A new clinical and molecular form of Unverricht–Lundborg disease localized by homozygosity mapping

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Summary
Progressive myoclonus epilepsy (PME) has a number of causes, of which Unverricht–Lundborg disease (ULD) is the most common. ULD has previously been mapped to a locus on chromosome 21 (EPM1). Subsequently, mutations in the cystatin B gene have been found in most cases. In the present work we identified an inbred Arab family with a clinical pattern compatible with ULD, but mutations in the cystatin B gene were absent. We sought to characterize the clinical and molecular features of the disorder. The family was studied by multiple field trips to their town to clarify details of the complex consanguineous relationships and to personally examine the family. DNA was collected for subsequent molecular analyses from 21 individuals. A genome-wide screen was performed using 811 microsatellite markers. Homozygosity mapping was used to identify loci of interest. There were eight affected individuals. Clinical onset was at 7.3 ± 1.5 years with myoclonic or tonic–clonic seizures. All had myoclonus that progressed in severity over time and seven had tonic–clonic seizures. Ataxia, in addition to myoclonus, occurred in all. Detailed cognitive assessment was not possible, but there was no significant progressive dementia. There was intrafamily variation in severity; three required wheelchairs in adult life; the others could walk unaided. MRI, muscle and skin biopsies on one individual were unremarkable. We mapped the family to a 15-megabase region at the pericentromeric region of chromosome 12 with a maximum lod score of 6.32. Although the phenotype of individual subjects was typical of ULD, the mean age of onset (7.3 years versus 11 years for ULD) was younger. The locus on chromosome 12 does not contain genes for any other form of PME, nor does it have genes known to be related to cystatin B. This represents a new form of PME and we have designated the locus as EPM1B.

Keywords: Epilepsy; myoclonus; gene mapping; photosensitivity

Abbreviations: PME = progressive myoclonus epilepsy; ULD = Unverricht–Lundborg disease

Introduction

Progressive myoclonus epilepsies (PME) are a group of rare disorders that were initially characterized into three groups on pathological grounds: Lafora disease, lipidoses, and the ‘degenerative’ forms (Hodskins and Yakovlev, 1930; Harriman et al., 1955). ‘Degenerative’ referred to forms where light microscopy of the brain revealed only neuronal loss and gliosis, without evidence of neuronal storage. With improved pathological, biochemical and clinical analyses, and application of molecular genetics, numerous specific forms of PME have been identified (Berkovic et al., 1986; Genton et al., 2002). All are characterized by myoclonus, epilepsy and progressive neurological degeneration. Unverricht–Lundborg disease (ULD) (Unverricht, 1891; Lundborg, 1903) and myoclonic epilepsy with ragged-red fibres (MERRF) are the most common degenerative forms.

ULD is an autosomal recessive disease that occurs worldwide, with clusters in the Baltic and Mediterranean regions. Onset is typically at around 10 years of age with myoclonic or tonic–clonic seizures. The myoclonic seizures tend to become more severe and disabling and there is an associated ataxia. Although dementia was thought to be part of the phenotype and is present in most other forms of PME, in well-treated contemporary ULD cases cognitive decline is minimal or absent (Koskineni et al., 1974; Berkovic et al., 1986; Genton et al., 1990; Genton et al., 2002). The disorder maps to chromosome 21 (EPM1) and is due to mutations in the cystatin B gene, an intracellular protease inhibitor (Pennacchio et al., 1996; Lehesjoki, 2003). The mutation found in most patients is an unstable dodecamer repeat located in the cystatin B promoter region (Lafreniere et al., 1997; Lalioti et al., 1997; Virtaneva et al., 1997). These mutations are associated with a deficiency of cystatin B messenger RNA in some cell types (Pennacchio et al., 1996; Lalioti et al., 1997; Lehesjoki, 2003). Other mutations described affect splicing, truncate the protein or are missense mutations resulting in loss of function of cystatin B. Cystatin B-deficient mice have a phenotype resembling ULD and show regional cell loss in the cerebellum and other regions mediated by apoptosis (Pennacchio et al., 1998; Lehesjoki, 2003).

