

Childhood absence epilepsy and febrile seizures: a family with a GABA_A receptor mutation

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Summary

Although several genes for idiopathic epilepsies from families with simple Mendelian inheritance have been found, genes for the common idiopathic generalized epilepsies, where inheritance is complex, presently are elusive. We studied a large family with epilepsy where the two main phenotypes were childhood absence epilepsy (CAE) and febrile seizures (FS), which offered a special opportunity to identify epilepsy genes. A total of 35 family members had seizures over four generations. The phenotypes comprised typical CAE (eight individuals); FS alone (15), febrile seizures plus (FS⁺) (three); myoclonic astatic epilepsy (two); generalized epilepsy with tonic–clonic seizures alone (one); partial epilepsy (one); and unclassified epilepsy despite evaluation (two). In three remaining individuals, no information was available. FS were inherited in an autosomal dominant

fashion with 75% penetrance. The inheritance of CAE in this family was not simple Mendelian, but suggestive of complex inheritance with the involvement of at least two genes. A GABA_A receptor $\gamma 2$ subunit gene mutation on chromosome 5 segregated with FS, FS⁺ and CAE, and also occurred in individuals with the other phenotypes. The clinical and molecular data suggest that the GABA_A receptor subunit mutation alone can account for the FS phenotype. An interaction of this gene with another gene or genes is required for the CAE phenotype in this family. Linkage analysis for a putative second gene contributing to the CAE phenotype suggested possible loci on chromosomes 10, 13, 14 and 15. Examination of these loci in other absence pedigrees is warranted.

Keywords: childhood absence epilepsy; epilepsy; GABA_A receptor; genetics; linkage analysis

Abbreviations: CAE = childhood absence epilepsy; FS = febrile seizures; GEFS⁺ = generalized epilepsy with febrile seizures plus; GTCS = generalized tonic–clonic seizures; IGE = idiopathic generalized epilepsy; JME = juvenile myoclonic epilepsy; MAE = myoclonic–astatic epilepsy

Introduction

Childhood absence epilepsy (CAE) is the prototype of idiopathic generalized epilepsy (IGE) accounting for 2–8% of patients with epilepsy (Livingston *et al.*, 1965; Cavazzuti, 1980; Olsson, 1988). Absences have an age-dependent expression, beginning in childhood and often resolving spontaneously by mid-adolescence. In 10–15% of CAE patients, there is a history of earlier febrile seizures (FS) (Livingston *et al.*, 1965; Penry *et al.*, 1975; Dieterich *et al.*, 1985; Loiseau, 1992; Berkovic, 1997). Afebrile generalized tonic–clonic seizures (GTCS) occur in 40% of individuals, usually after the onset of absences, and may continue into

adolescent or adult life (Livingston *et al.*, 1965; Dieterich *et al.*, 1985; Olsson, 1988).

CAE is genetically determined. Estimates of the risk of seizures in first-degree relatives of IGE probands vary from 4 to 10% (Metrakos and Metrakos, 1961; Doose *et al.*, 1973; Annegers *et al.*, 1982; Tsuboi, 1989). Within families of CAE probands, only about a quarter of affected relatives have the CAE phenotype, about half have other IGE subsyndromes and about a quarter have FS alone (Italian League Against Epilepsy Genetic Collaborative Group, 1993; Bianchi and the Italian League Against Epilepsy Genetic Collaborative

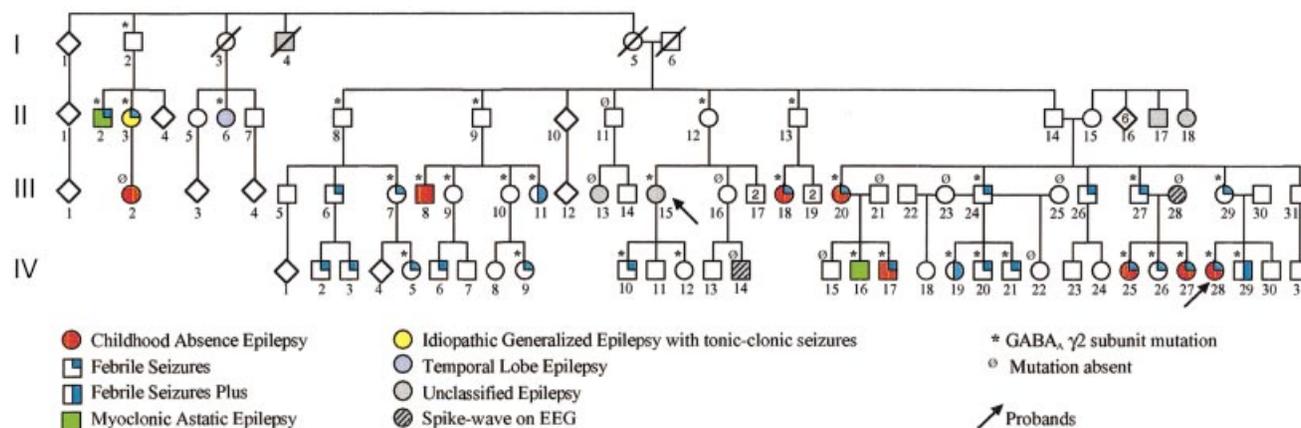


Fig. 1 Family pedigree. Pedigree of a four-generation family with childhood absence epilepsy, febrile seizures and other extended phenotypes. Some unaffected individuals have been omitted. The rest of the family members were not tested.

