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ORIGINAL INVESTIGATION

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Genome-wide linkage of febrile seizures and epilepsy to the FEB4 locus at 5q14.3-q23.1 and no *MASS1* mutation

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Abstract Febrile seizures (FS) represent the most common seizure disorder in childhood and contribution of a genetic predisposition has been clearly proven. In some families FS is associated with a wide variety of afebrile seizures. Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome with a spectrum of phenotypes including FS, atypical febrile seizures (FS+) and afebrile generalized and partial seizures. Mutations in the genes *SCN1B*, *SCN1A* and *GABRG2* were identified in GEFS+ families. GEFS+ is genetically heterogeneous and mutations in these three genes were detected in only a minority of the families. We performed a 10 cM density genome-wide scan in a multigenerational family with febrile seizures and epilepsy and obtained a maximal multipoint LOD score of 3.12 with markers on chromosome 5q14.3-q23.1. Fine mapping and segregation analysis defined a genetic interval of ≈33 cM between D5S2103 and D5S1975. This candidate region overlapped with a previously reported locus for febrile seizures (FEB4) in the Japanese population, in which *MASS1* was proposed as disease gene. Mutation analysis of the exons and exon–intron boundaries of *MASS1* in our family did not reveal a disease causing mutation. Our linkage data confirm for the first time that a locus on chromosome 5q14-q23 plays a role in idiopathic epilepsies. However, our mutation data is negative and do not support a role for *MASS1* suggesting that another gene within or near the FEB4 locus might exist.

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Introduction

Febrile seizures (FS) is the most common seizure disorder in childhood, affecting 2–5% of children under the age of 5 years in Caucasian populations (Hauser et al. 1996). The contribution of a genetic predisposition has been clearly documented by twin and family studies (Tsuboi and Endo 1991). Segregation analysis suggested a polygenic model in families of probands with a single febrile seizure, while in families with repeated episodes of FS, inheritance best fitted autosomal dominance with reduced disease penetrance (Rich et al. 1987; Johnson et al. 1996). Linkage analysis studies identified five chromosomal loci for FS: FEB1 at 8q13-q21, FEB2 at 19p, FEB3 at 2q23-q24, FEB4 at 5q14-q15 and FEB5 at 6q22-q24 (Wallace et al. 1996; Johnson et al. 1998; Kugler et al. 1998; Peiffer et al. 1999; Nakayama et al. 2000; Nabbout et al. 2002). In some families, FS is present as part of a broader phenotypic spectrum that also includes atypical febrile seizures (FS+) and afebrile generalized and partial seizures i.e. generalized epilepsy with febrile seizures plus (GEFS+) (Scheffer and Berkovic 1997). In GEFS+ families, mutations were identified in *SCN1B* on chromosome 19p13.1 (GEFS+1), *SCN1A* on chromosome 2q24 (GEFS+2) and *GABRG2* on chromosome 5q31-q33 (GEFS+3) (Wallace et al. 1998; Escayg et al. 2000; Baulac et al. 2001; Wallace et al. 2001). The disease phenotype within the GEFS+ families with identified mutations segregated in an

autosomal dominant pattern with reduced disease penetrance. Mutations in these three genes were found in several GEFS+ families, but in most families the causal gene has not yet been identified (Bonanni et al. 2004). In a large family with FS and familial temporal lobe epilepsy (TLE) a locus was found on chromosome 12q22-q23.3 (Claes et al. 2004).

Here, we report the results of a genome-wide scan in an extended Belgian–Dutch family with febrile seizures and epilepsy showing conclusive linkage with chromosome 5q14.3-q23.1 in a region that overlapped with the FEB4 locus previously reported in the Japanese population (Nakayama et al. 2000). The Monogenic Audiogenic Seizure Susceptibility 1 gene (*MASS1*) was suggested as the FEB4 gene, and is an alternative transcript (exons 5–39) of the larger Very Large G Protein-coupled Receptor 1 gene (*VLGR1*) (Nakayama et al. 2002; McMillan et al. 2002). Mutation analysis of all 90 exons and exon–intron boundaries of *VLGR1* in the linked Belgian–Dutch family did not reveal a disease causing mutation.

Patients and methods

Ascertainment and diagnosis of patients

We ascertained in Flanders, the Dutch-speaking region of Belgium (Belgian–Dutch) an extended family, EP122, with six patients in three successive generations for genome-wide linkage analyses (Fig. 2). Clinical information of the patients was obtained using a structured interview and/or medical records. Parents or older sibs were also interviewed to check the clinical information of the patients. The questions regarding epilepsy and febrile seizures can been found at the website http://www.molgen.ua.ac.be. Patients were diagnosed according to criteria of the commission on classification and terminology of the ILAE (Engel 2001). We summarized the clinical data of the patients in Table 1. Four patients were diagnosed with classical FS; none of them developed epilepsy later in life. The number of FS varied from 1 to 5 and the individual seizures were separated by more than 24 h. Two patients experienced unprovoked afebrile seizures without a history of febrile seizures. The epileptic phenotypes of both patients differ. Patient III-4 had complex partial seizures probably of temporal lobe origin. Seizures started shortly after birth and were controlled with anti-epileptic drugs. Patient III-11 experienced generalized tonic–clonic seizures and absences from the age of 3 years until 15 years of age. None of the family members had symptomatic epilepsy. In total DNA samples from 32 family members were obtained. Analysis of the segregation pattern supported an autosomal dominant inheritance, while the presence of three obligate carriers suggested a reduced disease penetrance.