During a systematic study of inherited epilepsies in Israel we encountered ULD with cystatin B mutations in an inbred Arab family (Mazarib et al., 2001). We subsequently studied a second large Arab family in a nearby town with a similar phenotype but, to our surprise, cystatin B mutations were not detected. This report describes the clinical features and outcome of homozygosity mapping and linkage analysis in this large family with complex consanguinity. We localized the gene for this novel form of PME, which we designate EPM1B.

Methods

Ascertainment and clinical studies

The family lives in an Arab town in the Western Galilee region of Israel and the proband and his sister were known to one of us (S.W.). The family was studied by multiple field trips to their town to clarify details of the complex consanguineous relationships and to personally examine the family. DNA was collected for subsequent molecular analyses from 21 individuals.

The study was approved by the Ethics Committee of the Tel Aviv Sourasky Medical Center and informed consent was obtained from participating subjects.

Molecular studies

Cystatin B mutation testing

Samples were examined for the dodecamer repeat expansion in the cystatin B gene as previously described (Mazarib et al., 2001). The polymorphic markers D21S1885, D21S2040, D21S1259, D21S1912 and PFKL (in centromere to telomere order) around the cystatin B gene were genotyped to exclude linkage to this region by observation of recombination events. Coding regions of the three cystatin B exons were PCR-amplified and sequenced in patients VI-9 and VI-11.

Genotyping, homozygosity mapping and linkage analysis

Five DNA samples from affected individuals (V-13, VI-7, VI-9, VI-11, VI-12) (Fig. 1) were submitted to the Australian Genome Research Facility for genotyping using the ABI Linkage Mapping Set v2.5–HD5. This mapping set contains 811 microsatellite markers with an average spacing of 5 cM.

The family in Fig. 1 contains several consanguineous marriages and EPM1B is segregating as an autosomal recessive disorder. Because of the time needed by LINKAGE to analyse such a pedigree (with multiple marriage loops and a large number of missing genotypes), the data were initially sorted using Microsoft Excel to identify homozygous markers. This rapidly mapped the gene. Following manual genotyping (Phillips et al., 1995) of additional markers in all 21 individuals available, linkage was performed using the LINKAGE package of programs (Lathrop and Lalouel, 1984; Cottingham et al., 1993; Schaffer et al., 1994). The exact relationship between members of the earliest generations could not be confirmed and these individuals were therefore considered unrelated. Parameters used were as follows: autosomal recessive, 100% penetrance, disease allele frequency of 0.0001 and equal marker allele frequencies. Marriage loops were broken at individuals IV-6, V-14 and V-16 using the MAKEPED component of the linkage package.

Results

Clinical genetic analysis

The family resided in the Western Galilee region of Israel and were said to have been there from the 12th century. Consanguineous marriages were common in this region and Fig. 1 shows the complex relationships, as determined by interviewing elderly relatives.

A total of eight affected individuals (four males) from four sibships were identified. There were no known affected individuals in earlier generations. Inspection of the pedigree strongly suggested an autosomal recessive mode of inheritance. There were multiple known consanguineous marriages...
Epileptology

The subjects were aged 17–37 years at the time of evaluation. No antecedents for epilepsy were identified. Mean age of seizure onset was 7.3 years (range 5–10 years). Onset was with myoclonic seizures ($n = 5$), tonic–clonic seizures ($n = 1$) or both ($n = 2$). In 4 cases the parents reported delayed walking in infancy with walking or running difficulties in childhood, before the recognition of myoclonic or tonic–clonic seizures. This was assumed to be from cerebellar ataxia, rather than unrecognized myoclonus, but no cases had been examined before seizure onset to verify this.

All developed myoclonic seizures with action myoclonus affecting the limbs and bulbar muscles and spontaneous myoclonic jerks sometimes visible in facial muscles and in the limbs at rest. Jerks were worse in the sun in all. Myoclonic seizures became progressively more severe over years, but the degree of disability varied between subjects. Three were confined to wheelchairs because myoclonic seizures were so frequent and severe. Treatment effects were difficult to assess, but some improvement on valproate and topiramate was reported.

