Original article

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Segregation of a haplotype encompassing *FEB1* with genetic epilepsy with febrile seizures *plus* in a Colombian family

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ABSTRACT – Febrile seizures and epilepsy are believed to be linked and some forms of epilepsy are associated with a history of febrile seizures (FS). Linkage analysis to seven known loci for FS and/or genetic epilepsy with febrile seizures *plus* (GEFS *plus*) was performed in a small Colombian family. Short tandem repeat (STR) markers were genotyped and two-point linkage analysis and haplotype reconstruction were conducted. A maximum LOD score of 0.75 at marker D8S533 for *FEB1* at a recombination fraction (θ) of 0 and a segregating haplotype were identified. *FEB1* was the first locus to be associated with FS and this is the second report to describe this association. Two genes in this region, *CRH* and *DEPDC2,* are good putative candidate genes that may play a role in FS and/or GEFS *plus*.

Key words: febrile seizure, autosomal dominant epilepsy with febrile seizures *plus*, genetic epilepsy, *FEB1*

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Febrile seizures (FS) are the most common convulsive events in humans. About 3-4% of children between 3 months and 6 years of age will experience at least one febrile seizure (Nakayama and Arinami, 2006), and of these, about 3-4% will develop epilepsy later in life (Nakayama and Arinami, 2006). Although this kind of seizure represents neuronal hyperexcitability that may occur more than once in a lifetime, it is not considered an epileptic syndrome since there is a precipitating event, such as fever. Environmental factors such as viral infections, as well as genetic factors, have been implicated in FS

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(van Zeijl *et al*., 2002). Family-based studies have shown that close relatives of patients have a higher risk of FS, compared to the general population (van Zeijl *et al*., 2002). In a similar way, high concordance rates of 35-69% and 14-20% have been reported in monozygous and dizygous twins, respectively. Although a genetic basis for FS is well established, controversy still exists regarding a pattern of inheritance (van Zeijl *et al*., 2002). Most studies support a polygenic or multifactorial model (Rich *et al*., 1987), but in families whose probands have suffered multiple seizures or whose family members are affected with different epilepsies, the inheritance pattern is consistent with a monogenic autosomal dominant model, with 65% penetrance (Johnson *et al*., 1996). In agreement with this observation, most described loci for FS have been identified by linkage analysis in large families in which FS segregates as an autosomal dominant trait. Currently, more than nine loci have been described for FS in families from different countries and some genes have been identified (reviewed by Kira *et al*. [2010]).

In contrast, generalised epilepsy with febrile seizures *plus* (GEFS *plus*), since its first description by Scheffer and Berkovic (1997), has been recognised as an epilepsy syndrome with an autosomal dominant inheritance pattern and incomplete penetrance (Scheffer and Berkovic, 1997). GEFS *plus* is a familial condition in which patients demonstrate FS and extended FS (FS *plus*), as well as different types of afebrile generalised or partial seizures. Considering that seizures may be either generalised or focal, this syndrome has also been referred to as "genetic epilepsy with febrile seizures plus", retaining the acronym, GEFS *plus*. As a predominantly Mendelian inherited condition, several loci have been identified for GEFS+, some of which are shared with FS, suggesting a common genetic basis for both conditions.

FEB1 was the first locus identified for FS and, since its first description by Wallace *et al*. (1996) in a large Australian family with autosomal dominant FS, this finding has not subsequently been reported prior to this study. Here, we present linkage to *FEB1* in a small Colombian family with GEFS *plus*.

Patients and methods

Family recruitment and clinical evaluation

The family reported here (Fam21) was recruited from the index case presented at the Paediatric Neurology Department at the San Vicente de Paul Hospital (Medellín, Colombia). At initial consultation, family seizure history and pedigree were determined. The status of other relatives was investigated at a later visit by the family by comprehensive neurological examination of available individuals. Blood samples (10 mL) were collected after informed consent was given and the study was approved by the Bioethics Committee at the Universidad de Antioquia. Genomic DNA was isolated by the phenol/chloroform standard method.

Genotyping

Short tandem repeat (STR) markers at seven loci, reported in patients with FS and/or GEFS *plus*, were investigated: *FEB1* (*D8S533*, *D8S1795*, *D8S1807* and *D8S279*), *FEB2* (*D19S216*, *D19S1034*, *D19S427* and *D19S177*), *SCN1A/SCN2A/FEB3* (*D2S2380*, *D2S354*, D2S382 and D2S2330), *SCN1B/GEFSP1* (*D19S248* and *D19S208*), *GABRG2/GEFSP3/FEB8* (*D5S2016* and *D5S2038*), *MASS1/FEB4* (*D5S1452* and *D5S1463*), and *FEB5* (*D6S262* and *D6S1656*). Oligonucleotide sequences may be provided on request.