Group, 1995). Although a dominant mode of inheritance was suggested initially (Metrakos and Metrakos, 1961), clinical analysis of twins and families, and molecular analyses strongly suggests that CAE and related IGE are inherited in a complex manner with involvement of more than one gene (Lennox and Lennox, 1960; Doose *et al.*, 1973; Andermann, 1982; Greenberg *et al.*, 1988a, 1992; Janz *et al.*, 1992; Italian League Against Epilepsy Collaborative Group, 1993; Bianchi and the Italian League Against Epilepsy Collaborative Group, 1995; Berkovic *et al.*, 1998).

A number of loci have been claimed for CAE. The first unconfirmed locus on chromosome 1 was reported in a Mexican family with a phenotype of CAE evolving into juvenile myoclonic epilepsy (JME) (Westling *et al.*, 1996). A locus on chromosome 8q24 has been reported in a large Indian family with a phenotype of persisting CAE and GTCS (Fong *et al.*, 1998). An earlier suggestion of a chromosome 8q locus, centromeric to the region indicated in the Indian family (Fong *et al.*, 1998), was reported by Zara *et al.* (1995) in families with mixed IGE subsyndromes. However re-analysis of these data using non-parametric linkage analysis did not produce a significant LOD score on chromosome 8 (Kruglyak *et al.*, 1996). Two other studies were performed in mixed IGE families looking at a possible locus on chromosome 8q, with opposing results (Sander *et al.*, 1998; Durner *et al.*, 1999). Sander *et al.* (2000) recently reported a genome-wide search for susceptibility loci in 130 families with mixed IGE and found evidence for epilepsy susceptibility loci on chromosome 14q23, 2q36 and 3q36. Similarly, Durner *et al.* (2001) performed a genome scan on 91 families comprising probands with JME, juvenile absence epilepsy (JAE) and epilepsy with GTCS alone. Analysis including all 91 families showed strong evidence for a chromosome 18 locus, common to all IGE. Together with the locus on chromosome 18, the analysis of subsets of the 91 families showed other positive LOD scores to other chromosomes depending on the IGE subsyndrome. Families with JME showed linkage to the previously reported chromosome 6 locus (Greenberg *et al.*, 1988b), and families with JAE and GTCS alone contributed

to a positive LOD score on chromosome 8 (Durner *et al.*, 2001). Grouping families with at least one member with absence seizures, two new putative loci were also reported in two regions of chromosome 5 (Durner *et al.*, 2001). A single case of absence epilepsy with episodic ataxia type II had a mutation in the calcium channel *CACNA1A* gene; whether this is relevant to his absences remains uncertain (Jouveneau *et al.*, 2001).

We recently described a GABA_A receptor γ2 subunit gene (*GABRG2*) mutation in a family with classical CAE associated with FS (Wallace *et al.*, 2001a). The mutation results in substitution of a highly conserved arginine for glutamine (R43Q) in the first of two benzodiazepine-binding domains of the protein, causing loss of benzodiazepine sensitivity *in vitro*. No other families with mutations in the benzodiazepine-binding domains have been described to date (Wallace *et al.*, 2001a).

We now present a detailed clinical and genetic analysis of this large pedigree that includes eight individuals with CAE. This permits an understanding of the phenotype-genotype correlation of this human GABA_A receptor mutation. Further, using the power of this large family, we explored the complex genetics of CAE and identified other putative loci.

Methods

Ascertainment of the family

The family was ascertained via two different probands (Fig. 1). The first proband (IV-28) was referred by Drs L. Shield and M. Mackay, Royal Children's Hospital, Melbourne, when she presented with onset of daily absences at age 4 years. The second proband (III-15), aged 35 years, was identified through the First Seizure Clinic at the Austin and Repatriation Medical Centre (ARMC), Melbourne. This study was undertaken with the approval of the Human Research Ethics Committee of the ARMC. All participating individuals and parents/guardians in the case of minors gave informed consent.

Clinical evaluation

Information was obtained on 220 family members from an outbred family, of whom 35 were reputed to have a history of seizures. Fifty-nine members within generations II–IV (Fig. 1) underwent a detailed personal interview using a validated seizure questionnaire (Reutens *et al.*, 1992). Extensive genealogical information was obtained. Previous medical records of affected individuals were reviewed where possible. Fifty members of the family were personally examined and underwent neurological examination. Forty-eight individuals had a 21-channel EEG recording. In all the subjects, the EEG was recorded during wakefulness, photic stimulation and 3 min of hyperventilation. Blood samples were taken for genetic analysis.

Classification of epilepsy phenotypes

Seizure types and epilepsy syndromes were classified according to the International Classification of Seizures (Commission on Classification and Terminology of the International League Against Epilepsy, 1981) and Epilepsy Syndromes (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) for each affected individual. Children with FS that persisted beyond 6 years of age or were associated with afebrile GTCS were designated as having febrile seizures plus (FS⁺) (Scheffer and Berkovic, 1997). Those individuals who had a history of seizures, but insufficient information to make an epilepsy syndrome diagnosis, were designated as having unclassified epilepsy. Those individuals in whom the history of seizures could not be verified by an eye-witness account were also considered as having unclassified epilepsy.

Genetic analysis

Mutational analysis of *GABRG2*(R43Q) on additional family members (I-2, II-2, II-3, III-2 and IV-16) not included in our initial report was performed by direct sequencing as previously described (Wallace *et al.*, 2001a).