Tonic–clonic seizures occurred in seven cases; these were often nocturnal and did not have an early morning peak. The tonic–clonic seizures were generally infrequent and partially controlled by anti-epileptic medication. Case VI-9 who was the mildest affected and aged 25 years at evaluation, had myoclonic seizures from aged 10 years but had never had tonic–clonic seizures. Neither absence seizures nor focal seizures were reported in any subject.

Apart from the presence of cerebellar signs, particularly involving gait and limb ataxia, there were no consistent neurological abnormalities. There was no hearing loss, optic atrophy, retinal abnormalities, sensory impairment and no consistent findings of pyramidal or extrapyramidal involvement. One individual had bilateral extensor plantar responses of uncertain cause. Deep tendon reflexes were preserved and in some individuals were slightly exaggerated. In 2 non-ambulant individuals there was mild distal leg wasting, pes cavus, shortening of the Achilles tendons interpreted as an effect of disuse.

There was no significant progressive dementia. The assessment of subtle cognitive changes was difficult because of language and cultural factors and because schooling was limited by the epilepsy and social factors. Formal neuropsychological evaluations were not performed. Individual VI-9, the most mildly affected, completed year 12 at school, appeared of normal intellect when examined at age 25 years but had difficulty writing because of action myoclonus. Individual VI-5, examined at age 33, was the most severely
affected and had lived in a hospice since age 27 years. She had normal orientation, naming and picture recognition, remembered three objects at 3 min, but had poor arithmetical skills and only correctly answered the first value with serial 7 testing.

**Clinical investigations**
EEG evaluation of a number of individuals revealed mild diffuse background slowing with generalized spike-wave or polyspike-wave discharges and photosensitivity in some records (Fig. 2). MRI of brain performed on VI-7 was normal (Fig. 3).

Muscle and skin biopsies on individual VI-7 were normal with no evidence of mitochondrial myopathy and no evidence of storage.

**Molecular genetic studies**
Testing for the dodecamer expansion of the cystatin B gene was negative. Sequencing revealed no mutations in the coding region of the cystatin B gene. Evaluation of polymorphic markers closely linked to cystatin B excluded linkage (data not shown). Genome-wide homozygosity mapping followed by linkage analysis of markers within the region identified by homozygosity mapping was carried out in order to establish a gene localization for EPM1B.

It is presumed that a single ancestor carried an EPM1B mutation that was passed through the pedigree. Because of consanguineous marriages, affected individuals have two copies of the same chromosomal region carrying the mutation (i.e. are autozygous with alleles identical by descent). This simplified the initial mapping as only five affected individuals needed to be genotyped in a genome screen and this gave us ample power to detect linkage by homozygosity mapping. Single homozygous markers were identified on several chromosomes; however, chromosome 12 was the only region which contained two consecutive homozygous markers (D12S1663 and D12S85). Genotyping of additional family members demonstrated significant linkage to D12S1663, with a maximum lod score of 6.32 (Table 1). D12S85 was uninformative because of the presence of only a single allele (data not shown). All available family members were subsequently genotyped for additional markers from the chromosome 12 region initially detected by homozygosity mapping.

![Fig. 2 EEG of individual V-13 at age 35 years.](image-url) He had myoclonic seizures from age 8 years and subsequent tonic–clonic seizures. Myoclonus was worse in the sunlight. There are generalized polyspike discharges at rest that are exacerbated by photic stimulation.
Table 1 Two point lod scores calculated from markers on chromosome 12p11-q13 ($\theta$ = recombination fraction). $Z_{\text{max}}$ ($\theta$) is the recombination fraction with the highest lod score.