We also analyzed 48 nuclear Belgian–Dutch families with FS only or FS associated with epilepsy. The families were selected through their proband, who had experienced one or more FS before the age of five years. Clinical information of each proband about frequency of seizures and association with fever was obtained from the parents, and probands were diagnosed according to the same criteria of the commission on classification and terminology of the ILAE (Engel 2001). In case of a positive family history, additional patients and relatives were collected for genetic studies. Additional patients were considered affected if they had at least one febrile and/or unprovoked afebrile seizure. Patients with symptomatic epilepsy were not included in this study. In total we obtained DNA samples of 333 family members, of whom 169 were patients (Table 2). In all probands mutations in *SCN1A*, *SCN1B* and *GABRG2* were excluded by sequence analysis of the exons and the intron–exon boundaries of genomic DNA (Audenaert et al., unpublished data). The Medical Ethical Committee of the University of Antwerp approved the study, and participants or their legal representatives signed informed consent.

Table 1 Clinical data of patients in family EP122

Patient	Age (year)	FS			AFS					AED treatment
		No. of FS	Age at FS onset (mo)	Age at last FS (mo)	AFS	Seizure type	Frequency of AFS	Age at AFS onset (mo)	Age at last AFS (yr)	
I-2	75	3	~ 24	~ 24	No	–	–	–	–	No
II-2	51	1	12	12	No	–	–	–	–	No
III-4	22	None	–	–	Yes	CPE	Numerous before medication, 1×/year with medication	< 1	22	Yes
III-10	19	1	13	13	No	–	–	–	–	No
III-11	17	None	–	–	Yes	GTCS, absences	2× GTCS, absences 1×/week	36	15	Yes (3–15 yr)
III-14	11	5	12	48	No	–	–	–	–	Yes (3–8 yr)

FS Febrile seizures, *AFS* unprovoked afebrile seizures, *GTCS* generalized tonic–clonic seizures, *CPE* complex partial epilepsy, *mo* months, *yr* years; *AED* antiepileptic drugs

Table 2 Characteristics of the 48 families with FS only or FS associated with epilepsy

Family structure	No. of families	No. of individuals	No. of patients	Phenotype of patients		
				FS	FS + AFS	AFS
≥3-generation	34	278	138	115	9	14
2-generation (≥2 affected offspring)	5	23	13	8	2	3
2-generation (1 affected offspring)	9	32	18	16	2	0

FS Febrile seizures, *AFS* unprovoked afebrile seizures

Genotyping

Family members of EP122 were genotyped for 382 autosomal microsatellite markers of the ABI Prism Linkage Mapping Set MD-10 (Applied Biosystems, Foster City, CA, USA) with an average marker distance of 10 cM. After amplification and pooling of the PCR products an ABI3700 automated sequencer (Applied Biosystems) was used for fragment length analysis. Data were analyzed using ABI Genescan 3.6 and ABI Genotyper 3.7 software. Eleven additional microsatellite markers in the candidate region between D5S647 and D5S2115 were selected from the Marshfield comprehensive genetic map (http://research.marshfieldclinic.org/genetics). Primer pairs for each marker were designed with a proprietary algorithm implemented in a PCR multiplexer program (Goossens et al., unpublished data). This resulted in two pools containing four and seven primer pairs that were each amplified in a single tube reaction without extensive optimization of PCR conditions.

Statistical analysis

Before conducting the genome-wide scan the genetic information content of the families was estimated with the software program S-link (Ott 1989; Weeks et al. 1990). All individuals with one or more febrile or unprovoked epileptic seizures were considered affected. We assumed an autosomal dominant inheritance pattern with reduced penetrance. The disease penetrance was set at 60% based upon the penetrance rates in GEFS + families reported in the literature (Singh et al. 1999) and we used an estimated disease gene frequency of 0.001. The simulations were calculated for a marker with eight equifrequent alleles at recombination fraction (θ) 0.05 of the disease locus. For family EP122 an average maximum logarithm of odds (LOD) score Z of 1.85 and a maximum achievable LOD score of 3.67 were generated over 250 replicates. We obtained a significant maximum achievable LOD score (>3) in only one (with nine affected individuals) of the 48 additional families with FS and epilepsy. The remaining families were smaller (maximum number of patients $=6$) and did not yield a maximum achievable LOD score >3. Nine families, of which only a trio was available for genetic testing, were ommitted from the linkage calculations and were only used for transmission disequilibrium testing (TDT). Parametric linkage analyses were performed in 39 families with the MLINK program for two-point linkage analysis and the LINKMAP program for multipoint linkage analysis, both from the FASTLINK computer package (version 5.1) (Cottingham et al. 1993). LOD scores were calculated with the same genetic model used for the simulation study. The marker map used for the multipoint analysis was based on the Marshfield comprehensive genetic map. In addition, parametric LOD scores were maximized for varying proportions (α) of linked families under the assumption of locus heterogeneity, using HLOD implemented in the software program GENEHUNTER (http://linkage.rockefeller.edu).

