5 years (2-22 y). Fourteen (37%) had a positive family history of epilepsy and/or FS. At the time of diagnosis all patients were developing normally; two of them had been born prematurely. Later, seven patients (17.5%) were diagnosed with the following: dyslexia (2 patients), visuospatial and visuoconstructive difficulties (1), attention deficit hyperactivity disorder (1), emotional problems (1), mild dysphasia (1) and mild mental retardation (1). Three of these seven had an individual learning programme in a special school for children with normal and subnormal intelligence. The children with dyslexia or emotional problems studied in regular schools.
The mean age at onset of AS was 5.5 years (range 1-8.5 years) (Figure 1). The mean age at the time of diagnosis was 6.5 years. The maximum delay between onset of seizures and diagnosis of epilepsy was six years. Four patients (10.5%) had FS, all of which occurred before the age of six. Almost all (95%) had frequent daily AS, one had AS every week and one had three observed AS, which lasted several minutes. One patient (2.5%) had MS as well. He not only experienced frequent AS, but also myoclonic jerks in his upper extremities, occasionally, for six months until he sought medical advice and was treated. His older sister, who is also included in the study, had CAE with AS, and altogether four GTCS. Six patients (16%) experienced GTCS (two had one, three fewer than ten and one over ten GTCS). Five of them experienced GTCS when already on AED. Only in one patient was GTCS the first manifestation of epilepsy. One patient from a family with familial febrile seizures experienced a prolonged, atypical FS at 8 months of age when he had exanthema subitum, a viral infection caused by the human Herpes virus 6. Later, at the age of three years, she had another FS. She developed typical AS at four years of age, which were successfully treated with ESM. At the age of nine, she experienced her first complex partial seizure (CPS). She had an allergic reaction when on CBZ but was later well controlled with clonazepam (CZP). ESM was the first AED for 33 (87%), and VPA was used as the first drug for four (10.5%) patients. One patient was not treated with AEDs, in accordance with his parents’ wishes. After a 1.5 year follow-up, he did not have any AS and his EEG was normal. Detailed information about medication and response is shown in Figure 2. Twenty-two (58%) patients had achieved remission with ESM monotherapy. Seven patients (18.5%) were in remission with VPA monotherapy. Combination therapy with VPA and clonazepam (CLB) was effective in two (5%), as was VPA and ESM also in two patients (5%). The mean age at discontinuation of AED for patients in remission was 12 years (range 7.5-22.5 years). The mean duration of AED treatment was 3 years (range 2.3-15.5 years). Medication had been discontinued in 32 (84%) of the patients with CAE. One patient had a relapse with GTCS six years later, but remission was achieved with phenytoin (PHT). At the time of the study, 34 patients (89.5%)
had been in remission for at least two years. An additional three patients had been seizure-free with AED for less than two years. Only one patient was found to have intractable epilepsy (2.5%). The onset of epilepsy had occurred at six years of age and later a diagnosis of attention deficit hyperactivity disorder was made. He suffered from AS and GTCS. He had been followed for 22 years and had received mono- and polytherapy with ESM, VPA, phenobarbital (PB) and PHT.

**Eyelid myoclonia with absence epilepsy**

Two female patients (2.8%) were diagnosed with eyelid myoclonia and absences (EMA). They were both initially misdiagnosed as having tics. The eyelid myoclonias and AS had begun at the age of four in one and at the age of six in the other. Both had normal development and they studied in regular schools. One had a younger sister with CAE and older family relatives with FS. The other is the only epileptic patient in her family. ESM was the initial drug for one and VPA for the other. Polytherapy with ESM and VPA, as well as VPA and nitrazepam (NZP) was administered for both because of continuous eyelid myoclonias and AS. Despite medication and good compliance they both continued to experience eyelid myoclonia and AS. One also had one GTCS at the age of ten years. She continued on medication (VPA). The other wanted to discontinue medication and continued to experience eyelid myoclonia and occasional AS. She did not have any GTCS. They had been followed for 7 and 13 years respectively at the time of the study.

**Juvenile absence epilepsy**

We identified 18 patients (25.5%) with JAE. They were followed for a mean period of 15 years (3-53 y). Four (22%) of them had a family history of epilepsy. Only one patient had had FS twice, during the second year. All had normal development when the diagnosis of epilepsy was made. One was a twin and had been born prematurely. Later one patient was transferred from a regular school to a special school for children with normal and subnormal intelligence, because of behavioural problems. The mean age at onset of epilepsy was 11 years (range 9-14 years) (figure 1). Diagnosis was made at a mean age of 12 years (range 9-17 years). Fourteen patients (78%) had daily AS, two (11%) weekly and one (5.5%) had had only three observed, prolonged AS before the onset of treatment. This information was not available for one patient. None reported MS. Only one had GTCS as the first manifestation of epilepsy and even when on AED, he continued to have GTCS. During AED treatment, six other patients had GTCS (three patients 1-2 GTCS, two 10 GTCS and one > 15 GTCS). ESM was the first AED in seven (39%), VPA in five (28%), CBZ and PHT together in three (17%) patients. Detailed medication history and response rates are given in Figure 2. Five patients (28%) had achieved remission during ESM.
monotherapy and seven (39%) with VPA monotherapy. VPA combined with ESM was efficient in one (5.5%). In one patient, AS and GTCS were well controlled with a combination of VPA and CBZ. Additionally, one patient had been seizure-free for almost two years on ESM monotherapy. Medication had been slowly discontinued in 11 (61%) patients, who had been seizure-free for more than two years. The mean age at discontinuation of medication was 16.5 years (range 15-19.3 years). In these patients, the mean duration of AED treatment was 4.7 years (range 3.8-8.3 years). Three patients relapsed. One was again controlled with VPA; one patient had AS, of which she was not aware, but AS were observed during the follow-up interview; and one was treated with CBZ and still had had approximately one GTCS every year. At the follow-up, the majority of JAE patients (76%) had been seizure-free for more than two years. Three patients (16.5%) had intractable seizures. All intractable patients reported GTCS (two > 10 GTCS) and rhythmic random closing of the eyes, which was also described in their medical records. They all had brief AS, but two of the three also had prolonged AS. Rhythmic eyelid closing or prolonged AS was not reported by patients in remission or detected in their medical records. Intractable patients, followed for a mean of 56 years (20-53 years), had received mono- and polytherapy with VPA, ESM, PB, CBZ and PHT.
Juvenile myoclonic epilepsy