We also re-analysed our whole genome screen of this family, with only individuals with CAE considered affected. All other individuals with seizures were classified as ‘unknown’ for this analysis. The analysis was performed following our digenic model of IGEs (S. F. Berkovic, C. Marini, R. H. Wallace, F. L. Phillips, J. C. Mulley, I. E. Scheffer, unpublished results). This model predicts that the IGE subsyndromes are determined by a combination of mutations in two ion channel subunit genes. In its basic form, the digenic model is a simple two-locus heterogeneity model. The digenic model incorporates the presence of a number of loci and the IGE subsyndromes are due to various combinations of two such loci. Having found one gene segregating with individuals with CAE, FS and FS⁺, we predicted that a second locus would be largely restricted to those with the CAE phenotype.

After obtaining whole-genome screening data (provided by the Australian Genome Research Facility) from the newly ascertained individuals listed above, we performed a CAE affected-only analysis on the entire family. Individuals with FS or FS⁺ were coded as unknown in the linkage analysis (performed using FASTLINK v4.0). Two-point LOD scores were calculated assuming 50% penetrance and a 2% phenocopy rate. Additional markers were genotyped in regions that could not be excluded.

Results

Genealogy

Ancestors of the family migrated to Australia from England and Ireland in the 18th century. All living members of the family resided in Melbourne, rural Victoria or New South Wales. There was no consanguinity in the family. From the genealogical and clinical information obtained, an extensive pedigree was constructed (Fig. 1).

Epilepsy phenotypes

Thirty-five individuals had a history of seizures. Their clinical details are presented in Table 1.

Childhood absence epilepsy (n = 8, see Fig. 1)

The first proband and seven other individuals of two consecutive generations had CAE (III-2, III-8, III-18, III-20, IV-17, IV-25, IV-27 and IV-28). Six were females and two were males, aged from 3 to 44 years. The mean age of onset of absences was 37 months, ranging from 10 months to 6 years (median 33 months). Typical absences were described with loss of awareness and staring for 5 to 10 s occurring many times per day. Individual IV-25 had daily absences with loss of awareness, occasional oral automatisms, infrequent falls and incontinence; duration was <10 s. Individual III-8 had absences with tonic/atonic components and marked clinical photosensitivity lasting <1 min. The mean age of offset of absences was 6 years (range 3–12 years). Individual IV-27 aged 4.5 years had ongoing absences.

All but two individuals (III-2 and III-8) had FS prior to the onset of absences. FS were simple, lasting <5 min in all. The mean number of FS was seven (ranging from one to 25); the median was four. One child (IV-17) had a febrile seizure lasting 2–3 min, affecting predominantly the left side, followed by a left Todd’s paresis. Four individuals (III-2, III-8, III-20 and IV-17) also had later afebrile GTCS petering out by 14 years of age. Three members (III-2, III-8 and IV-17) had learning problems.

Typical 3 Hz spike–wave activity was documented on EEG studies of five out of eight members with a clinical diagnosis of CAE (III-2, III-8, III-18, IV-27 and IV-28) (Fig. 2). One individual aged 8 years (IV-25) had bilateral centro-temporal spikes; at the time of the study, her absences were controlled

Table 1 Clinical details of affected family members

Pedigree reference	Age*	Neurological examination	Seizure onset	Seizure offset	Seizure type	EEG	Epilepsy syndrome	GABRG2(R43Q) mutation
II-2	41	Mild ID	6 months	29 years	FS, A, GCTS, MJ	Bi T S	MAE	Positive
II-3	40	N	1 year	25 years	FS, GTCS	3 Hz GSW	IGE with GTCS	Positive
II-6	54	Psychosis	7 years	–	CPS, GTCS	RT spikes	TLE	Positive
III-2	17	LD	5 years	12 years	A, GTCS	3 Hz GSW	CAE	Negative
III-6	41	N	2 years	3 years	FS	Not done	FS	Not tested
III-7	38	N	18 months	18 months	FS	N	FS	Positive
III-8	43	LD	6 years	15 years	A, GTCS	GSW	CAE	Positive
III-11	38	N	2 years	7 years	FS	LT spikes	FS+	Positive
III-13	28	N	14 years	23 years	GTCS, 1 uncl. sz	N	Uncl.	Negative
III-15	35	N	35 years	35 years	GTCS, 1 unw sz	LT theta	Uncl.	Positive
III-18	23	N	5 years	12 years	FS, A	3 Hz GSW	CAE	Positive
III-20	37	N	10 months	13 years	FS, A, GTCS	N	CAE	Positive
III-24	35	N	11 months	28 months	FS	LT SW	FS	Positive
III-26	32	N	6 months	6 months	FS	not done	FS	Not tested
III-27	31	N	6 months	3–4 years	FS	N	FS	Positive
III-28	30	N	–	–	–	GSW, PS+	EEG trait	Negative
III-29	28	N	13 months	3 months	FS	N	FS	Positive
IV-2	19	N	18 months	18 months	FS	Not done	FS	Not tested
IV-3	17	N	6 months	6 months	FS	Not done	FS	Not tested
IV-5	10	N	6 months	1 year	FS	N	FS	Positive
IV-6	12	N	14 months	14 months	FS	N	FS	Not tested
IV-9	16	N	24 months	30 months	FS	N	FS	Positive
IV-10	12	N	18 months	18 months	FS	N	FS	Positive
IV-14	3	N	–	–	–	3 Hz GSW	EEG trait	Negative
IV-16	12	Mild ID	7 months	9 years	A, DA,GTCS, MJ	GPSW, PS+	MAE	Positive
IV-17	5	LD	10 months	no	FS, A, GTCS	N	CAE	Positive
IV-19	15	LD	11 months	5 years	FS, GTCS	N	FS+	Positive
IV-20	14	N	6 months	2 years	FS	N	FS	Positive
IV-21	11	N	10 months	14 months	FS	N	FS	Positive
IV-25	6	N	3 years	4 years	FS, A	Bi C-T spikes	CAE	Positive
IV-26	5	N	12 months	3 years	FS	N	FS	Positive
IV-27	3	N	18 months	no	FS, A	3 Hz GSW	CAE	Positive
IV-28	3	N	21 months	3 years	FS, A	3 Hz GSW	CAE	Positive
IV-29	1	N	8 months	no	FS, afebrile tonic sz	N	FS+	Positive