In the 48 FS and GEFS + families TDT and nonparametric LOD scores (NPL Z_{all}) were calculated with GENEHUNTER. Allele frequencies of the microsatellite markers were estimated from genotypes of 74 unrelated spouses.

Mutation analysis

The exon-intron boundaries of *VLGR1* were defined by aligning genomic (accession number NT_006713) and cDNA sequences (accession number NM_032119) retrieved from Genbank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide). PCR amplifications were performed with flanking intronic primers designed using the software tool SNPbox (Weckx et al. 2005). In total, 92 primer pairs were used to amplify all exons of *VLGR1* (primer sequences are available upon request). PCR fragments were sequenced with BigDye Terminator v3.1 Cycle Sequencing kit from Perkin-Elmer Applied Biosytems. Sequences were analyzed on an ABI3700 automated sequencer with the Sequencing Analysis 5.0 software (Applied Biosystems).

Results

Genome-wide scan in family EP122

In the genome-wide linkage analysis in family EP122, four neighboring markers located on chromosome 5q14.3-q23.1, containing the FEB4 locus, yielded positive LOD scores ($Z>1.5$), with D5S644 obtaining the highest two point LOD score of 3.03 in the absence of recombination ($\theta=0$). None of the other genome scan markers obtained LOD scores higher than 1.5. Linkage

analysis of markers located within the other loci for FS, GEFS+ and FS associated with TLE (FEB1, FEB2, FEB3, FEB5, GEFS+1, GEFS+2, GEFS+3, chromosome 12q22-q23.3) resulted in conclusively negative LOD scores (<−2) (data not shown). Eleven additional markers from the region between D5S647 and D5S2115 were analyzed and two of them (D5S1463 and D5S2100) also yielded a two point LOD score above 3. The highest multipoint LOD score of 3.12 was reached in the region between D5S2498 and D5S644 (Fig. 1). Segregation analysis with markers located between D5S647 and D5S2115 identified a disease haplotype spanning a region of 33.6 cM between D5S2103 centromeric and D5S1975 telomeric based upon meiotic recombinations in patients III.4 and III.14 respectively (Fig. 2).

Mutation analysis

The candidate region defined in family EP122 overlapped at the centromeric site with the previously reported FEB4 locus over a distance of 19.2 cM between D5S2103 and D5S2501, and comprised *MASS1* (Fig. 3). We sequenced all exons of *MASS1* as well as the additional exons of *VLGR1*, 90 in total, on genomic DNA of three patients and one at risk individual not segregating the disease haplotype. We identified 38 single nucleotide polymorphisms (SNPs) of which eight were present in all patients because they were contained within the disease haplotype (Table 3). To rule out that one of these SNPs was a disease causing mutation, we investigated their prevalence in control individuals. All eight were intronic SNPs and have been reported previously (http://ncbi.nlm.nih.gov/SNP) (Sherry et al. 2001). In addition, in 95 unrelated control individuals randomly selected from the Belgian–Dutch population their segregating allele frequency varied from 11 to 69%.

Table 3 SNPs in *VLGR1* segregating with FS and epilepsy in family EP122

SNP	Intron	dbSNP	Segregating allele frequency
c.2016+53delA	10	rs3838659	0.13 (-)
c.2241-19G>T	11	rs1344030	0.12 (T)
c.2367+8C>T	12	rs2366773	0.69 (T)
c.2553+209G>A	13	rs2366774	0.68 (A)
c.2735-34C>G	14	rs16868901	0.11 (G)
c.2898+85A>G	15	rs16868903	0.11 (G)
c.2899-42T>C	16	rs2366776	0.13 (C)

Contribution of the FEB4 locus in the Belgian–Dutch population

To assess the contribution of the FEB4 locus to FS and epilepsy in the Belgian–Dutch population, we genotyped members of 48 Belgian–Dutch families with only FS and with FS and epilepsy for six markers—D5S1463, D5S2100, D5S2498, D5S644, D5S433 and D5S2501—within the linked haplotype of EP122 and located in the centromeric region shared with FEB4. The