We identified 13 patients (18.5%) with JME. They were followed for a mean of 17 years (7-31 y). Over half of these patients (54%) had a positive family history of epilepsy. None had a personal or family history of FS. One had her first seizure, GTCS, during a fever at the age of 10 years. Two patients had learning and attention difficulties, but had attended regular schools. The mean age at onset of seizures was 11 years (figure 1) and, of diagnosis, 13 years. The maximum delay between onset of seizures and diagnosis was eight years. Seven patients (54%) had < 10 GTCS. The remaining six patients who had ≥ 10 GTCS were difficult to treat. On the other hand, two patients with only one GTCS were also intractable. They had frequent AS and MS.

For eight patients (61.5%), VPA was the initial drug. Two patients (15%) had been initially treated with ESM since the age of 4.3 and 10.8 years. They both had only AS at that time. The first one was initially diagnosed as having CAE. From the age of 11 she had MS and from the age of 13 she also had GTCS. Two patients (15%) had been initially treated with CBZ. One patient (7.5%) was initially treated with phenobarbital (PB). Later, all patients had been treated with VPA monotherapy or with VPA in combination with other AEDs (figure 2). One patient had to discontinue VPA because of elevated transaminases. Medication was slowly discontinued in three patients, resulting in relapse. Of the three patients, two became seizure-free again following VPA monotherapy. The third patient decided to stay off medication and had occasional MS after awakening. At the follow-up, 38% of the JME patients had been in remission for at least two years with VPA monotherapy. Sixty-two percent of patients were intractable. They had been followed for a mean period of 20 years (11-31 years). Polytherapy had been used for seven of eight intractable patients. Combinations of VPA and ESM, PHT, PB, CBZ or CZP did not increase the number of seizure-free patients. The severity of their seizures varied from occasional MS and GTCS to frequent AS and MS.

Discussion

We assembled our study population by reviewing nearly 20,000 EEG reports obtained between 1903 and 1992. Almost all EEG recordings in this region are performed in TaUH. During this period, about 1000 EEGs, mainly on adults, had been recorded in a private EEG laboratory. In Finland, all newly diagnosed children with epilepsy are referred to the paediatric department or to the paediatric neurology unit. We may therefore assume that all diagnosed paediatric and nearly all adult patients from this area were included. Due to the subtle nature of AS and MS, it is possible that there exist undiagnosed individuals who have not been bothered by these symptoms and have not sought medical advice.

We identified 82 patients with tentative IGE and 75 of them could be evaluated. Four patients had MAE, which belongs to the group of cryptogenic epilepsies. Of the remaining 71 patients, 53.5% had CAE, 25.3% had JAE, 18.5% had JME and 2.8% had EMA. We did not diagnose any patient with GTCS on awakening, although, four patients reported that awakening (data not shown) also precipitated GTCS. These four patients had AS and two had also MS, which were the most frequent types of seizure. They were diagnosed as having JAE (2 patients) and JME (2 patients). The usefulness of GTCS on awakening as a specific syndromic entity has been discussed [4, 5].

According to a prospective Finnish study, with 30 years of follow-up, the majority of patients with onset of epilepsy in childhood have a very favourable, long-term prognosis [6]. In that study, the remission rate was 92% in patients with idiopathic epilepsy. Patients were not subclassified, and for example, benign partial epilepsy with centro-temporal spikes with almost a 100% remission rate has a positive impact on the total outcome [7]. In our study, the remission rate, whether on- or off-medication, was 89.5% in CAE and 78% in JAE, followed for mean 10.5 years (2-22 y) and 15 years (3-53 y) respectively. A similar 91% seizure-freedom after 3.5 years follow-up was reported among patients who had only AS [8]. Wirrell et al. [9] report that 70% of patients diagnosed with absence epilepsy (CAE and JAE) were seizure-free ≥ 1 year with or without medication after a median 14 years’ follow-up. Based on the same study population and retrospective method, the group reports that 65% of CAE patients were in remission without AED after a mean 14 years’ follow-up [10]. Our result of 68.5%, after 10.5 years’ follow-up, compares well. Seizures in JME patients are more difficult to treat. Our remission rate obtained with conventional AED treatment was 38%, which is very similar to that reported in Cleveland and Engelsen’s study [11]. They report that 41% of JME patients had been seizure-free for more than one year. Others report a 50% remission after 1-9 years’ follow-up when conventional AED therapy was used [12]. Refractory seizures in patients with idiopathic epilepsy are a recognised problem [10, 13-16]. The occurrence of many seizures before initiation of therapy or an inadequate response to initial treatment with AED, predicted intractability in a prospective study by Kwan and Brodie [16]. A retrospective study focusing on children with absence epilepsy shows that a failure with the first AED predicts the risk of progress toward JME and intractable epilepsy [9]. We had one patient with onset AS at four years of age. She failed on the first medication, ESM, which is a drug of choice for AS in young children. Later she also failed on VPA. Near to puberty, she developed MS and GTCS. In our study, risk for intractability was a greater, especially if the first medication had been PHT, CBZ or PB. Only 5 out of 10 patients who had received PHT, CBZ or PB as a first medication achieved remission later (figure 2). They were all later treated with VPA, which is the drug of choice for JME and JAE. All responders with JME in our study received VPA monotherapy. Intractability among 26% of the patients with IGE has been reported [16]. Twenty percent of our IGE patients had intrac-
table seizures. We noticed that patients with different IGE syndromes seemed to have variable risks of developing intractable seizures. 2.5, 16.5 and 62% of patients with CAE, JAE and JME respectively had intractable epilepsy. Seven of the 11 intractable JAE and JME patients had been diagnosed with epilepsy between 1961 and 1975. The first medication in these patients was not VPA, as this has only been available in Finland since 1973. The knowledge of JME and its optimal treatment increased during the early 1980's [17]. All CAE patients were initially treated with either ESM or VPA. In the JAE group, 39% of the patients had received CBZ or PHT as the first drug. This might reflect that in older children AS were mixed with absence seizures originating from the temporal lobe. There are two other possible explanations for intractability other than non-optimal treatment. Non-compliance is a known reason [18]. The drug concentrations had been monitored in all patients, but unfortunately this data was not collected systematically for this survey. Genetic differences which affect the bioavailability of AEDs are a recently identified reason for intractability [19, 20].