These markers were amplified by PCR under standard reaction conditions using F-fluorescent oligonucleotides. Typing was performed in an ABI-310 genetic analyser (Foster City, CA, USA). All genotypes were independently scored by two investigators using Genescan, Genotyper, and Genemapper (Applied Biosystems) software (Foster City, CA, USA).

Linkage and haplotype analysis

In order to identify non-Mendelian features, genotypes were investigated using Pedcheck software (O'Connell and Weeks, 1998). Two-point linkage analyses were performed, assuming a dominant model for FS/GEFS+ at different phenocopy and penetrance rate values and a frequency of 0.003 for the disease allele in the MLINK program of the LINKAGE package (Lathrop *et al*., 1984). All individuals with a diagnosis of FS or GEFS+ were considered to be affected. Haplotypes were reconstructed using Simwalk2 (Sobel and Lange, 1996) and visualised by Haplopainter (Thiele and Nürnberg, 2005) software.

Results

Fam21 was composed of 17 individuals, however, only 9 are presented who were relevant to the genetic analysis (*figure 1*). Two of these individuals, II:4 and III:1, presented with onset of FS at 18 and 15 months, respectively. Individual II:4 presented with more than five FS, while III:1 has so far presented with two FS. Age at last FS was 10 years and 22 months for individuals II:4 and III:1, respectively. Individual II:4 also experienced afebrile partial seizures (<5) at 5-10 years old. The types of seizure were complex focal (II:4) and tonic

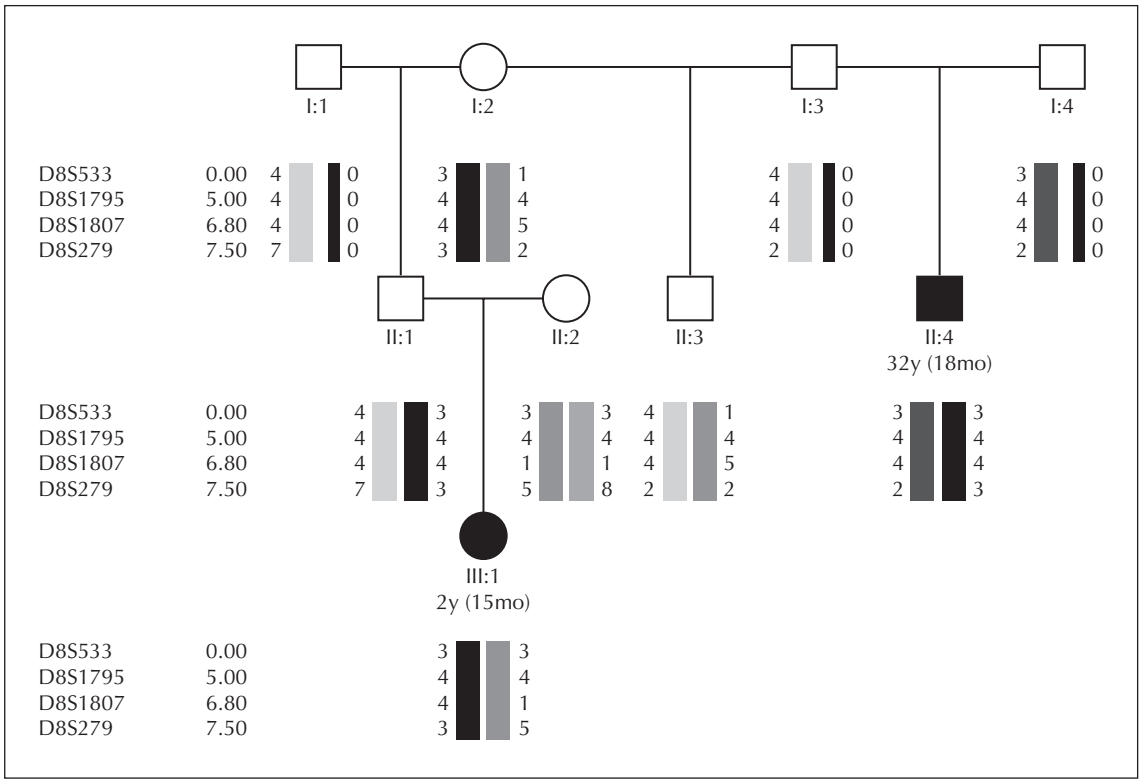

Figure 1. Pedigree of Fam21 Filled symbols indicate individuals with FS+ or FS; clear symbols represent unaffected individuals. The marker and distance in cM, according to Marshfield, is presented on the left-hand side. Bars indicate haplotypes Numbers flanking the bars refer to the alleles for the markers evaluated for *FEB1*. The darkest bar corresponds to the haplotype associated with the condition (*i.e.* 3-4-4-3). Indicated age corresponds to age at first FS. Individual I:2 married three different spouses (I:1, I:3 and I:4).

generalised (III:1). To date, individual III:1 has not developed afebrile seizures. None of the individuals had mental retardation.