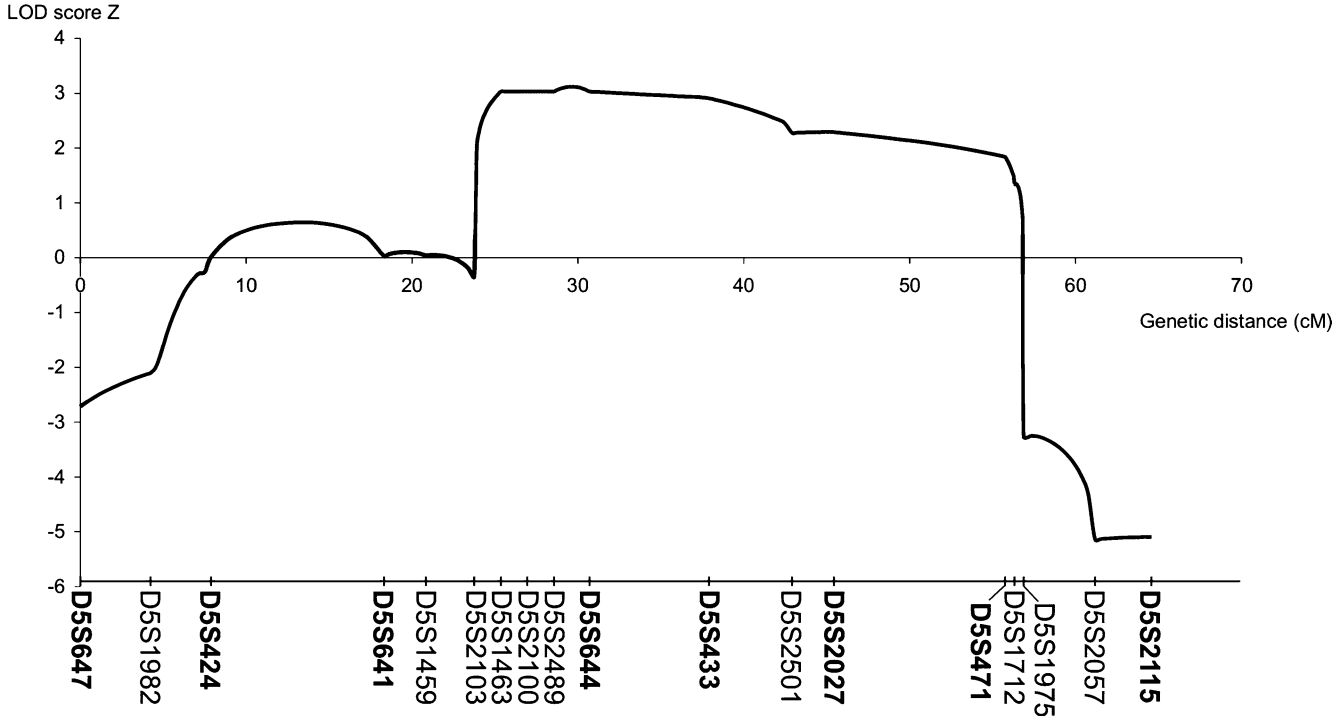

Fig. 1 Multipoint LOD scores for markers on chromosome 5q12-q31. Microsatellite markers included in the 10 cM genome-wide scan are in *bold*. Genetic localization of the markers was based on the Marshfield comprehensive genetic map