CAE = childhood absence epilepsy; FS = febrile seizures; FS+ = febrile seizures plus; MAE = myoclonic–astatic epilepsy; TLE = temporal lobe epilepsy; IGE = idiopathic generalized epilepsy; GTCS = generalized tonic–clonic seizures; A = absences; CPS = complex partial seizures; GSW/GPSW = generalized spike/polyspike waves; MJ = myoclonic jerks; DA = drop attacks PS+ = photic sensitivity; ID = intellectual disabilities; LD = learning difficulties; Bi = bilateral; C-T = centro-temporal; LT = left temporal; RT = right temporal; N = normal; uncl. = unclassified; unw = non-witnessed; *in years, at the beginning of the study

on sodium valproate and could not be elicited by hyperventilation. Two subjects (III-20 and IV-17) had normal EEG recordings at the time of the study (37 and 5 years of age, respectively) and were in remission; earlier records were unavailable.

Of the eight CAE cases, all but one (III-2) had the GABRG2(R43Q) mutation. Interestingly, individual III-2 did not have FS.

Febrile seizures alone (n = 15, see Fig. 1)

There were five females and 10 males whose mean age was 21 years (range 6–41 years) at the time of the study. The mean number of FS was four, ranging from one to 20 (median two). The mean age of onset for FS was 14 months

(median 12 months), and that for offset was 26 months (median 18 months).

EEG was performed in 11 cases (aged from 5 to 38 years, mean 19, median 14) and was normal in 10; only individual III-24 showed left temporal sharp waves. Of the 15 cases with FS alone, 10 underwent DNA testing and all had the GABRG2(R43Q) mutation.

In addition to these 15 individuals, 11 others had seizures with fever, including six with later CAE (see above), three with FS+, one with myoclonic–astatic epilepsy (MAE) and one with IGE with GTCS only (see below).

Febrile seizures plus (FS+) (n = 3, see Fig. 1)

A 39-year-old woman (III-11) had four FS between the ages of 2 and 7 years. A 2-year-old boy (IV-29) had clusters of 10

