Suggestion of a major gene for familial febrile convulsions mapping to 8q13–21

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Abstract

Febrile convulsions affect 2 to 5% of all children under the age of 5 years. These convulsions probably have a variety of causes, but a genetic component has long been recognised. A large and remarkable family is described in which febrile convulsions appear to result from autosomal dominant inheritance at a single major locus. A gene for febrile convulsions was excluded from regions of previously mapped epilepsy genes and extension of exclusion mapping, using microsatellite markers, to the entire genome implied that a locus on chromosome 8q13–21 may be involved. Linkage analysis of markers on chromosome 8 gave a multipoint lod score of 3.40, maximised over different values of penetrance and phenocopy rate, for linkage between the gene for febrile convulsions and the region flanked by markers D8S553 and D8S279. This lod score was calculated assuming the disease has a penetrance of 60% and a phenocopy rate of 3%. Although there was no indication of linkage other than to markers on chromosome 8, linkage remains suggestive rather than significant because of the maximisation procedure applied. The support for linkage involving a major gene, as opposed to an alternative hypothesis of a complex inheritance pattern, relied upon the assumption of low penetrance.

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Key words: febrile convulsions; chromosome 8; genetic linkage.

Febrile convulsions are seizures usually occurring between 3 months and 5 years of age, associated with fever but without evidence of intracranial infection or other defined cause.¹ They account for the majority of all childhood convulsive disorders, with 2–5% of all children likely to have at least one febrile convulsion.² Although these seizures are benign, in some cases (<4%) afebrile seizures may occur at a later date.³ A family history of febrile convulsions is often found, which could indicate a common environmental or a genetic basis. A range of genetic models has been proposed to explain the reported family history of febrile convulsions including autosomal recessive, autosomal dominant, and polygenic or multifactorial inheritance.⁴ Even if the majority of genetically determined febrile seizures arise from polygenic inheritance, study of rare families in which the disorder is caused by a major

locus may enable its identification by linkage analysis.

Linkage between febrile convulsions and a particular chromosomal region has not yet been established. This is in part owing to difficulty in identifying single large families suitable for a successful linkage study. During a twin study of the epilepsies, a large Australian family with an autosomal dominant pattern of febrile convulsions was ascertained.⁵ Segregation of the phenotype in a single family provides a unique opportunity to identify the chromosomal location of a gene for this important childhood disorder. This eliminates interfamilial genetic heterogeneity as a confounding effect in linkage analysis. Nevertheless, the possibility of intrafamilial genetic heterogeneity remains, and the sensitivity of the analysis is reduced by incomplete penetrance and the possible presence of phenocopies. Despite this, genetic linkage analysis suggested that a locus on chromosome 8q13–21 may be associated with febrile convulsions in this family.

Materials and methods

THE FAMILY

Linkage analysis was performed on a large Australian family spanning three generations (fig 1). Family members underwent a structured interview, developed from a previously published seizure questionnaire⁶ with additional specific questions regarding onset, timing, frequency, and circumstances of febrile seizures.

Twenty-three subjects in this pedigree were identified with seizures and four were excluded from the analysis. Subject IV-8 had seizures associated with an early fatal childhood encephalopathy and was regarded as having a different condition. Subject III-26 had febrile convulsions but is unrelated to the test family; therefore he and his two affected children (IV-37, IV-38) were also omitted.

The other 19 subjects were considered as affected. All but one (II-1) had known febrile seizures. She is now aged 72 years, and therefore information regarding her early childhood may have been inaccurate. As she had a son with definite febrile seizures she was regarded as an obligate carrier of febrile seizures although she was not affected herself.

Of the 18 subjects with documented febrile seizures, 10 had a single attack, two had two attacks, and six had three or more attacks. The age of onset of the febrile seizures was 5 to 72 months (mean 17.6 (SD 16.1) months, median 12.5). One person (II-4) was reported to have had a febrile seizure at the age of 6 years; all

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Pedigree showing a large Australian family with an autosomal dominant pattern of febrile convulsions. Chromosome 8 marker haplotypes are shown, which cover approximately 8 cM in the region 8q13-8q21, between the markers D8S533 and D8S279.

the others had an age of onset at 3 years or younger. The mean age of cessation of febrile seizures was 23 (SD 17) months and the median age of cessation was 18 months. The convulsions were generally brief although six subjects attended hospital for their attacks. Most attacks occurred in relation to suspected upper respiratory tract infections.