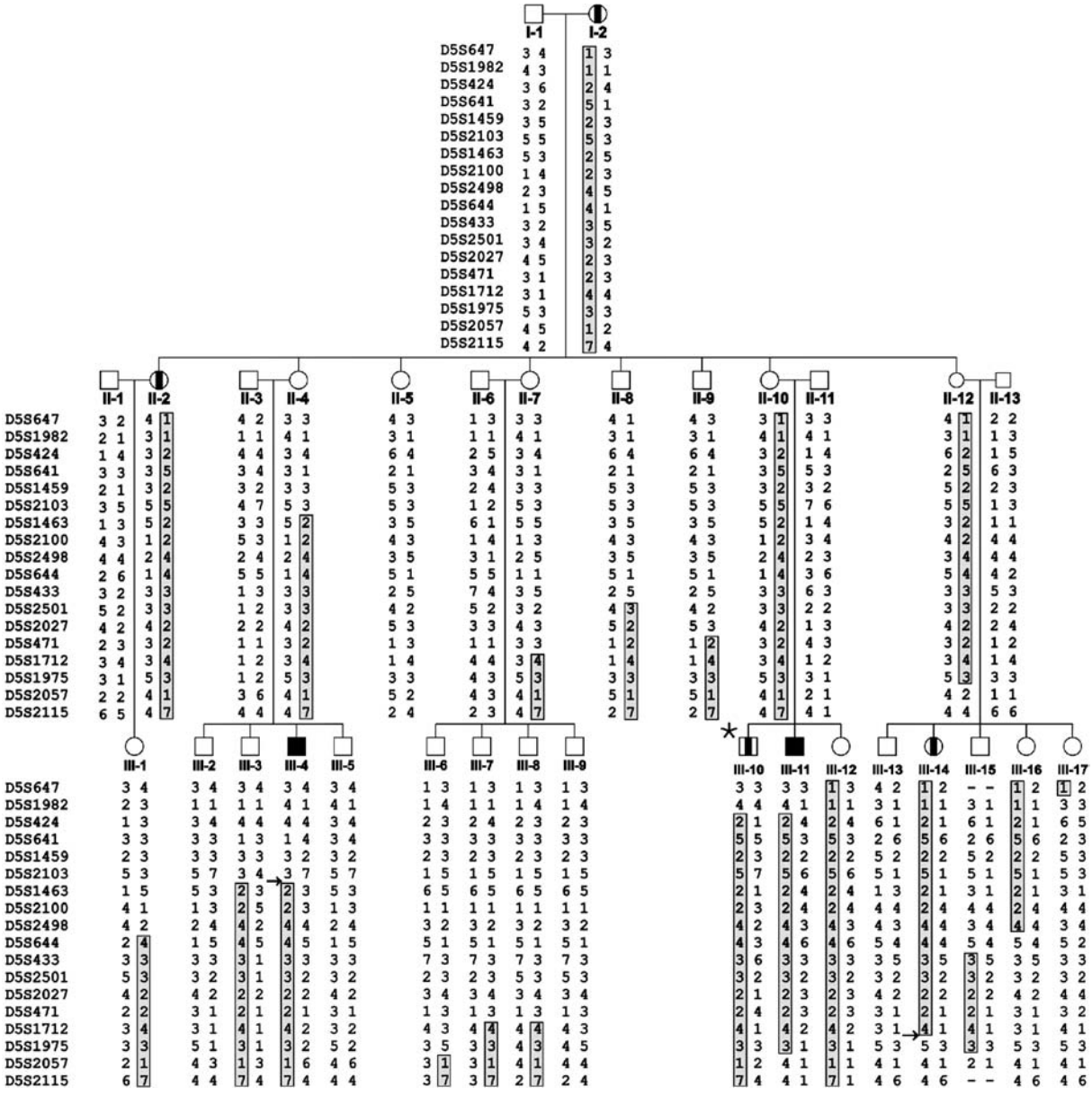

Fig. 2 Pedigree of family EP122. *Fully shaded square* man with a febrile seizures, *half shaded square* man with febrile seizures, *half shaded circle* woman with febrile seizures. The proband is indicated with an *asterisk*. The alleles on the disease haplotype are *boxed*. The *arrows* indicate the recombinations defining the minimal candidate region

combined parametric LOD scores throughout the entire FEB4 locus were extremely negative (< -40) in the sample of 39 families, assuming genetic homogeneity. Moreover, there was no evidence in favor of linkage, even when the LOD scores were maximized regarding a presumed proportion of linked families ($\alpha = 0$, HLOD = 0). Likewise, the non-parametric linkage (NPL) analysis resulted in negative NPL Z_{all} scores (corresponding $P > 0.5$). In separated analysis of the families, 7 out of 39 families showed positive LOD scores for markers within the FEB4 locus. However, the maximum LOD score obtained was only 0.73. Since these LOD scores were not conclusive, the cosegregation of the disease with a haplotype on chromosome 5q14.3-q23.1 could be coincidental. No haplotype sharing was identified between these seven families or with the FEB4 linked family EP122. A transmission disequilibrium test was performed with the 48 probands and their parents. In total, 53 alleles were analyzed but none reached a significant *P*-value.

Discussion

We ascertained a three generation Belgian–Dutch family with FS and epilepsy. Families in which FS, FS+ and generalized epilepsy co-occur are often diagnosed as GEFS+ (Scheffer and Berkovic 1997). However this