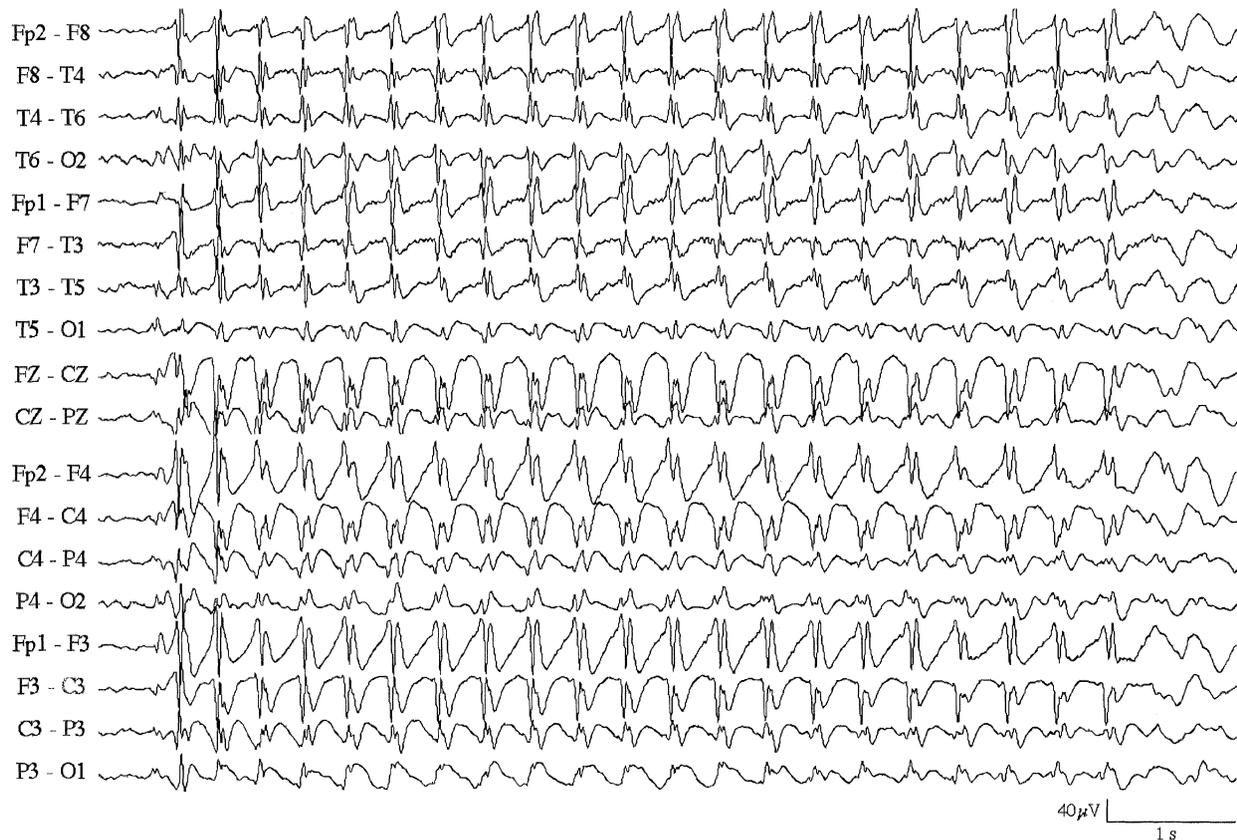


Fig. 2 EEG of a the 3-year-old female (IV-27) with CAE showing typical high amplitude 3 Hz spike and wave with predominance over the anterior regions, after 1 min of hyperventilation. The discharge lasted ~6 s, during which she had a clinical absence seizure

or more GTCS, associated with illness. The majority of GTCS were febrile, but some were afebrile. As he was only 2 years old at the time of the study, his epilepsy syndrome may not have fully evolved. A 15-year-old girl (IV-19) had a total of nine GTCS, five with fever and four without, between the ages of 11 months and 5 years 6 months.

EEG was normal in two and showed left temporal epileptiform activity in one (III-11). All three had the *GABRG2*(R43Q) mutation.

Myoclonic–astatic epilepsy (MAE) (n = 2, see Fig. 1)

A 41-year-old-man (II-2) had ~10 FS from 6 to 12 months. Daily absence seizures with head nods began at 2 years, followed 3 years later by GTCS and occasional jerks in the morning. He was treated originally with phenobarbital and then with phenytoin and carbamazepine. He had ~50 GTCS over a period of 20 years. His epilepsy was controlled at age 29 when sodium valproate was introduced. He had normal development but had difficulty in secondary school. Apart from borderline intellectual function, and facial coarseness, ataxia and nystagmus probably due to chronic phenytoin intoxication, his neurological examination was unremarkable. The

report of an EEG performed at the age of 13 years described spikes during photic stimulation; the actual record was destroyed. The EEG at 41 years showed a slow background with bitemporal independent epileptiform activity. The *GABRG2*(R43Q) mutation was present.

A 14-year-old boy (IV-16) had seizure onset at 8 months with episodes of staring and unresponsiveness for a few seconds occurring 25–30 times a day. A month later, these episodes became associated with generalized stiffening, eyes rolling back, grey colour of the face and incontinence followed by falling. The episodes lasted <1 min and then he would resume his activities immediately. They occurred ~5 times per day. The introduction of nitrazepam stopped the attacks. At around 2 years, marked photosensitivity became evident and staring and drop attacks would only occur in front of the television. At 6 years, myoclonic jerks and GTCS, provoked by photic stimulation, occurred. His seizures were controlled from 9 years on sodium valproate. He had normal early developmental milestones. Concerns regarding learning were evident from kindergarten. WISC III at 10 years showed a borderline intellect with significant difficulties in the verbal domain. His EEGs showed generalized 2–3 Hz spike–waves during rest, and brief discharges of generalized polyspike–waves during rest and photic stimulation. The *GABRG2*(R43Q) mutation was present.

Table 2 Results from CAE affected-only analysis calculated assuming 50% penetrance and a 2% phenocopy rate

Chromosome	Marker	Maximum LOD score	Recombinants
5q33	D5S422	1.34	III-2
10q21	D10S1652	1.27	III-2, III-8
13p11–q12	D13S221	1.97	IV-17
14q22–q23	D14S258	1.36	III-2, III-8
15q11–q13	D15S1002	1.31	III-2, III-18

Generalized epilepsy with tonic–clonic seizures alone (n = 1, see Fig. 1)

A 40-year-old woman (II-3) had 3–4 FS at ~1 year of age. She was well until 13 years, when afebrile tonic–clonic seizures occurred. Prior to the seizure, she would look vague, and on one occasion was witnessed having manual automatisms. Auras, isolated absences and myoclonus were denied. She had several seizures over a period of 12 years. The last one was at the age of 25 years, and she remains on phenytoin and sodium valproate. Her EEG at the age of 7 years showed a 2.5–3 Hz generalized spike–wave during hyperventilation. She had the *GABRG2*(R43Q) mutation.

Partial epilepsy (n = 1, see Fig. 1)

A 55-year-old woman (II-6) developed complex partial seizures of temporal lobe origin at 8 years. Occasional secondary generalized seizures occurred. No details of her early childhood history could be obtained, so the occurrence of FS or absences was not known. Her course was complicated by a chronic paranoid psychosis that required frequent hospitalization and ultimate institutionalization. Her EEG showed epileptiform spikes over the right temporal region. An MRI showed several small foci in the cerebral white matter, most probably due to chronic small vessel disease. No epileptogenic lesions, including hippocampal sclerosis, were identified. She had the *GABRG2*(R43Q) mutation.