None of the refractory CAE, JAE, JME or EMA patients had received lamotrigine (LTG). It has been reported to reduce or completely control seizures in IGE syndromes [21-23]. Since LTG has only been available in Finland since November 1995, this study population had not been treated with LTG. Polytherapy was required in 10.5 and 11% of patients with CAE and JAE, respectively, to achieve cessation of seizures, but none of the patients with JME reached freedom from seizures with polytherapy. The limited efficacy of polytherapy in general, has recently been discussed [16].

All intractable JAE patients had GTCS and rhythmic random eyelid closing. This phenomenon was clinically different from the eyelid myoclonus in EMA patients [24]. Unfortunately, no EMGs had been performed on these patients. In CAE patients, myoclonic manifestations were not associated with intractability [25].

We tried to use the frequency of AS as one criterion for distinguishing between CAE and JAE patients. Information was available in the medical records about the frequency of AS on a scale such as: “frequent/many, or some AS daily or AS seen almost every day or they are seen weekly”. Most CAE patients (95%) had frequent daily AS and most JAE patients (78%) had daily AS before the treatment. Other investigators have also reported daily AS in adolescent [26] and adult [27] patients with JAE. We followed the example of Loiseau et al. [28] who considered the age of nine years as the criterion for dividing subjects between CAE and JAE. Two separate peaks of the onset of epilepsy in CAE and JAE were evident in our study (figure 1), as shown also by Olsson [29] and Janz [30].

Over half (54%) of the patients with JME had a positive family history of epilepsy, which was less frequent in the families of the patients with CAE and JAE. Earlier studies report variable (25-65.9%) rates of positive family history [31-33]. We found that 10.5% of patients with CAE, as compared to 5 and 0% of patients with JAE and JME, had a personal history of FS. A positive family history of FS was reported in 10.5% of patients with CAE and in none of the families of patients with JAE or JME. Our finding is in agreement with that of Rocca et al. [34], who showed that FS is a risk factor for AS. FS was thought to represent an early manifestation of a convulsive diathesis or a manifestation of pre-existing brain dysfunction. Scheffler and Berkovic [35], who described the syndrome of generalised epilepsy with febrile seizures plus (GEFS+), have confirmed the first hypothesis through genetic studies. Gene mutations causing GEFS+ and CAE with FS have been identified in chromosomes 19, 2 and 5 [36-38]. We identified six (8%) patients with FS followed later by AS. Two of them also had family members, who had experienced FS. Two other families were identified where the proband had CAE and three to eight first-to-third degree relatives had experienced FS or generalised seizures. The phenotype in these families was less severe than the phenotype in Australian GEFS+ families [35]. The genetic origin of idiopathic epilepsy and FS in two of these four families was confirmed by linkage analysis on chromosome 5 [39].

Because of its retrospective nature, our study has limitations, firstly, in data collection. Medical records contain information about the diagnosing process, treatment and follow-up, but this documentation is not primarily aimed at scientific research and it does not necessarily contain the details researchers would wish to find after several years. For example, information about the frequency of AS or seizure-provocative factors was, in many cases, insufficient. Secondly, we reviewed almost 20000 EEG reports and the patients were identified according to these reports. We did not review the original EEG recordings. It is possible that a detailed analysis of EEG might have provided information that could have modified the diagnosis in some patients. Despite the limitations described above, we believe that the data collection is comparable to other studies performed earlier [8-10, 15, 29]. We interviewed most of the patients personally and one relative of one third of the patients. We obtained accurate information about the outcome, and the long-term follow-up allowed us to collect prevalence data of epilepsy in other family members.