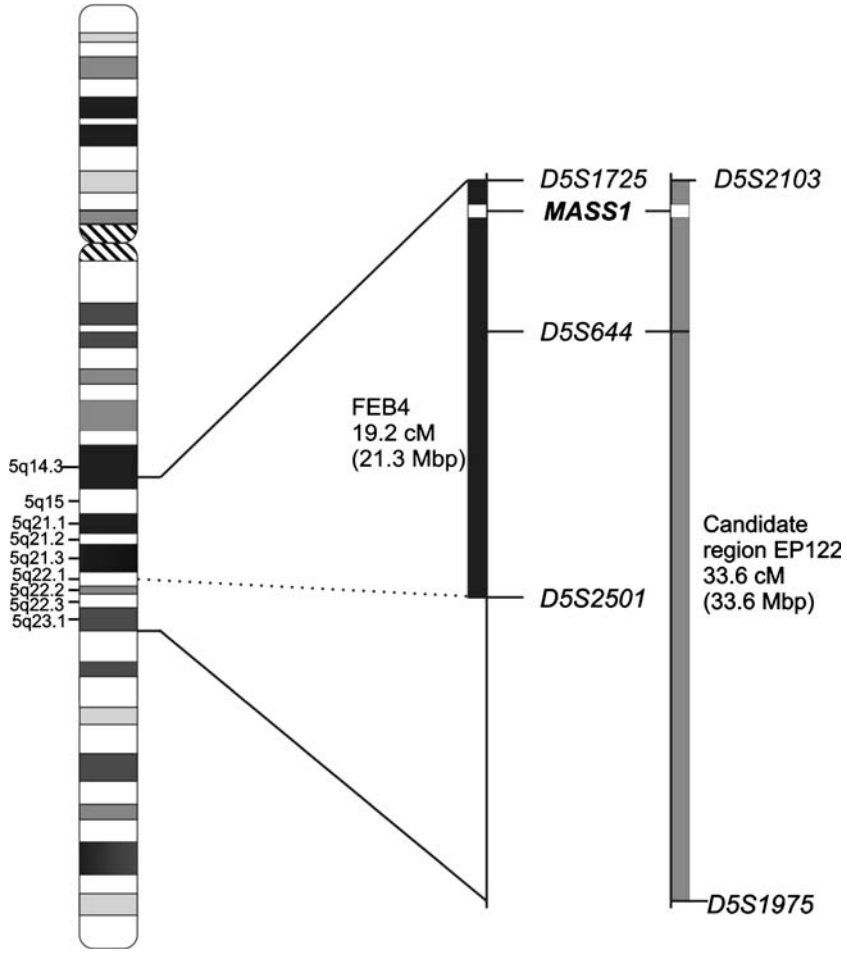

Fig. 3 Schematic presentation of the FEB4 locus and the candidate region defined in family EP122 (Nakayama et al. 2000). Markers D5S1725 and D5S2103 are located at the same genetic map position. D5S644 yielded significant LOD scores in both studies. The localization of the markers was based on the Marshfield comprehensive genetic map. *MASS1* was localized based on the physical map of the UCSC genome browser (http://genome.ucsc.edu)

particular family there are no individuals with FS+ which is characteristic of GEFS+. Because there are no standard criteria for evaluating a GEFS+ phenotype we leave the discussion open whether this family can be diagnosed as GEFS+ or not.

We performed a genome-wide scan in the family and obtained conclusive evidence for linkage to chromosome 5q14.3-q23.1 in a region of 33.6 cM between markers D5S2103 and D5S1975. The boundaries of the linkage interval were based upon recombinations in patients and the disease locus included the complete FEB4 locus. Segregation analysis identified several recombinants in unaffected at risk individuals. The distal segments of the candidate region were more often shared between patients and unaffected family members. If the causal gene were located in the distal part of the region, the disease penetrance in this family would be much lower than the 60% reported in the literature (Singh et al. 1999). Therefore, it is more likely that the disease causing mutation is localized in the proximal part of the candidate region, within the FEB4 locus. We analyzed the 35 exons of *MASS1*, previously suggested as the putative FEB4 gene in the Japanese population, as well as the additional 65 exons of *VLGR1* for mutations in family EP122. We identified eight intronic SNPs cosegregating with the disease in the family. All eight SNPs were previously reported and had segregating allele frequencies ranging between 11 and 69% in a Belgian–Dutch control population. These observations make it unlikely that one of these SNPs could be a disease causing mutation. Further genetic analysis of six microsatellite markers (average spacing >3 cM) localized within the FEB4 locus showed no evidence for linkage in a sample of 39 Belgian–Dutch families with FS and epilepsy. Likewise a transmission disequilibrium test performed with the 48 probands and their parents did not detect allele sharing, excluding a close genetic relationship with EP122 as well as among the families. These data suggest a minor role for FEB4 in the Belgian–Dutch population. However, markers at a higher genetic density will be necessary to observe a potential, though genetically distant founder effect in this population.

The FEB4 locus was previously identified in a single extended Japanese family and further support for this