Unclassified epilepsy (n = 5, see Fig. 1)

A 36-year-old woman (III-15, second proband) had two GTCS at the age of 35 years. Her EEG within 24 h of the seizures did not show epileptiform abnormalities and her MRI was normal. Her mother was alive and confirmed that childhood seizures did not occur. The *GABRG2*(R43Q) mutation was present.

A 30-year-old woman (III-13) had three GTCS, two at 14 years of age and the third at 24 years. Several EEGs including a sleep study failed to show any epileptiform activity; her MRI was normal. The *GABRG2*(R43Q) mutation was absent.

Three family members (I-4, II-17 and II-18) in the first and second generations were identified as having a history of seizures. Insufficient information was available to diagnose an epilepsy syndrome and they were considered unaffected for the analysis.

EEG studies in unaffected individuals (n = 23)

EEG studies were also performed on 23 clinically unaffected family members (nine married-in individuals) including seven children aged 3–15 years (mean 8 years) and 16 adults aged 28–67 years (mean 45 years). A 3-year-old boy (IV-14) showed 2.5–3 Hz generalized spike–waves. He did not have the *GABRG2*(R43Q) mutation. Of the adults, only a married-in individual (III-28) showed generalized spike–wave discharges during photic stimulation without clinical correlate. She did not have the *GABRG2*(R43Q) mutation. None of the other family members had epileptiform discharges.

Genetic analysis

Genetic analysis of the pedigree was not consistent with X-linked or mitochondrial inheritance, as male to male transmission occurred. Autosomal recessive inheritance was also excluded, as affected members were found in three consecutive generations. FS appeared to have an autosomal dominant mode of inheritance with 75% penetrance. Bilineal inheritance was observed in one branch of the family (II-14 and II-15 offspring); however, this could not be investigated further because individuals II-17 and II-18 did not consent to the study. Two married-in individuals (III-21 and III-28) had a family history of seizures.

Genotyping and identification of *GABRG2* mutation

Family members (I-2, II-2, II-3, III-2 and IV-16) not included in our initial report (Wallace *et al.*, 2001a) were genotyped and examined for the *GABRG2* mutation. Their mutation status is shown in Fig. 1 and discussed under the phenotypes described above. Of those with the *GABRG2* mutation, 64% had FS (alone or as a precursor of other syndromes), 21% had CAE and 15% had a GEFS+ phenotype.

Affected-only analysis for CAE

Genome-wide linkage analysis was performed on the whole family assuming only individuals with CAE were affected (see Methods). Under this model, several regions where linkage to CAE could not be excluded (LOD scores >1.0) were detected (Table 2). After further genotyping in selected regions and re-analysis, no single region of the genome was found to be linked to every CAE case in this pedigree. The original linkage to chromosome 5q was detected and, as mentioned above, III-2 was a recombinant and did not carry

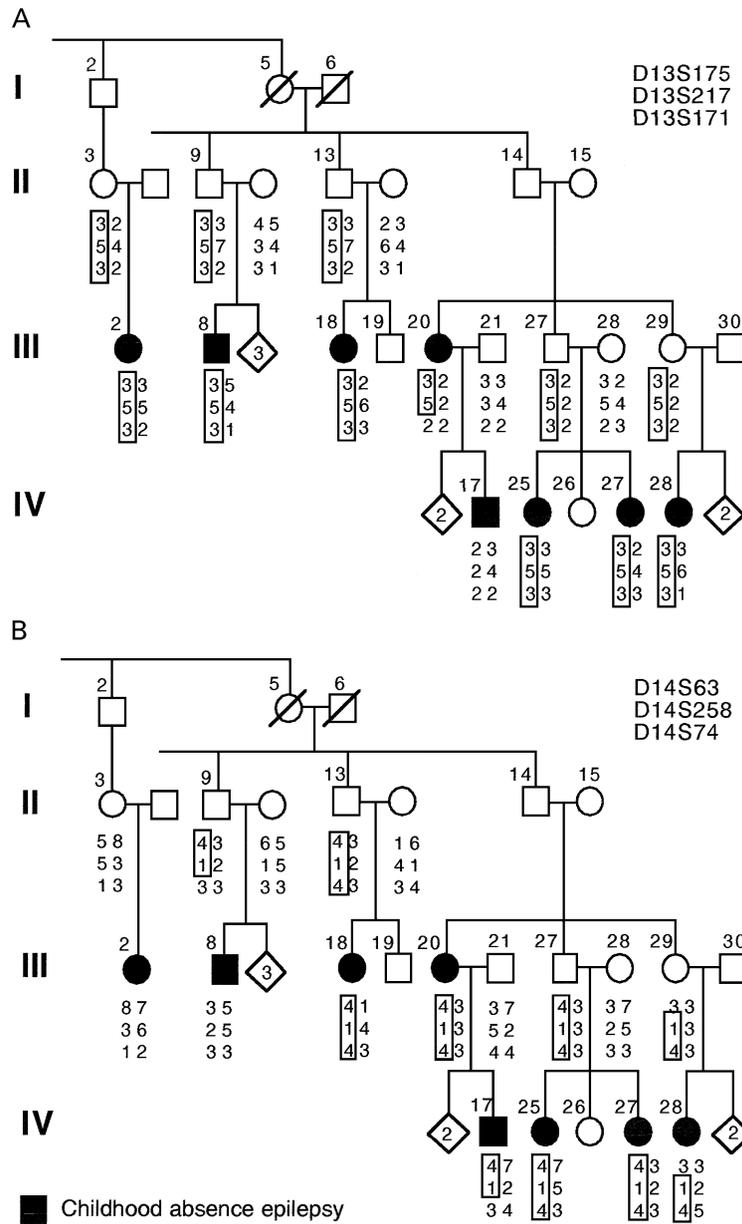


Fig. 3 Affected-only analysis of CAE; other individuals with seizures were regarded as unaffected. Haplotypes are shown for two of the loci identified by linkage analysis. **(A)** Chromosome 13p11–q12: individual IV-17 is the only recombinant at this locus. **(B)** Chromosome 14q22–q23: individuals III-2 and III-8 are recombinants at this locus.