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Suggestive evidence for a new locus for epilepsy with heterogeneous phenotypes on chromosome 17q

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Introduction

Childhood absence epilepsy (CAE) is a classical subtype of idiopathic generalized epilepsy (IGE), and febrile seizures (FS) is a common seizure type in relatives of CAE probands (Italian League Against Epilepsy Genetic Collaborative Group, 1993). While both CAE and FS have a polygenic basis in most patients (Andermann and Metrakos, 1972; Doose et al., 1983) mutations of major effect have been reported. Mutations in GABRG2, GABRB3, CLCN2 and CACNA1H have been described in rare families with CAE and FS or other generalized epilepsy phenotypes (Wallace et al., 2001; Kananura et al., 2002; Marini et al., 2003; Haug et al., 2003; Chen et al., 2003; Heron et al., 2007; Tanaka et al., 2008). De novo mutations in JRK/JH8 and GABRA1 have been reported in two patients with CAE (Moore et al., 2001; Maljevic et al., 2006).

Currently, ten loci for familial FS are known (Wallace et al., 2001; Nakayama and Arinami, 2006; Hedera et al., 2006; Nabbout et al., 2007; Dai et al., 2008). Heterozygous mutations in the SCN1A and GABRG2 genes have been identified in two families with phenotypes varying from simple to complex FS (Mantegazza et al., 2005; Audenaert et al., 2006). The generalized epilepsy febrile seizure plus syndrome (GEFS+) is the first epilepsy syndrome connected to these same genes (Helbig et al., 2008). Recently, mutations in the human seizure-related 6 gene, SEZ-6, were reported in patients with complex FS (Yu et al., 2007).

In this paper we describe the clinical and genetic features in a Finnish family in which the proband was diagnosed with CAE. Four other family members had FS. Additionally two family members experienced episodes resembling aura originating from temporal lobe. We present data suggesting a novel locus for seizures on 17q12-q24, the underlying mutation remaining unknown. Our data support the existence of the previously implied modifier locus for seizures on 18p11-q11 and another on 5q11.2.

Methods

Family ascertainment and diagnostic classification

A non-consanguineous Finnish family (Fig. 1) was identified through a proband with CAE ascertained in an earlier study (Sirén et al., 2002). We interviewed the patients and their family members, collected clinical information and EEG reports from patient records and performed sleep deprivation EEG on nine individuals. In order to evaluate the long-term outcome a follow-up telephone interview was performed 10 y after initial data collection. Individuals with suspected focal seizures were interviewed by using a seizure questionnaire (Reutens et al., 1992). Unaffected spouses were interviewed in order to identify possible bilinear inheritance and gather eye witnesses’ descriptions of suspected focal seizures.

Epileptic seizures and syndromes were classified according to the International League Against Epilepsy criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981; Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Individuals were considered affected in linkage calculation Scheme 1, if they had a diagnosis of epilepsy, if they had a medical record of FS or if at least one close relative confirmed that they had an episode compatible with FS. In linkage calculation Scheme 2 family members who described episodes compatible with aura originating from temporal lobe were also considered affected. A written informed consent was obtained from 25 family members, nine of whom were affected. Venous blood samples were collected from 25 individuals to extract DNA and/or RNA. The Ethics Committee of Tampere University Hospital, Tampere, Finland and the Ethics Committee of the Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland approved the study protocol.
Suggestive evidence for a new locus for epilepsy with heterogeneous phenotypes on chromosome 17q 67

Figure 1  Haplotype reconstruction on chromosome 17q12-q24. Haplotypes for alleles at 22 microsatellite markers are shown under each individual. The disease-associated haplotype is boxed. Recombinations between D17S1818 and D17S1814 in individual 10 and between D17S840 and D17S515 in individual 8 define the minimum critical region. Symbols with a black corner correspond to affected and white symbols to unaffected individuals. The seizure types are indicated on the figure. A question mark (?) inside the individual’s symbol denotes an unknown affectedness status. The proband is marked with an arrow. The black bar inside the marker map defines the area with the location score of 2.7.

Genotyping

A genome-wide scan (GWS) was performed on 19 individuals using 366 autosomal polymorphic DNA markers from Weber set 9 (Research Genetics Inc., Huntsville, AL, USA) amplified in standard conditions using Red Hot™ DNA polymerase (ABgene, Epsom, UK). Gel electrophoresis and genotype analysis were done with a dual laser automated fluorescent DNA sequencer (LI-COR 4200) and fragment analysis with Gene ImagIR 4.03 (LI-COR Inc., Lincoln, NE, USA). Regions with a multipoint location score ≥1.5
were fine-mapped using markers from www.ncbi.nlm.nih.gov/projects/genome/guide/human with AmpliTaq Gold (PerkinElmer Inc., Waltham, MA, USA) reagents, an automated DNA sequencer (ABI 3730xl DNA Analyzer) and Genemapper 4.0 genotyping software (Applied Biosystems, Foster City, CA, USA).

Linkage analysis

Multipoint location scores after GWS were calculated with SimWalk2 version 2.91 (Sobel and Lange, 1996). The heterogeneous seizure phenotype was analyzed as an autosomal dominant trait with incomplete disease penetrance of 70%, estimated based on the observed inheritance in the family. The phenocopy rate was defined as 3%, since the frequency of FS in general population ranges from 2 to 5%. The disease gene frequency was set at 0.001. The recombination fraction was considered to be equal in males and females. Allele frequencies were extracted from the family data. Two-point LOD scores were calculated with program ANALYZE (Hiekkalinna et al., 2005) for fine-mapped loci with a multipoint location score \( \geq 1.5 \). Family members 1, 2 and 5 (Fig. 1) were considered unknown in linkage calculations because no definitive clinical data were available. The expected maximum two-point LOD score in the family, calculated for the same model parameters using a simulation program for linkage analysis (SLINK; Ott, 1989) was 3.0 (calculation Scheme 1) and 3.2 (Scheme 2) for multiallelic markers. Haplotypes were constructed by Simwalk2.