Linkage analysis of the condition to STRs at loci reported for FS and/or GEFS+ was performed. LOD scores for all loci tested, with the exception of *FEB1*, were predominantly negative (data not shown). At *FEB1*, a maximum LOD score value of 0.75 was obtained at a recombination fraction (θ) of 0 for marker *D8S533*, considering a penetrance and phenocopy rate of 90 and 0%, respectively. Positive values were observed for other markers at this locus *(table 1)*.

Table 1. Two-point LOD scores for *FEB1*

Marker	Recombination Fraction (θ)				
	0.0	**0.1**	**0.2**	**0.3**	**0.4**
D8S533	0.75	0.58	0.41	0.23	0.09
D8S1795	0.0	0.0	0.0	0.0	0.0
D8S1807	0.24	0.17	0.1	0.05	0.01
D8S279	0.46	0.35	0.24	0.15	0.07

Haplotype analysis in the family revealed a haplotype characterised by alleles 3-4-4-3 at markers *D8S533-D8S1975-D8S1807-D8S279*. This haplotype segregated with the disease and was also present in obligate carriers (*figure 1*). No recombinants were observed in this family for *FEB1*.

Discussion

This report of a small Colombian family is the second reported study to link *FEB1* to GEFS *plus*. The maximum LOD score in Fam21 was 0.75. Although under the threshold value of 3 for linkage statistical significance, a value of this magnitude is indicative of possible linkage since the reduced value may reflect the small size and number of affected individuals of Fam21. Nonetheless, this LOD score is not so dissimilar to the ELOD (expected LOD score) of 0.9, identified using simulations (data not shown).

The family presented is small and the study therefore has limited power, thus, a significant LOD score for each marker at a locus is not possible. However, since we found a set of STRs with positive LOD scores, all of which together formed a haplotype with zero recombinants, it is easier to accept that the positive LOD scores

suggest true linkage. The difference between the ELOD and LOD scores may be due to differences in allele frequencies. Such frequencies were assumed to be 0.25 in the simulations and 1/*n* for the real marker data, where *n* is the number of alleles.

Besides this positive LOD score, haplotype analyses have allowed us to identify a haplotype which segregates with affection status. Methods based on linkage disequilibrium, such as haplotype analyses, depend on the identity of individuals descended from a common founder with a specific mutation (Botstein and Risch, 2003). When a mutation occurs, it associates with a set of alleles at different loci. As recombination events take place over generations, new alleles will be introduced to the initial block. For those alleles close to the mutation, recombination events occur at a very low rate and the alleles will segregate with the mutation as well as the disorder, if deleterious. In the light of this, in our study, a mutation is expected at some point within the region of interest which is responsible for the phenotype observed in Fam21.

FEB1 is a region of 9.2 cM, which comprises 27 reported genes (Wallace *et al*., 1996). Considering expression pattern and function in the brain (Brunson *et al*., 2001; Storey *et al*., 2002), three of these, *CRH,* calbindin and *PREX2*, may be good candidate genes that play a role in FS and/or GEFS+, of which the former two were suggested by Wallace *et al*. (1996). The three gene products are involved in neurodevelopment. *CRH,* which encodes for the corticotropin releasing hormone, may have an important role in the pathogenesis of different conditions such as infantile spasms, Lennox-Gastaut syndrome, and myoclonic progressive epilepsy (Brunson *et al*., 2001). This gene has also been implicated in the aetiology of autosomal dominant nocturnal frontal-lobe epilepsy (ADNFLE) (Combi *et al*., 2008). Based on the expression atlas reported in the UCSC genome browser (www.genome.ucsc.edu), four additional candidate genes locate to this chromosome region. These include *RIPK2*, *NECAB1*, *OTUD6B*, and *RUNX1T1*. An exhaustive examination of the candidate region, perhaps using next-generation sequencing techniques, may help to identify the gene(s) involved in FS/GEFS+.

Although *FEB1* has been evaluated in all genetic studies of FS and GEFS *plus* since the initial study of Wallace *et al*. (1996), none have replicated the finding of *FEB1* linkage. Taken together, our positive LOD score and the segregating haplotype in the analysed Colombian family provide a second report in the literature of the association between the *FEB1* locus and GEFS+. Since one of the members of Fam21 presented with afebrile seizures and febrile seizures beyond 6 years of age, our study reinforces the retrospective understanding that the family studied by Wallace *et al*. was in fact also a GEFS+ family, and not a "pure" FS family.

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