<table>
<thead>
<tr>
<th>Marker</th>
<th>$\theta$</th>
<th>0.0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
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<tr>
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<td>2.91</td>
<td>3.99</td>
<td>4.22</td>
<td>3.94</td>
<td>3.05</td>
<td>2.00</td>
<td>0.93</td>
<td>0.05</td>
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<tr>
<td>D12S2080</td>
<td>5.73</td>
<td>5.62</td>
<td>5.16</td>
<td>4.59</td>
<td>3.40</td>
<td>2.20</td>
<td>1.02</td>
<td>0.00</td>
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<tr>
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<td>1.53</td>
<td>1.50</td>
<td>1.36</td>
<td>1.17</td>
<td>0.78</td>
<td>0.42</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>D12S1663</td>
<td>6.32</td>
<td>6.20</td>
<td>5.73</td>
<td>5.11</td>
<td>3.84</td>
<td>2.52</td>
<td>1.20</td>
<td>0.00</td>
</tr>
<tr>
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<td>3.96</td>
<td>3.63</td>
<td>3.21</td>
<td>2.34</td>
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<td>0.70</td>
<td>0.00</td>
</tr>
<tr>
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<td>0.82</td>
<td>1.86</td>
<td>2.00</td>
<td>1.68</td>
<td>1.13</td>
<td>0.54</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Distance (in centimorgans) between markers is D12S345 – 0.55 – D12S2080 – 2.74 – D12S1048 – 0.0 – D12S1663 – 6.16 – D12S1701 – 1.35 – D12S1661.

(Table 1 and Fig. 4), giving further support for localization of EPM1B to chromosome 12p11-q13. The upper boundary of the EPM1B region is defined by a recombination between D12S345 and D12S2080 in individual V-17 (Fig. 4) and the lower boundary by recombinations between D12S1701 and D12S1661 in individuals VI-5 and VI-11. The region between D12S345 and D12S1661 spans approximately 15 megabases.

Discussion

The clinical phenotype of this family bears a strong similarity to classical ULD (Table 2). Classical ULD (EPM1) was excluded by the molecular studies. There were subtle points of difference, particularly the earlier mean age of onset and perhaps a slightly more severe course, but in individual patients this would be difficult to discern. We do not have autopsy or biopsy brain material to exclude the possibility of an unusual form of Lafora disease or a lipid storage condition, but the lack of other clinical signs, the preservation of intellect and normal skin and muscle biopsies would make such disorders quite unlikely. We suspect, but cannot yet prove, that this condition would fall into the so-called degenerative group of PME (Berkovic et al., 1986).

It is of interest that, in the Israeli Arab population, where consanguineous marriages are common (Jaber et al., 2000), two clinically similar but genetically distinct forms of PME exist. Families with EPM1 (Mazarib et al., 2001) and EPM1B (this paper) were identified in towns separated by approximately 20 km. This emphasizes the potential confounding effect of genetic heterogeneity in attempts to localize genes by homozygosity mapping in inbred populations, as previously discussed (Miano et al., 2000). However, haplotype analysis of the linked chromosome 12 pericentromeric region clearly demonstrates genetic homogeneity of this family with EPM1B diagnosed from one town.

The locus for EPM1B was mapped to the pericentromeric region of chromosome 12. This region currently contains 47
known genes and numerous predicted genes (NCBI Map Viewer: www.ncbi.nlm.nih.gov/mapview), none of which have an obvious relationship to pathways involving cystatin B biology. Thus, identification of the EPM1B gene may reveal a novel pathway causing the PME syndrome.

Cystatin B mutations are found in most clinically diagnosed ULD cases (Pennacchio et al., 1996; Lafreniere et al., 1997; Lalioti et al., 1997; Virtanева et al., 1997; Lehesjoki, 2003). There remains a residuum of cases, generally sporadic or from small families, where cystatin B abnormalities are not found (this study, for example). It is possible that, once the gene is identified in the present inbred family, it may account for this residuum. This could complete the molecular characterization of the condition described by Unverricht and Lundborg over a century ago (Unverricht, 1891; Lundborg, 1903).

Acknowledgements
Grant support is gratefully acknowledged from the National Health and Medical Research Council, the American Epilepsy Society (Research Initiative Award) and Bionomics Ltd.

References