Mutation screening

Nine genes (Table 1) encoding brain-expressed ion-channel proteins were sequenced from the 17q12-q24 region. In addition, RPP8 and SLC25A9, identified as candidates from the microarray-based expression analysis (see Section "mRNA expression profiling") and residing on 17q12-q24 and IMPA2 from 18p11-q11 were sequenced. For sequencing at least two patients and one unrelated Finnish control were used. Alterations identified only in patient samples were analyzed in all family members. All family members were also screened for the triplet repeat variation near the transcription start point of the carbonic anhydrase X (CA10) gene, expansion of which has previously been implied as a candidate for neurological disorders (Kleiderlein et al., 1998).

Exons and exon—intron boundaries were sequenced from genomic DNA extracted with standard methods. Exon—intron structures were determined from the ENSEMBL database (http://www.ensembl.org/index.html) and primers were designed using exonPrimer (http://lgh.gsf.de/lgh/ExonPrimer.html) and Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) programs. Primer sequences are available upon request. PCR was performed in standard conditions using AmpliTaq Gold (PerkinElmer, Waltham, MA, USA) reagents. PCR products were purified and sequenced using ExoSAP-IT (USB, Cleveland, OH, USA), BigDye® Terminator v3.1 Cycle Sequencing Kit and an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed with Sequencher program (Gene Codes, Ann Arbor, MI, USA). Identified variants were compared to SNP database (http://www.ncbi.nlm.nih.gov/SNP/) using SNP BLAST. The effect of coding SNPs was predicted by SNPs3D software (www.snp3d.org). The effect of intronic SNPs was predicted with ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi).

Array-based comparative genomic hybridization

Array-based comparative genomic hybridization was performed in patient 8 (Fig. 1) by GeneDx inc. using GenomeDx microarray v3.0 containing \( \sim 105,000 \) oligonucleotide probes.

mRNA expression profiling

Microarray-based mRNA expression profiling was performed in three affected (10, 12, and 14) and three unaffected (23, 24, and 25) age- and gender-matched family members in order to identify differently expressed genes in the chromosome 17q12-q24 region. Total RNA was extracted from whole blood with PAXgene Blood RNA Kit (Qia-gen Finland, Helsinki, Finland). The microarray experiments were performed as described earlier (Laaksonen et al., 2006) by using Sentrix® Human-6 Expression BeadChips (illumina, San Diego, CA, USA). The gene expression microarray data were analyzed using R [1] and bioconductor softwares (Gentleman et al., 2004). After quality inspections the data were quantile normalized. The statistical analyses were carried out using Limma package (Smyth, 2004). The differentially expressed genes in each comparison were selected requiring \( |\log 2 \text{ fold-change}| >0.5 \) (fold-change 1.41) and \( p\)-value <0.05.

Results

Phenotypes

The proband (individual 12, Fig. 1) had CAE with typical absence seizures and bilateral, generalized 3 Hz spike and wave discharges in her EEG. Three patients (10, 11, and 14) experienced simple FS only and two patients (8 and 15) complex FS (Table 2).

Two individuals (4 and 6) spontaneously described having experienced episodes of déjà vu (4) and increased sensitivity to sensation and hearing (6). In individual 4, déjà vu symptoms started without warning and disappeared after a brief duration. She experienced a feeling of familiarity: "I have seen this before. I have experienced this before. I know what will happen next. I have been here before although I visit this place first time in my life."

In individual 6, the sensory episodes tended to appear when he was relaxing. They always consisted of a strange taste in his mouth and he had an odd feeling in his tongue. He felt and heard his heart beating strongly. He heard the noise of circulation and the wall clock ticking loudly. The sensations were more acute throughout his body. The whole event normally lasted 2—3 min, but could occasionally last longer. In both individuals (4 and 6) the episodes occurred since the early teens repeatedly once in one to two months. In adulthood the episodes became more infrequent occurring once or twice a year. Later, the episodes appeared very rarely. No eye witness reports were available.

Individual 2 reported of an un witnessed episode of unconsciousness at the age of 22 y. Furthermore, his spouse described of repeated episodes of unresponsiveness, paleness and nausea after his thirties. The duration and frequency remained unclear. Individual 2 did not recall these episodes. We considered individual 2 unknown for linkage analysis because the data were indeterminate.

None of the spouses had a history of seizures. Sleep deprivation EEGs performed on nine individuals were normal (Table 2).