region was provided by nonparametric linkage analysis in 39 nuclear families. Together the linkage data defined a region between D5S1725 and D5S2501 that spanned 19.2 cM (Fig. 3). Significant linkage was obtained in the family sample with D5S644 ($P = 5.4\times10^{-6}$) (Nakayama et al. 2000). The FEB4 locus was reported previously as a FS locus; however, the phenotype in the large Japanese family included FS and afebrile seizures, like family EP122. Therefore FEB4 should be considered as a locus for FS and epilepsy. *MASS1* was proposed as the FEB4 gene based on two lines of evidence. First, a homozygous deletion of nucleotide c.6213G in *mass1* was reported in the Frings mouse strain, a genetic model for audiogenic seizures (Skradski et al. 2001). The paralog of *mass1* maps to human chromosome 5q14, within the FEB4 locus. Second, one heterozygous nonsense mutation c.7955 C > A, at codon 2652 (S2652X), segregated with the disease in a single nuclear Japanese family with two affected sibs (Nakayama et al. 2002). The absence of a *MASS1* mutation in family EP122 can be interpreted in several ways. The remote possibility of a mutation present in regulatory sequences such as the promoter, which we have not analyzed, cannot be excluded. Also, we cannot exclude the posibility that the region on chromosome 5q14.3-q23.1 contains two genes in which mutations can be associated with FS and epilepsy. However the failure to detect a *MASS1* mutation in EP122 adds to the growing evidence that mutations in another gene than *MASS1* might be the cause of FEB4. First, the *mass1* mutation in the Frings mouse is a homozygous out of frame deletion leading to premature termination of the encoded protein (Skradski et al. 2001). Homozygous *Vlgr1* knockout (*Vlgr1*$^{-/-}$) mice lacking exons 2–4 of *Vlgr1* also showed audiogenic seizure susceptibility. This deletion of exons 2–4 induced a frame shift followed by a premature stopcodon and the predicted generation of a 14 amino acid peptide, if any. The initiation codon of *Mass1*, located at exon 5 of *Vlgr1*, is not affected in the *Vlgr1*$^{-/-}$ mice and the expression of *Mass1* is probably preserved. Therefore it was predicted that complete absence of functional Vlgr1 protein and not Mass1 leads to audiogenic seizures in mice (Yagi et al. 2005). The nonsense mutation detected in the Japanese family, affected both *MASS1* and *VLGR1*, and was present in heterozygous state. Although not examined, the authors suggested that this mutation might lead to a loss-of-function and that haploinsufficiency of *MASS1* or *VLGR1* might cause a convulsive disorder in humans (Nakayama et al. 2002). Recent evidence however has raised doubts about this hypothesis. Four truncating *VLGR1* mutations, predicted to result in loss-of-function alleles, caused in compound heterozygous state the recessive Usher Syndrome type II. Three of these truncating mutations also affected the *MASS1* transcript. Interestingly, neither the Usher syndrome patients nor their heterozygous parents developed FS or epilepsy (Weston et al. 2004). Second, the only *MASS1* mutation, assumed to cause an epileptic phenotype in human, was detected in a single nuclear Japanese family. Its predicted pathogenic nature is therefore not supported by conclusive linkage between the mutation and the disease, but based solely upon its absence in control individuals thereby not excluding the possibility of a rare polymorphism. Remarkably no *MASS1* mutation was reported in the single large family that was instrumental in identifying the FEB4 locus. Furthermore, no additional *MASS1* mutations were reported despite analysis of 48 families contributing to the FEB4 linkage and association analysis with nine missense polymorphisms in *MASS1* did not lead to a significant result (Nakayama et al. 2002).

In summary, we conclude that a gene other than *MASS1* is probably underlying the FEB4 locus as well as the linkage in family EP122. Within the region overlapping between FEB4 and the EP122 candidate region, 85 genes are located, none of them encodes for a subunit of an ion channel. Some of the genes located in the region, including *sialyltransferase 8D* and *ephrin A5*, may play a role in the development of the central nervous system and are good functional candidate genes. The controversy about the involvement of *MASS1* in FEB4 closely resembles what we recently observed in the molecular genetic studies of inherited peripheral neuropathies. *KIF1B* was claimed as the gene for Charcot-Marie-Tooth type 2A (CMT2A) based on the analysis of a hetero- and homozygous knockout mouse model and the observation of a mutation in a single nuclear family (Zhao et al. 2001). Subsequently, not only were mutations in *KIF1B* excluded in families showing conclusive linkage to the CMT2A locus but all were shown to segregate a mutation in *Mitofusin* (Züchner et al. 2004). Nevertheless, the linkage in family EP122 confirms that a gene at 5q14.3-q23.1 is responsible for FS and epilepsy. Identification of this gene will largely contribute to our understanding of the genetic etiology of idiopathic epilepsies.

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References

Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E (2001) First genetic evidence of GABA (A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 28:46–48

Bonanni P, Malcarne M, Moro F, Veggiotti P, Buti D, Ferrari AR, Parrini E, Mei D, Volzone A, Zara F, Heron SE, Bordo L, Marini C, Guerrini R (2004) Generalized epilepsy with febrile

seizures plus (GEFS+): clinical spectrum in seven Italian families unrelated to SCN1A, SCN1B, and GABRG2 gene mutations. Epilepsia 45:149–158

Claes L, Audenaert D, Deprez L, Van Paesschen W, Depondt C, Goossens D, Del-Favero J, Van Broeckhoven C, De Jonghe P (2004) Novel locus on chromosome 12q22-q23.3 responsible for familial temporal lobe epilepsy associated with febrile seizures. J Med Genet 41:710–714

