Genetic mapping of a new Lafora progressive myoclonus epilepsy locus (EPM2B) on 6p22

E M Chan, D E Bulman, A D Paterson, J Turnbull, E Andermann, F Andermann, G A Rouleau, A V Delgado-Escueta, S W Scherer, B A Minassian

Background: Lafora disease is a progressive myoclonus epilepsy with polyglucosan accumulations and a peculiar neurodegeneration with generalised organellar disintegration. It causes severe seizures, leading to dementia and eventually death in early adulthood.

Methods: One Lafora disease gene, EPM2A, has been identified on chromosome 6q24. Locus heterogeneity led us to search for a second gene using a genome wide linkage scan in French-Canadian families.

Results: We mapped a second Lafora disease locus, EPM2B, to a 2.2 Mb region at 6p22, a region known to code for several proteins, including kinesins. Kinesins are microtubule dependent motor proteins that are involved in transporting cellular components. In neurones, they play a major role in axonal and dendritic transport.

Conclusion: Analysis of the present locus in other non-EPM2A families will reveal whether there is further locus heterogeneity. Identification of the disease gene will be of major importance towards our understanding of the pathogenesis of Lafora disease.
degree cousins. Although the exact pedigree structure is unknown in the fourth family, LD6, consanguinity was confirmed through a review of church records. Linkage to the EPM2A locus on 6q24 was excluded in these families, and sequencing of the EPM2A gene revealed no mutations.

All patients followed a typical clinical course with disease onset within a year around the 12th birthday. All had LB on

Table 1  Two-point lod scores for microsatellite markers on 6p22

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sex averaged distance (Kosambi)(M)</th>
<th>Lod score at h values of</th>
<th>Z Max</th>
<th>ℓ Max</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S1653</td>
<td>26.71</td>
<td>0.43</td>
<td>1.07</td>
<td>1.41</td>
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<tr>
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<td>1.52</td>
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<td>1.58</td>
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<tr>
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<td>31.01</td>
<td>16.37</td>
<td>1.82</td>
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<tr>
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<td>18.00</td>
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<td>42.27</td>
<td>20.73</td>
<td>4.14</td>
<td>4.37</td>
</tr>
</tbody>
</table>

*Map locations from the Center for Medical Genetics (http://research.marshfieldclinic.org/genetics/); the sequence of all primers for all markers are in Genbank.
†Physical distance from University of California, Santa Cruz (www.genome.ucsc.edu/) human genome assembly browser (November 2002).
biopsy or at autopsy (fig 1). As an illustrative example, patient A (fig 2) developed normally until the age of 12 years, when he had his first generalised tonic-clonic seizure, followed soon after by the appearance of myoclonic jerks. EEG showed multiple spike and polyspike-slow wave generalised discharges enhanced by photic stimulation. With seizure intractability and mental decline, a liver biopsy was performed (fig 1A), and the diagnosis of LD was made. By the age of 17 years, the patient was bedridden, tube fed and demented. He died at 20 years of age. Brain autopsy was declined, but histology of the spinal cord revealed numerous pathognomonic neuronal LB (shown in reference 2). Patient A had a first cousin who died from LD at 19 years of age. Their fathers are brothers, their mothers sisters, and the fathers are first degree cousins of the mothers (fig 2).

The genome wide linkage scan was performed using Weber panel version 8A (www.marshfieldclinic.org/research/genetics). A total of 387 fluorescently labelled, highly polymorphic microsatellite markers were included in the screening set, with an average heterozygosity of 0.77 calculated from CEPH families. Markers had an average intermarker spacing distance of approximately 10 centimorgans (cM). Chromosomal regions that generated a maximum two point lod score of >1.0 with the disease were genotyped using additional flanking markers. Linkage analysis was performed, specifying an autosomal recessive mode of inheritance with no phenocopies allowed, and a disease allele frequency of 0.001. Marker allele frequencies for the genome wide linkage scan were calculated using the pedigrees being analysed (n = 9-18), and a panel of ninety F-C control individuals were genotyped for five markers at the 6p22 region. Pairwise linkage analysis was calculated using the programs MLINK and ILINK.14–16 In the analysis, inbreeding loops were broken according to Terwilliger and Ott.17 The lod scores were calculated for recombination fractions ranging from 0 to 0.5 and under the assumption that there was no sex difference in recombination rates. Multipoint linkage analysis was performed using GeneHunter (version 2.1_r3 beta)18 19 with inbreeding loops kept intact. Intermarker, sex averaged distances were obtained from published maps.20