Linkage analysis

The seizure phenotype in the family showed a dominant inheritance pattern with reduced penetrance. In the
Table 1  Sequenced candidate genes on 17q12-q24 and 18p11-18q11.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Chromosome</th>
<th>Protein name</th>
<th>Expression and function</th>
<th>OMIMA</th>
<th>Accessionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACNB1</td>
<td>17</td>
<td>Calcium channel, voltage dependent, beta 1 subunit</td>
<td>Brain and skeletal muscle isoforms. Able to regulate CACNA1A function.</td>
<td>114207</td>
<td>NM_000723.3, NM_199247.1</td>
</tr>
<tr>
<td>KCNH4</td>
<td>17</td>
<td>Potassium voltage gated channel, subfamily H (eag-related), member 4</td>
<td>Expressed only in brain. May be involved in cellular excitability of neurons in the human central nervous system.</td>
<td>604528</td>
<td>NM_012285.1, NM_198376.1, NM_938406.1</td>
</tr>
<tr>
<td>CACNA1G</td>
<td>17</td>
<td>Calcium channel, voltage dependent, alfa 1G subunit</td>
<td>Expressed dominantly in brain. Modulates generation of GABA-B-receptor mediated spike and wave discharges in the thalamocortical pathway.</td>
<td>604065</td>
<td>NM_061896.3, NM_198376.1, NM_938406.1</td>
</tr>
<tr>
<td>KCNH6</td>
<td>17</td>
<td>Potassium voltage gated channel, subfamily H (eag-related), member 6</td>
<td>Expressed in the brain. May differentially control the firing of neurons engaged in several networks.</td>
<td>608168</td>
<td>NM_030779.2, NM_173092.1</td>
</tr>
<tr>
<td>CACNG5</td>
<td>17</td>
<td>Calcium channel, voltage dependent, gamma 5 subunit</td>
<td>Expression in adult and fetal brain. May regulate function of other voltage dependent calcium channels.</td>
<td>606405</td>
<td>NM_014404.1, NM_145811.1</td>
</tr>
<tr>
<td>CACNG4</td>
<td>17</td>
<td>Calcium channel, voltage dependent, gamma 4 subunit</td>
<td>Expressed in brain. Mice with mutations in Cacng4 have increased seizure activity.</td>
<td>606404</td>
<td>NM_014405.2</td>
</tr>
<tr>
<td>CACNG1</td>
<td>17</td>
<td>Calcium channel, voltage dependent, gamma 1 subunit</td>
<td>Expressed in fetal and adult brain. Integral membrane protein.</td>
<td>114209</td>
<td>NM_000727.2</td>
</tr>
<tr>
<td>KCNJ16</td>
<td>17</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 16</td>
<td>Expressed in brain. Forms a functional brain potassium channel by interacting with PSD95. Controls negatively KCNJ2 channel activity.</td>
<td>605722</td>
<td>NM_018658.1</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>17</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 2</td>
<td>Expressed in brain. Forms a functional brain potassium channel.</td>
<td>600681</td>
<td>NM_000891.2</td>
</tr>
<tr>
<td>RPIP8</td>
<td>17</td>
<td>Rap2-interacting protein</td>
<td>Expressed in brain. Interacting with RAP2A which is expressed in excitatory synapses.</td>
<td>605448</td>
<td>NM_006695.3</td>
</tr>
<tr>
<td>SLC25A39</td>
<td>17</td>
<td>Solute carrier family 25, member 39</td>
<td>Mitochondrial carrier protein. Brain-expressed SLC25 members are important for neurons in energy production and neuronal signaling. No studies of tissue expression on member 39.</td>
<td>610820</td>
<td>NM_016016</td>
</tr>
<tr>
<td>IMPA2</td>
<td>18</td>
<td>Myo-inositol monophosphatase-2</td>
<td>Expressed in brain. Possible SNP association with febrile seizures.</td>
<td>605922</td>
<td>NM_014214.1</td>
</tr>
</tbody>
</table>

Table 2  Summary of clinical characteristics and EEG findings of family members.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/gender</th>
<th>Febrile seizures</th>
<th>Duration/min</th>
<th>Epilepsy phenotyping</th>
<th>Seizure frequency</th>
<th>EEG</th>
<th>Epilepsy syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Onset/end</td>
<td></td>
<td>Onset</td>
<td>Ictal phenomenology/duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>76/f</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Undefined</td>
</tr>
<tr>
<td>2</td>
<td>74/m</td>
<td>ND</td>
<td>~40 y</td>
<td>Unresponsive, motionless, staring and pale/N</td>
<td>ND</td>
<td>ND</td>
<td>Undefined</td>
</tr>
<tr>
<td>3</td>
<td>68/f</td>
<td>0</td>
<td></td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>Undefined</td>
</tr>
<tr>
<td>4</td>
<td>51/f</td>
<td>0</td>
<td>Teenage</td>
<td>Délavu/1/2 min</td>
<td>Several times yearly until ~25 y, since then twice a year</td>
<td>ND</td>
<td>Possible benign TLE</td>
</tr>
<tr>
<td>5</td>
<td>49/m</td>
<td>ND</td>
<td>Teenage</td>
<td>Strange taste, increased sensation in his tongue, auditory and haptic sense, feeling of time passing fast/1–2 min</td>
<td>Once in a month until mid twenties, later once a year</td>
<td>Normal</td>
<td>Possible benign TLE</td>
</tr>
<tr>
<td>6</td>
<td>45/m</td>
<td>0</td>
<td>Teenage</td>
<td>Impairment of consciousness</td>
<td>Daily frequent 3 Hz generalized spike-wave discharges</td>
<td>ND</td>
<td>Complex FS</td>
</tr>
<tr>
<td>7</td>
<td>45/f</td>
<td>0</td>
<td>10 mo</td>
<td>&gt;30</td>
<td>—</td>
<td>Normal</td>
<td>Complex FS</td>
</tr>
<tr>
<td>8</td>
<td>37/m</td>
<td>1</td>
<td>18 mo</td>
<td>&lt;1</td>
<td>—</td>
<td>ND</td>
<td>Simple FS</td>
</tr>
<tr>
<td>9</td>
<td>39/f</td>
<td>0</td>
<td>16 mo</td>
<td>&lt;1</td>
<td>—</td>
<td>ND</td>
<td>Simple FS</td>
</tr>
<tr>
<td>10</td>
<td>4/f</td>
<td>1</td>
<td>8 y</td>
<td>Impairment of consciousness</td>
<td>Daily frequent 3 Hz generalized spike-wave discharges</td>
<td>ND</td>
<td>Complex FS</td>
</tr>
<tr>
<td>11</td>
<td>18/m</td>
<td>1</td>
<td>5–10</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple FS</td>
</tr>
<tr>
<td>12</td>
<td>17/f</td>
<td>0</td>
<td>5–10</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>13</td>
<td>13/m</td>
<td>0</td>
<td>5–10</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>14</td>
<td>9/f</td>
<td>3</td>
<td>5–10</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>15</td>
<td>7/m</td>
<td>3</td>
<td>2 to &gt;30</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>16</td>
<td>5/m</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>17</td>
<td>68/f</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>18</td>
<td>61/m</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
<tr>
<td>19</td>
<td>43/f</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Normal</td>
<td>Simple and complex FS</td>
</tr>
</tbody>
</table>