Cottingham RW Jr, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Engel J Jr (2001) International League Against Epilepsy (ILAE), a proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE task force on classification and terminology. Epilepsia 42:796–803

Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A (2000) Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet 24:343–345

Hauser WA, Annegers JF, Rocca WA (1996) Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. Mayo Clin Proc 71:576–586

Johnson EW, Dubovsky J, Rich SS, O'Donovan CA, Orr HT, Anderson VE, Gil-Nagel A, Ahmann P, Dokken CG, Schneider DT, Weber JL (1998) Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the Midwest. Hum Mol Genet 7:63–67

Johnson WG, Kugler SL, Stenroos ES, Meulener MC, Rangwalla I, Johnson TW, Mandelbaum DE (1996) Pedrigree analysis in families with febrile seizures. Am J Med Genet 61:345–352

Kugler SL, Stenroos ES, Mandelbaum DE, Lehner T, McKoy VV, Prossick T, Sasvari J, Swannick K, Katz J, Johnson WG (1998) Hereditary febrile seizures: phenotype and evidence for a chromosome 19p locus. Am J Med Genet 79:354–361

McMillan DR, Kayes-Wandhover KM, Richardson JA, White PC (2002) Very large G protein-coupled receptor-1, the largest known cell surface protein, is highly expressed in the developing central nervous system. J Biol Chem 277:785–792

Nabbout R, Prud'homme JF, Herman A, Feingold J, Brice A, Dulac O, LeGuern E (2002) A locus for simple pure febrile seizures maps to chromosome 6q22-q24. Brain 125:2668–2680

Nakayama J, Fu YH, Clark AM, Nakahara S, Hamano K, Iwasaki N, Matsui A, Arinami T, Ptacek LJ (2002) A nonsense mutation of the MASS1 gene in a family with febrile and afebrile seizures. Ann Neurol 52:654–657

Nakayama J, Hamano K, Iwasaki N, Nakahara S, Horigome Y, Saitoh H, Aoki T, Maki T, Kikuchi M, Migita T, Ohto T, Yokouchi Y, Tanaka R, Hasegawa M, Matsui A, Hamaguchi H, Arinami T (2000) Significant evidence for linkage of febrile seizures to chromosome 5q14-q15. Hum Mol Genet 9:87–91

Ott J (1989) Computer-simulation methods in human linkage analysis. Proc Natl Acad Sci USA 86:4175–4178

Peiffer A, Thompsom J, Charlier C, Otterud B, Varvil T, Pappas C, Barnitz C, Gruenthal K, Kuhn R, Leppert M (1999) A locus for febrile seizures (FEB3) maps to chromosome 2q23–24. Ann Neurol 46:671–678

Rich SS, Annegers JF, Hauser WA, Anderson VE (1987) Complex segregation analysis of febrile convulsions. Am J Hum Genet 41:249–257

Scheffer IE, Berkovic SF (1997) Generalized epilepsy with febrile seizures plus a genetic disorder with heterogeneous clinical phenotypes. Brain 120:479–490

Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29:308–311

Singh R, Scheffer IE, Crossland K, Berkovic SF (1999) Generalized epilepsy with febrile seizures plus: a common childhood-onset genetic epilepsy syndrome. Ann Neurol 45:75–81

Skradski SL, Clark AM, Jiang H, White HS, Fu YH, Ptacek LJ (2001) A novel gene causing a medelian audiogenic mouse epilepsy. Neuron 31:537–544

Tsuboi T, Endo S (1991) Genetic studies of febrile convulsions: analysis of twin and family data. Epilepsy Res Suppl 4:119–128

Wallace RH, Berkovic SF, Howell RA, Sutherland GR, Mulley JC (1996) Suggestion of a major gene for familial febrile convulsions mapping to 8q13–21. J Med Genet 33:308–312

Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC (1998) Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat Genet 19:366–370

Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF (2001) Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49–52

Weckx S, De Rijk P, Van Broeckhoven C, Del-Favero J (2005) SNPbox, a modular software package for large scale primer design. Bioinformatics 21:385–387

Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. Am J Hum Genet 47:A204

Weston MD, Luijendijk MWJ, Humphrey KD, Möller C, Kimberling WJ (2004) Mutations in the VLGR1 gene implicate G-protein signalling in the pathogenesis of Usher syndrome type II. Am J Hum Genet 74:357–366

Yagi H, Takamura Y, Yoneda T, Konno D, Akagi Y, Yoshida K, Sato M (2005) Vlgr1 knockout mice show audiogenic seizure susceptibility. J Neurochem 92:191–202

Zhao C, Takita J, Tanaka Y, Setou M, Nakagawa T, Takeda S, Yang HW, Terada S, Nakata T, Takei Y, Saito M, Tsuji S, Hayashi Y, Hirokawa N (2001) Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. Cell 105:587–597

Züchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schröder JM, Vance JM (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nat Genet 36:449–451