the *GABRG2*(R43Q) mutation. A LOD score of 1.97 was obtained on chromosome 13 and one recombinant (IV-17) was present (Table 2 and Fig. 3A). Three other possible loci were identified where the LOD score was >1, on chromosomes 10, 14 and 15, and all three had two recombinants (Table 2 and Fig. 3B).

Discussion

This large family, where a pathogenic *GABA_A* receptor subunit gene defect has been found, provides insights into the

molecular relationships of FS, FS⁺ and CAE. The size of this family has also allowed us to probe the difficult area of the complex genetics of CAE. Not only do we have strong evidence that the *GABA_A* receptor gene contributes to this phenotype, but we have tentative evidence for a second locus.

Febrile seizures and *GABRG2* mutation

FS are the most common form of childhood seizures, affecting 2–5% of all children (Hauser *et al.*, 1985; Johnson *et al.*, 1996). FS have a major genetic component with

dominant inheritance in some families, but complex inheritance is probably operative in the majority of cases (Rich *et al.*, 1987; Johnson *et al.*, 1996). Three putative loci have been published for FS on chromosomes 8q13–q21, 19p and 5q14–q15 (Wallace *et al.*, 1996; Johnson *et al.*, 1998; Nakayama *et al.*, 2000). No specific genes were identified until we reported a mutation in *GABRG2* on chromosome 5q32–34 in this family where FS is inherited as an autosomal dominant trait with 75% penetrance (Wallace *et al.*, 2001a). All 10 tested individuals with FS alone in our family had the mutation. Additionally, all 11 individuals with FS as a precursor to other syndromes had the mutation.

Generalized epilepsy with febrile seizure plus (GEFS⁺)

GEFS⁺ was described recently as a familial syndrome with a heterogeneous spectrum of phenotypes including FS, FS⁺ and MAE (Scheffer and Berkovic, 1997). Mutations have been found in the genes encoding β and α subunits of the neuronal sodium channel genes (*SCN1B*, *SCN1A* and *SCN2A*) in a number of large pedigrees (Wallace *et al.*, 1998, 2001b, 2002; Escayg *et al.*, 2000, 2001; Sugawara *et al.*, 2001).

Simultaneously with our description of the *GABRG2*(R43Q) mutation in this kindred, a French family with GEFS⁺ was also found to have a different *GABRG2* mutation (Baulac *et al.*, 2001). Interestingly, in our pedigree, there were three individuals with FS⁺ and two with mild MAE, confirming that *GABRG2* mutations can also contribute to GEFS⁺ phenotypes.

These clinical and molecular findings thus challenge us to ask what is the relationship between classical FS and GEFS⁺, and how this family should be classified. In GEFS⁺ families, individuals with a phenotype indistinguishable from classical FS are observed, and some have sodium channel mutations. However, the distinguishing clinical feature of previously described GEFS⁺ families is the common occurrence of the more extended phenotypes such as FS⁺, where seizures with fever extend beyond age 6 years and/or are associated with afebrile tonic–clonic seizures (Scheffer and Berkovic, 1997). In published families, individuals with the extended phenotypes account for 55–89% of cases with identified mutations within each family (Scheffer and Berkovic 1997; Baulac *et al.*, 1999; Moulard *et al.*, 1999; Singh *et al.*, 1999; Escayg *et al.*, 2001; Wallace *et al.*, 2001b). In the present family, amongst those with the mutation, the proportion of those with extended phenotypes was 24% (five extended phenotypes, versus 10 with classical FS plus six with classical FS evolving into CAE).

One interpretation is that the *GABRG2*(R43Q) mutation is a gene for classical FS although it also contributes to the GEFS⁺ spectrum, presumably due to modifying genes. An alternative explanation is that the mutation is fundamentally for GEFS⁺ but where the predominant phenotype is simple FS.

Our recent findings of a small GEFS⁺ family (Harkin *et al.*, 2002) with a truncation mutation in *GABRG2* may clarify this dilemma. The mutation in the present family affects the extracellular benzodiazepine-binding domain, with no impairment of currents to GABA *in vitro* (Wallace *et al.*, 2001a), whereas the mutations in the French and Australian GEFS⁺ families are located in the extracellular and intracellular loops of the mature $\gamma 2$ subunit and cause severe reduction of the current in response to GABA (Baulac *et al.*, 2001; Harkin *et al.*, 2002). Thus, we hypothesize that mutations in the benzodiazepine-binding domains predominantly cause classical FS, while mutations that reduce the response to GABA lead to GEFS⁺. A precedent for this is the phenotype–genotype correlation with the calcium channel subunit *CACNA1A*, where mutations at different sites cause a spectrum of overlapping phenotypes of migraine, ataxia and even seizures (Ophoff *et al.*, 1998). Further analysis of GABA receptor genes in carefully characterized families may clarify the clinical and molecular relationships of GEFS⁺, IGE and classical FS phenotypes.