ND, no data; CAE, childhood absence epilepsy; TLE, temporal lobe epilepsy. Unaffected family members 23, 24 and 25 whose samples were used for mRNA expression profiling are not included in the table.
Table 3  Maximum two-point LOD score values in parametric linkage analysis of the 17q12-q24 region.

<table>
<thead>
<tr>
<th>Name</th>
<th>LOD score</th>
<th>Theta(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D17S1294</td>
<td>0.565712</td>
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\(^a\) Theta, recombination fraction.

GWS, with linkage calculation Scheme 1, multipoint location scores \(\geq 1.5\) were obtained on 5p13.1-q13, 17q12-q24, 18p11-q11 and 18q21. With linkage calculation Scheme 2 the loci on chromosomes 17q12-q24, 18p11-q11 and 18q21 reached a location score of \(\geq 1.5\). After fine mapping all four loci by genotyping 4–16 additional markers the chromosome 18q21 was excluded.

In the GWS, the highest multipoint location scores of 2.5 and 2.7 were obtained between markers D17S1299 and D17S2193 with calculation Schemes 1 and 2, respectively. Fine mapping with 16 microsatellite markers defined an approximately 37 cM (\(\sim\)34 Mb) region by recombinations between markers D17S1818 and D17S1814 in patient 10 and between markers D17S840 and D17S515 in patient 8 (Figs. 1 and 2). The highest two-point LOD scores were 2.6 and 2.8 at marker D17S1660 (at recombination fraction, theta 0.0) with Schemes 1 and 2, respectively (Table 3). All affected individuals carry the disease haplotype. Individuals 1 and 2 who were obligate carriers, but who were considered unknown in linkage analysis, and one unaffected individual (16), are carrying the disease haplotype.

In the GWS, a multipoint location score of 1.7 with calculation Scheme 1 was obtained on chromosome 5p13.1-q13. After fine mapping with twelve markers the region of interest was 4 cM (\(\sim\)2.5 Mb) defined by recombinations between D5S623 and D5S628 in patient 14 and between markers D5S2076 and D5S664 in patient 10 (Supplemental data: Fig. S1). The haplotype analysis revealed that all affected individuals, three labeled unknown (1, 2, and 5) and one unaffected individual (21) carried the disease-associated haplotype (Fig. 2). The highest two-point LOD scores were 2.0 and 2.1 at two markers D5S628 and D5S2076 at theta 0.0 with calculation Schemes 1 and 2, respectively (data not shown).

The region on chromosome 18p11-q11 showed location scores of \(\geq 1.5\) in the GWS and after fine mapping with ten markers. The region of interest on was 24 cM (\(\sim\)14 Mb) defined by recombinations between D18S843 and D18S464 in patients 11 and 12 and between markers D18S877 and D18S1149 and D18S877 in patient 10 (Supplemental data: Fig. S2). The hap-
lotype analysis revealed that all affected individuals, three labeled unknown (1, 2, and 5) and four unaffected individuals (16, 18, 19, and 21) carried the disease-associated haplotype (Fig. 2). The highest two-point LOD scores calculated with Schemes 1 and 2 were 1.3 and 1.5 for $D18S1153$ at theta 0.0 (data not shown).

**Mutation screening**

Nine ion-channel genes expressed in the brain were sequenced from the chromosome 17q12-q24 region (Table 1). In addition, we selected two genes, RPIP8 and SLC25A39 from six significantly up- or down-regulated genes identified with microarray-based gene expression profiling for sequence analysis from the same region. The choice of the genes was based on known gene characteristics (Table 4).

All 126 coding exons of the selected 11 genes on were sequenced. We detected 53 polymorphic variants (Supplemental data Table S1). Of these, 43 matched sequences in the reference SNP database (http://www.ncbi.nlm.nih.gov/SNP/). Five variants were identified only in the control sample sequenced. Five variants were identified only in the patient samples screened and therefore the whole family was analyzed for the presence of the alteration by sequencing. None of the variants were confirmed to be disease-associated.

The size of the triplet repeat region in CA10 was found to be 127 bp in all family members, corresponding to the reported normal variation (127—133 bp) (Kleiderlein et al., 1998).

Three SNPs were detected in the IMPA2 gene on the 18p11-q11 locus. One SNP co-segregated with the disease-associated haplotype, but was also found in unaffected family members and spouses.

**Array-based comparative genomic hybridization**

In array-based comparative genomic hybridization no clinically relevant copy number variations were identified in the genome and no abnormalities in the four regions of interest (average probe resolution in the analysis 37 kb).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Differently expressed genes on the chromosome 17q12-q24 region with &gt;1.41 fold-change and $p$-value &lt;0.05.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbol</td>
<td>Protein name</td>
</tr>
<tr>
<td>MKS1</td>
<td>Meckel syndrome, type 1</td>
</tr>
<tr>
<td>RPIP8</td>
<td>RAP2-interacting protein 8</td>
</tr>
<tr>
<td>SLC25A39</td>
<td>Solute carrier family 25, member 39</td>
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<tr>
<td>PHOSPHO1</td>
<td>Phosphatase, orphan 1</td>
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<tr>
<td>MMD</td>
<td>Monocyte to macrophage differentiation associated protein</td>
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<td>HOXB2</td>
<td>Homeobox-B2</td>
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</table>


$^b$ GENEID, the GeneID number (www.ncbi.nlm.nih.gov/sites/entrez?Db=gene) A literature reference is given in parenthesis, when data on expression and function was not obtained solely from OMIM or GENEID databases.