RESULTS
The genome wide scan of 387 markers was initiated in the F-C families, and after 303 markers were genotyped, linkage to the D6S1969 microsatellite on chromosome 6p22 was detected with a two-point lod score of 2.84 at a recombination fraction of θ = 0.00. To fine map the putative LD locus, 25 additional microsatellites spanning a region of 16 cM were genotyped in the four families. This resulted in maximum two-point lod scores ranging from 0.89-6.35 for 25 microsatellites at θ = 0.001-0.219 (table 1). The EPM2B markers
are new microsatellites identified in this study (Genbank accession numbers: EPM2B-1, BVO12563; –2, BVO12564; –3, BVO12565; –3, BVO12566; –4, BVO12567; BVO12568). Multipoint linkage analysis was analysed by GeneHunter using the 26 markers spanning a genetic distance of 16 cM. This generated the same maximum homogeneity and heterogeneity lod scores of 5.37 across 13 markers in the critical interval (fig 3) and produced a heterogeneity parameter, \( x \), of 1.00 across this region.

Haplotype analysis revealed a critical recombinant between D6S1567 and EPM2B-1 in the paternal chromosome of individual B, establishing the telomeric boundary of the disease gene region at D6S1567 (fig. 2). This person is unaffected and is 15 years older than the age of disease onset in these families. The centromeric boundary of the critical interval is defined by a recombination event observed in the maternal chromosome of individual C between D6S1588 and D6S1668. Regions of homozygosity shared by affected individuals in all four families allowed further fine mapping of the centromeric border. A nine marker haplotype (D6S1567, EPM2B-1, EPM2B-2, D6S1688, EPM2B-3, GATA137G09, D6S285, EPM2B-4, EPM2B-5) is identical and homozygous in all patients. Loss of homozygosity in the affected individuals in family LD beyond EPM2B-5 places the centromeric boundary at the next marker, EPM2B-6. The final critical interval is therefore between D6S1567 (telomeric) and EPM2B-6 (centromeric), spanning a distance of 2.235.308 bases. A five marker haplotype present in all affected individuals in families LD7, LD27 and LD28 at markers D6S1567, D6S285, D6S1599, D6S1665, and GATA137G06 spanning our critical interval was found to be absent in 180 F-C control genomes; no significant pairwise linkage disequilibrium was observed between these markers (\( P > 0.17 \), alleles <20% frequency were pooled).

**DISCUSSION**

We have identified a second LD gene locus, named EPM2B, at 6p22 based on a study of LD families from a F-C isolate. Affected individuals from these families are probably segregating a single mutation. All four families are of exclusively F-C descent, and all originate from the same region of east Quebec (lower Saint-Lawrence, southern shore; P. Rioux et al, in preparation), their linkage to the new locus is highly significant (two-point maximum lod score = 5.2), and all share the same nine marker disease haplotype. All but two of the patients in this study have died since they were recruited.

The current genetic mapping data localises the EPM2B locus to a 2.2 Mb critical region. Known proteins encoded in this region include the acute myeloid leukaemia associated protein, DEK,25 thyopurine s-methyl transferase (TPMT), responsible for the TPMT deficiency trait,22 and KIF13A, a kinesin heavy chain protein similar to *Drosophila* kinesin-73.26 Kinesins are microtubule dependent motor proteins that are involved in transporting cellular components. In neurons, they play a major role in axonal and dendritic transport.27 Hypothetical proteins in the region include a zinc finger, RING domain containing protein (AL132329) and an amino oxidase domain containing protein (FLJ34109). Mutation analysis of all known and putative genes in the region is in progress.

EPM2A disclosed a novel RER associated glyco-branching enzyme, tyrosinase, phosphatase and recently its first interacting partner. The EPM2B protein should further unravel this biological pathway and elucidate the cellular mechanisms underlying the unique RER associated disturbance of glyco-branching metabolism, the non-apoptotic neurodegeneration, and the florid neuronal hyperexcitability and intractable epilepsy of LD.

Analysis of the present locus in other non-EPM2A families will reveal whether there is further locus heterogeneity. Identification of the EPM2B gene will improve patient diagnosis, prenatal diagnosis and genetic counselling for this devastating epilepsy.

**ACKNOWLEDGEMENTS**

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