Three of the 19 affected subjects had afebrile seizures. Subject II-6 had complex partial seizures and secondarily generalised seizures from the age of 39 years, associated with left hippocampal sclerosis on magnetic resonance imaging. She is treated with carbamazepine but continues to have approximately 10 seizures per year. She had had approximately six febrile seizures from the age of 7 months until 1 year of age. The longest attack was reported to last approximately five minutes. Subject II-1 had two nocturnal seizures at the age of 48 and 50 years and was on carbamazepine. Subject III-24 had two seizures at the age of 17 years following a leptospirosis infection. He is now aged 29 and has had no further seizures, and is not on medication.

GENOTYPING

The genome search for the location of the febrile convulsion gene began with the analysis of markers in candidate regions, based on previously mapped epilepsies.⁷ The search was then extended by choosing additional loci at approximately 20 cM intervals along the remaining chromosomes. Highly polymorphic microsatellite markers were chosen primarily from the Genethon linkage map.⁸ Amplification of 100 ng of DNA was performed using conditions described by Phillips *et al.*⁹ Products were separated on 5% denaturing poly-

acrylamide gels and visualised by autoradiography.

LINKAGE ANALYSIS

Genotyping was initially carried out using only members of the family affected with febrile seizures and their parents (where available). This saved considerable time because linkage to many loci could be excluded rapidly. When linkage to a marker was not excluded using only the affected members, the entire family was genotyped.

The disorder, defined as one or more febrile seizures,¹ was initially analysed as an autosomal dominant trait with 80% penetrance, assuming the frequency of the affected allele to be 0.0001. Two point linkage analysis was performed using the program MLINK, from the LINKAGE package.¹⁰ The lod scores generated by this program were further examined using the EXCLUDE program¹¹ to summarise the exclusions generated by multiple two point lod scores. Any regions of the genome not adequately excluded were highlighted and remained areas of possible linkage assuming autosomal dominant inheritance.

Once the gene was assigned to a chromosome, two point lod scores (using data from the entire family) were recalculated using various conditions. These included a range of penetrances from 60% to 80%, and inclusion of a 3% phenocopy rate. Multipoint lod scores were calculated for the same conditions described above using LINKMAP from the LINKAGE package.¹² Four microsatellite markers were used in each LINKMAP run; for each subsequent run one new marker was incorporated into the analysis, and one marker from the previous run dropped. In this way the disease

Table 1 Summary of results generated by the EXCLUDE program. (A) Initial genome search (275 markers, 80% penetrance); (B) result of genome search with 290 markers (80% penetrance); (C) column B reanalysed at 60% penetrance with a 3% phenocopy rate; (D) only subjects who experienced multiple febrile convulsions included (80% penetrance)

Chromosome	Percent probability of a locus being on a given chromosome			
	A	B	C	D
1	0.13	0.00	0.00	0.29
2	7.91	0.00	0.00	82.06
3	0.22	0.00	0.00	1.23
4	0.01	0.00	0.00	0.00
5	1.62	0.00	0.00	1.74
6	0.67	0.00	0.00	0.25
7	0.04	0.00	0.00	0.10
8	8.42	99.96	100.00	3.24
9	0.10	0.00	0.00	1.35
10	0.72	0.00	0.00	7.95
11	0.00	0.00	0.00	0.18
12	33.40	0.00	0.00	0.56
13	0.01	0.00	0.00	0.50
14	46.21	0.02	0.00	0.00
15	0.24	0.00	0.00	0.23
16	0.12	0.00	0.00	0.04
17	0.01	0.00	0.00	0.00
18	0.00	0.00	0.00	0.03
19	0.13	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
21	0.01	0.00	0.00	0.16
22	0.03	0.00	0.00	0.07

locus was analysed relative to a window of four marker loci at a time.