Suggestive evidence for a new locus for epilepsy with heterogeneous phenotypes on chromosome 17q 73

Discussion

We describe a family with heterogeneous phenotypes including FS, CAE, and possible TLE, where linkage analysis suggests the presence of an underlying gene on chromosome 17q12-q24 with two possible modifier loci on chromosomes 5 and 18.

The affected members of the family presented a benign epilepsy phenotype. Most of the affected individuals had FS, while one family member only had CAE. Two individuals (4 and 6) had phenotypes which can be normal physiologically phenomena or manifestations of benign temporal lobe epilepsy (TLE). We considered their symptoms compatible but not diagnostic to focal seizures originating from temporal lobe in calculation Scheme 1, but diagnostic in Scheme 2. Patients with focal seizures only have been described in families with the majority of patients having IGE or FS and in the GEFS+ families (Abou-Khalil et al., 2001; Marini et al., 2004). In benign familial TLE, simple focal seizures can consist of psychic (e.g., déjà vu), special sensory (e.g., somatosensory, gustatory, auditory and visual) or autonomic symptoms. Among family members with benign familial TLE, mild symptoms were often ignored and regarded as normal phenomena (Berkovic et al., 1996).

The GWS results in the family suggested the presence of a novel susceptibility locus on chromosome 17q12-q24. The obtained two-point LOD score of 2.8 is near the maximum achievable in this family.

Even after refined mapping, the region of interest remained large and contains over 500 genes. Among these, we chose for sequencing the nine ion-channel encoding genes, which are expressed in the brain. Although a significant number of sequence alterations were identified in these genes none of them fulfilled the criteria for a possible causative mutation. It remains possible that such an alteration exists in a gene region that was not covered by our mutation screening strategy, or in one of the other genes which were not tested.

As the identified locus on chromosome 17 contained a high number of genes, we performed mRNA expression profiling in order to illuminate additional potential candidate genes for further sequence analysis. Whole blood represents an alternative tissue for neurogenetic research given the non-accessibility of patient brain tissue for gene expression analysis. Correlation of gene expression in blood and certain brain regions has previously been shown for several genes expressed in both tissues (Sullivan et al., 2006). Based on the array results and in silico analyses we selected brain-expressed genes RPIP8 and SLC25A39 for sequencing. However, no disease-associated variant was identified. Of the four remaining genes that showed altered expression in patients, two were not obvious candidates for FS and epilepsy as they are expressed either in mineralizing cells (PHOSPHO1) or participate in macrophage maturation (MMD). In the light of a recent report in which dysregulation of an early developmental gene, early growth response 1 (EGR1), was implicated in idiopathic absence epilepsy (Helbig et al., 2008b), the two other genes HOXB2 and MKS1 might be interesting candidate genes as they participate in early brain development (Geisen et al., 2008; Kyttila et al., 2006). We tested the three affected individuals (10, 12, and 14) participating in the microarray study for the Finnish MKS1 founder mutation and found them to be negative (data not shown). The use of mRNA profiling from blood as a surrogate for other tissues has limitations. The chip used contained 1500 different chromosome 17 transcripts of which more than one third, including the nine sequenced ion-channel genes, had a fairly low expression level in blood. Neuronal ion-channel genes related to epilepsy in humans may have much higher expressions in brain tissue and, thus, differences in expression levels can not be detected by using blood cell mRNA as a surrogate marker (Helbig et al., 2008b).

Chromosome 17q is enriched in segmental duplications which can potentially lead to the loss or gain of a dosage-sensitive gene or to disruption of a gene or its regulatory elements (Zody et al., 2006). Therefore, an array-based comparative genomic hybridization was performed on one individual. However, no copy number variation was found in the linked locus on 17q12-q24 or elsewhere in the genome.

Two other regions showed positive linkage evidence even after fine mapping, the LOD scores, however, remaining much lower than on the chromosome 17q region. Of these the region on chromosome 5q has not previously been implicated in epilepsy. On the contrary, linkage of FS to chromosome 18p11 with an association of a common haplotype in the IMPA2 gene to FS has been described in Japanese families (Nakayama et al., 2004). Moreover, a possible modifier locus on 18p, including IMPA2 was recently reported for FS and CAE (Nabbout et al., 2007). This region also showed positive linkage evidence in our study and could not be excluded after fine mapping. Neither our study, nor that of Nabbout et al. (2007), however, observed a mutation or co-segregation of the IMPA2 SNP haplotype with FS.

Interestingly, combined analysis of disease-associated haplotypes covering the three regions with positive linkage evidence (Fig. 2) is compatible with polygenic inheritance in the family, despite the apparently autosomal dominant inheritance pattern. Contribution for all three loci seems to be necessary for the clinical seizure manifestation irrespective of the seizure phenotype. The clinical variability in seizure phenotypes and absence of overt seizures in the two obligate carriers imply the contribution from yet other, genetic and/or environmental factors for the clinical outcome. Identification of more families with similar benign phenotypes that are linked to the susceptibility loci identified here are needed to reveal the underlying genetic alterations giving rise to the increased seizure susceptibility.

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Appendix A. Supplementary data


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