Childhood absence epilepsy: a specific phenotype?

In this large family, eight individuals had CAE. The phenotype was classical CAE with pyknoleptic absences affecting more girls (six out of eight), associated with typical 3 Hz spike and wave, and remission before puberty. Unusual features were the age of onset of absences before 4 years in five out of eight cases and the earlier occurrence of FS in six.

Although CAE is a well-recognized entity, it does not have crisp phenotypic boundaries and it is unclear whether CAE, as currently defined, comprises one or a number of entities (Berkovic *et al.*, 1987). CAE may overlap with JAE or evolve into JME. Similarly, subtypes have been posited including a form with only self-remitting absence seizures, a subtype with absences and GTCS, a form with absences, GTCS and myoclonic jerks, and also eyelid myoclonia with typical absence epilepsy (EMA) (Appleton *et al.*, 1993; Fong *et al.*, 1998). Doose and colleagues described two types of absence epilepsy of childhood onset: one beginning before 5 years, associated with other seizure types such as myoclonic–astatic seizures, often refractory and with developmental delay and, a second with absences alone, later onset and more favourable course (Doose and Baier, 1973; Doose *et al.*, 1989). The molecular and neurobiological distinction of these clinically posited subtypes presently is unknown.

The genetic relationship between FS and CAE is undoubted. Most studies show that FS occurs in 10–15% of CAE cases (Livingston *et al.*, 1965; Penry *et al.*, 1975; Dieterich *et al.*, 1985; Loiseau, 1992; Berkovic, 1997). In multiplex families with CAE and other IGEs, 25% of affected relatives have FS alone (Italian League Against Epilepsy Genetic Collaborative Group, 1993; Bianchi and the Italian League Against Epilepsy Genetic Collaborative Group,

1995). In our family, the frequency of FS was unusually high in relatives. Also, the observation that six out of eight CAE patients had preceding FS was unusual.

This family also sheds light on the relationship of GEFS⁺ to classical IGE. Previously we described two large GEFS⁺ families where single individuals had typical IGE; those members subsequently were found not to have sodium channel mutations causing GEFS⁺, suggesting that the clinical association was fortuitous (Wallace *et al.*, 1998, 2001b). In the present family, however, GEFS⁺ phenotypes of FS⁺ and MAE were associated with the *GABRG2*(R43Q) mutation, suggesting that there is a genetic relationship between IGE and GEFS⁺ phenotypes in some circumstances. This overlap is supported further by the finding of the mutation in II-13 who had IGE with preceding FS, and in III-8 with CAE without prior FS.

Genetic basis for CAE

The *GABRG2*(R43Q) mutation was found in seven out of eight CAE subjects in this pedigree. The causative role of mutations in *GABRG2* for CAE is also supported by the recent finding of a small German family with CAE and FS in which a splice site mutation of the *GABRG2* gene was found (Kananura *et al.*, 2002). One CAE individual from our family (III-2), who did not have preceding FS, did not have the mutation. She is from a distant branch of the family and was only recently assessed, and thus was not included in the initial linkage study. Presumably, other genes were responsible for her phenotype.

Unlike FS, here inherited in an autosomal dominant fashion, CAE has complex inheritance. This large pedigree allowed testing of a digenic model. We hypothesized that the CAE phenotype in seven individuals of this family was due to the combination of the *GABRG2*(R43Q) mutation with a second gene segregating with the CAE phenotype. In the individual III-2 lacking the *GABRG2*(R43Q) mutation, the putative second gene segregating with CAE is combined with another mutation with the equivalent effect of the *GABRG2*(R43Q) mutation. The unusual density of CAE in this pedigree raised the possibility that we might identify a single second locus.

The affected-only analysis of CAE failed to reveal a single locus segregating with CAE. The most promising finding from a linkage point of view was on chromosome 13q where there was only a single recombinant (IV-17). However, this region is not known to harbour ion channel subunit genes, and no other obvious candidates have emerged. Weaker linkage evidence for a region on 15q was found where there were two recombinants (III-2 and III-8). This region is of interest because it includes other GABA_A subunit genes, but mutation screening has been negative to date. Haplotype analysis of the locus on chromosome 14q22–q23 also revealed two recombinants. The *GPHN* gene, which codes for gephyrin, maps to this region on chromosome 14q. Gephyrin is essential for clustering and localizing GABA receptors in the plasma

membrane (Essrich *et al.*, 1998) and therefore considered a candidate for family members with CAE that map to chromosome 14q22–q23.

The clinical and molecular analysis of this family suggests that the *GABRG2*(R43Q) mutation is a cause of FS and may contribute to GEFS⁺. There is evidence that CAE is explained by an oligogenic or perhaps digenic model (Greenberg *et al.*, 1988a, 1992; S. F. Berkovic, C. Marini, R. H. Wallace, F. L. Phillips, J. C. Mulley, I. E. Scheffer, unpublished results). One of those genes in this family is *GABRG2*, but even in such a high density pedigree there may be a complex interplay of multiple mutations. Nevertheless, this family has provided clues to the putative location of other CAE genes that require further evaluation in other pedigrees.

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