Results

Initially, areas corresponding to previously mapped epilepsies were examined (6p, 8p, 8q24, 10q, 20q13.2, and 21q22.3). None of the markers in these chromosomal regions indicated linkage to the febrile convulsion gene. Two point lod scores of 275 markers distributed throughout the genome were then analysed using the EXCLUDE program. This calculated the percent probability of a locus being on a given chromosome, with the results presented in a graphic format. The most likely positions for the febrile convulsion gene, by exclusion, were chromosomes 2, 8, 12, and 14 (table 1A) assuming autosomal dominant inheritance of a major locus.

Additional markers were then genotyped from chromosomes 2, 8, 12, and 14, and their lod scores added to the original data. The resulting EXCLUDE map then showed a single peak on chromosome 8, with a probability of 99.96% that the febrile convulsion gene was in this area (table 1B). The pairwise lod scores of markers covering this region gave a maximum of 2.91 at a recombination frequency of 0.05 for the marker D8S543 (table 2A). This lod score was calculated using only the affected members of the family. When these markers were reanalysed including all family members, the lod score for marker D8S543 dropped to 1.16 (table 2B). The maximum lod score obtained when the entire family was genotyped was 2.25 at a recombination frequency of 0 for marker D8S530 (which maps to the same region as D8S543). Penetrance is unknown, hence 80%, as used in this stage of the analysis, must be considered as a very rough approximation.

Based on the assumption that the gene is located on chromosome 8, all of the 16 affected subjects tested had the same genotype, and 13 unaffected subjects were apparent carriers. Three of these (II.1, III.5, III.12) are obligate carriers. This indicated a penetrance of around 60%, rather than 80% as first assumed. Based on this finding the markers in table 2B were reanalysed at 60%, 70%, and 80% penetrance (table 3). The two point lod score rose from 2.25 at 80% penetrance to 2.82 at 60% penetrance.

Another factor that may be affecting the analysis is the presence of phenocopies. Given that the occurrence of febrile convulsions in the population is around 3%, there is the possibility of two or three phenocopies in this large family (88 subjects). One affected subject (III.26) was obviously a phenocopy because he was not related to the family of interest. Although this person and his children were omitted from the overall study, his spouse and two affected children had the chromosome 8 marker haplotype associated with the disease genotype. This suggested that the mother could be a carrier and that her children's febrile convulsions could be the result of inheritance of a gene from their mother. Subject III.32, who had only a single convulsion, may also be a phenocopy because his unaffected father (II.10) does not have the marker haplotype associated with the disease.

To account for possible phenocopies, the data were reanalysed at 60%, 70%, and 80% penetrance with a 3% phenocopy rate (table 3). The inclusion of a phenocopy rate in the two point analysis decreased the lod score, because the affected subjects are now considered possible phenocopies. At 60% penetrance the multipoint lod score determined by LINKMAP analysis rose to 3.40 when a 3% phenocopy rate was included. To ensure the rest of the genome was still excluded when a penetrance of 60% and a 3% phenocopy rate was used, two point lod scores of 290 markers were reanalysed using these conditions. The lod scores were then examined using the EXCLUDE program (table 1C) which showed chromosome 8 was the only likely position of a gene for febrile convulsions in this family, when the penetrance is 60% and the phenocopy rate is 3%. When subject III.32 was omitted from the analysis the lod score rose to 3.41 at D8S543 (60% penetrance). This procedure selectively chooses only those parts of the family that fit the data and therefore cannot be considered proof of linkage. It does, however, give an indication of how this single person has affected the probability that the gene responsible for febrile convulsions is located on chromosome 8. The multipoint lod score of 3.40 previously determined, including III.32 in the analysis, remains suggestive of linkage.

The analysis was also affected by the fact that several of the markers were uninformative for large parts of the family. At present, no additional markers known to us map to this region of chromosome 8. From these data it can only be concluded that this is the most likely position of the gene responsible for febrile

Table 2 Two point lod scores between the febrile convulsion gene and markers on chromosome 8q13–21 (80% penetrance). These markers span a region of approximately 16 cM. (A) Lod scores calculated using affected family members only; (B) lod scores calculated using the entire family

Locus	θ								
	0.0	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θ_{max}
(A) Affected only									
GGAA8G07	-5.27	-2.36	-0.68	0.09	0.53	0.45	0.14	0.54	0.22
D8S260	0.62	0.61	0.58	0.53	0.40	0.25	0.11	0.62	0.00
D8S510	-4.41	-2.18	-0.90	-0.39	-0.01	0.09	0.07	0.09	0.30
D8S512	-0.45	0.43	0.91	0.97	0.80	0.50	0.17	0.97	0.10
D8S544	-2.69	-0.56	0.45	0.67	0.53	0.20	0.04	0.68	0.12
D8S533	-4.21	-0.67	1.00	1.50	1.54	1.03	0.05	1.60	0.16
D8S553	-2.26	-0.42	0.65	0.96	0.96	0.68	0.31	1.01	0.16
D8S543	2.11	2.62	2.91	2.76	2.23	1.47	0.62	2.91	0.05
D8S530	2.52	2.47	2.26	2.00	1.45	0.90	0.39	2.52	0.00
D8S279	-0.77	0.77	1.72	1.90	1.65	1.08	0.41	1.90	0.10
D8S541	-2.28	-0.61	0.64	1.10	1.14	0.76	0.27	1.20	0.16
D8S286	-4.88	-1.83	-0.25	0.45	0.81	0.66	0.31	0.81	0.20
(B) Entire family									
GGAA8G07	-8.89	-4.60	-1.97	-0.73	0.27	0.48	0.24	0.48	0.30
D8S260	0.54	0.55	0.59	0.61	0.55	0.40	0.20	0.61	0.10
D8S510	-7.22	-3.60	-1.70	-0.85	-0.14	0.09	0.10	0.10	0.40
D8S512	-2.84	-2.01	-1.19	-0.75	-0.30	-0.12	-0.06	-0.06	0.40
D8S544	-6.79	-4.33	-2.72	-1.86	-0.97	-0.50	-0.23	-0.23	0.40
D8S533	-7.93	-3.48	-1.23	-0.20	0.63	0.73	0.40	0.75	0.25
D8S553	-2.51	-0.22	1.02	1.46	1.56	1.20	0.61	1.59	0.15
D8S543	-0.88	-0.39	0.39	0.85	1.15	0.99	0.50	1.16	0.21
D8S530	2.25	2.25	2.23	2.13	1.76	1.22	0.59	2.25	0.00
D8S279	-4.19	-2.41	-0.88	-0.11	0.53	0.58	0.27	0.61	0.25
D8S541	-5.42	-3.34	-1.38	-0.38	0.41	0.51	0.26	0.53	0.27
D8S286	-9.35	-5.40	-2.81	-1.44	-0.21	0.20	0.20	0.24	0.35

Table 3 Summary of the maximum lod scores calculated assuming various conditions

Penetrance (%)	Phenocopy rate (%)	Two point		Multipoint	
		Max lod	Marker	Max lod	Marker
60	–	2.82	D8S530	1.84	D8S553-543
60	3	2.53	D8S543	3.40	D8S553-543
70	–	2.64	D8S530	1.22	D8S553-543
70	3	2.24	D8S530	2.88	D8S553-543
80	–	2.25	D8S530	0.84	D8S553-543
80	3	1.86	D8S530	1.91	D8S553-543

convulsions in this family, based on the assumption that a major gene with autosomal dominant inheritance produces the phenotype of one or more febrile convulsions.

One study suggested that families in which people experience single febrile convulsions were best described by the polygenic model of inheritance, and that in families with multiple febrile convulsions the data were consistent with a single major locus model.¹³ Considering this, members of the febrile convulsions family shown in the figure were reanalysed with only those people who experienced unequivocal multiple febrile convulsions coded as affected. Subjects who experienced single convulsions were designated unknown when determining lod scores. Using these conditions the EXCLUDE map suggested a localisation to chromosome 2 as more likely than to chromosome 8 (table 1D). This likelihood reflects the fact that the chromosome 8 markers were less informative than the chromosome 2 marker in this smaller part of the family, and does not exclude the chromosome 8 region. The maximum lod score at this position on chromosome 2 is 1.32 at a recombination frequency of 0.05 for marker D2S142. The small family now defined by this revised and very stringent clinical criteria no longer has the power to detect linkage, with the maximum possible lod score obtainable for a completely linked and fully informative marker being 1.6. The chromosome 2 data are thus meaningless as an

indication for the location of a major gene or of a modifier gene for febrile convulsions.

Discussion

Febrile seizures are common and the proportion which have a genetic basis are probably genetically heterogeneous. In families where some people have later afebrile seizures, at least two clinical patterns are seen. First, febrile seizures may occur in families where there is a high frequency of later generalised epilepsy syndromes. Second, febrile seizures may occur in families where some subjects have temporal lobe epilepsy with associated hippocampal sclerosis. The current family fits into the second group. Our linkage results suggest that a major gene for this pattern of familial febrile convulsions is located on chromosome 8. Exclusion of chromosomal regions to which other epilepsies map indicate that febrile convulsions are not allelic to these disorders. This is not surprising since the epilepsies mapped so far, including three idiopathic generalised and two idiopathic partial epilepsies,⁷ are clinically very different from febrile convulsions.

Exclusion mapping throughout the human genome showed that the most likely position of the febrile convulsion gene is chromosome 8q13–21. All of the affected subjects tested have the same genotype at this position (III-32 was not tested), highly supportive of the assumption that the gene is in this area. However, 13 unaffected subjects (including three obligate carriers) also have the same genotype, suggesting a low penetrance of 60 to 65%. When the possibility of phenocopies was taken into consideration, by incorporating a 3% phenocopy rate, the maximum multipoint lod score was 3.40 between D8S553 and D8S279 (60% penetrance). The region on chromosome 8 flanked by these markers covers approximately 8 cM on the Genethon linkage map.⁸ Maximisation of the lod score over different penetrance and phenocopy values can inflate the lod score. On that basis, these linkage data must be regarded as suggestive rather than significant.

If a major gene for febrile convulsions in this family maps to this region of chromosome 8 it might be identified by the positional candidate approach.¹⁴ Candidate genes in this region of chromosome 8 include corticotrophin releasing hormone¹⁵ and calbindin.¹⁶ Using rat models, studies showed that both of these genes may have some involvement in seizures.^{17,18} Other candidate genes, on the basis of neural expression, include peroxisomal assembly factor-1¹⁹ and peripheral myelin protein-2.²⁰

Detection of linkage would support a hypothesis of autosomal dominant inheritance of a major gene in this family, with the phenotypic manifestations of one or more febrile convulsions. This is conditional upon one or more modifier loci mapping elsewhere, or an unknown environmental effect modifying the expression of a major locus on chromosome 8. If further linkage analyses applied to other families fails to confirm that a febrile convulsion gene maps to chromosome 8, then there are

two possibilities. One is that febrile convulsions are genetically heterogeneous. The other possibility is that the disorder may not be the result of inheritance of a single major locus. The lod score of 2.25 obtained for D8S530 (at 80% penetrance) may merely represent a chance finding, given the large number of two point lod scores that were determined. Some syndromes are presumed to result from the additive effect of a small number of genes. Such a polygenic aetiology is implicated in families with a history of single febrile convulsions.¹³ Alternatively, febrile convulsions may result from a combination of genetic and environmental causes (multifactorial inheritance) or environmental factors alone. With the common occurrence of febrile convulsions in the human population (2 to 5%), the possibility of phenocopies within this family must also be considered. Another possibility is that the Genethon map does not extend to the telomeres of all chromosomes, and that a major gene for febrile convulsions in this family maps to one of these regions of high recombination not covered by the Genethon map.

The first gene responsible for an idiopathic epilepsy has recently been identified.²¹ Localising genes responsible for febrile seizures and epilepsies will have important consequences for diagnosis and treatment. Management and prophylaxis of febrile seizures remains controversial. The establishment of a linkage marker, and eventually a specific gene, would have direct implications in accurate counselling of parents, and in planning rational trials of prophylactic therapy.

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