Identification of a recombination event narrowing the Lafora disease gene region

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Abstract

Patients affected with progressive myoclonus epilepsy of the Lafora type present during late adolescence with a characteristic EEG pattern and Lafora bodies seen on skin biopsy. The critical region for the Lafora gene has been localised to chromosome 6q24 flanked by the dinucleotide repeat markers D6S292 and D6S420. This study for linkage of markers from the candidate gene region was performed in a previously unpublished family affected with Lafora disease. EEG and skin biopsy evaluation for Lafora bodies were performed on five of eight family members followed for seizure activity. Haplotype and linkage analysis of DNA from five family members were carried out using the nine dinucleotide repeat markers reported in the common region of homozygosity by Serratosa *et al* in 1995. The present study of an additional family affected by Lafora disease has narrowed the 17 cM critical region for the Lafora

disease gene on chromosome 6q24 to a 4 cM region flanked by markers D6S308 and D6S311.

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Patients affected with autosomal recessive progressive myoclonus epilepsy (PME) of the Lafora type (Lafora disease) present during late adolescence with myoclonic seizures.¹ A characteristic electroencephalographic (EEG) pattern and periodic acid-Schiff (PAS) positive inclusions (Lafora bodies) are seen in the myoepithelial cells of the secretory acini of the apocrine glands or in the cells of the eccrine duct on skin biopsy.^{2,3} This is followed by neurological deterioration with death occurring within 10 years of onset. The Lafora disease gene probably encodes a protein important in carbohydrate metabolism as the Lafora bodies result from the abnormal accumulation of polyglucosans in the cytoplasm of cells in the central and peripheral nervous system.^{4,5} Using linkage analysis and homozygosity mapping, Serratosa *et al*⁶ defined a 17 cM region on chromosome 6q23-25 flanked by the dinucleotide repeat markers D6S292 and D6S420 that contained the putative Lafora disease gene (fig 1). We report the narrowing of the candidate gene to a 4 cM region by linkage analysis of markers in a previously unpublished family affected with Lafora disease (fig 2). Subjects II.1, II.2, II.3, and II.4 were evaluated for seizures. Two of the six offspring (II.1 and II.6) were diagnosed by clinical diagnostic criteria including EEG and Lafora bodies seen on skin biopsy. One subject (II.3) had a stereotactic brain biopsy for diagnosis because two skin biopsies had been normal. Subjects II.2 and II.4, aged 30 and 28 years respectively, had similar generalised paroxysmal sharp EEG activity but were negative for Lafora bodies in axillary skin biopsies. Both are mentally normal and are over the age of onset for Lafora disease. High resolution cytogenetic evaluation showed that both II.2 and II.4 had a normal karyotype.

Genomic DNA was extracted from peripheral blood of I.2, II.1, II.2, and II.4. A paraffin embedded brain biopsy specimen was used to obtain DNA from II.3. Haplotype and linkage analysis of DNA from five family members were carried out using the nine dinucleotide repeat markers (D6S292, D6S403, D6S308,

Figure 1 Schematic representation of chromosome 6q, showing the relative location of the polymorphic markers. The genetic map is based on data from the 1993-1994 Génethon human genetic linkage map.*

Figure 2 Pedigree of Lafora disease family. The results of dinucleotide repeat marker analysis from the Lafora disease gene critical region on chromosome 6q are listed below each subject. Markers are ordered from centromere to telomere. Brackets around results of I.1 indicate the inferred haplotypes. The recombination event in II.4 occurred in I.2 between markers D6S308 and D6S310. The haplotype of each parent segregating with the disease gene is blackened.

D6S310, D6S471, D6S314, D6S453, D6S311, and D6S420) reported in the common region of homozygosity on chromosome 6q.⁶ Based on the analysis of four offspring, the paternal haplotypes were inferred (fig 2, I.1). The maternal and paternal blackened haplotype segregated with the disease. The resulting haplotypes for the offspring II.1, II.2, II.3, and II.4 are shown in fig 2. The alleles for markers D6S403 and D6S308 of II.4 were associated with the affected haplotype of the mother whereas markers telomeric to D6S310 were from the normal maternal haplotype. Thus, II.4 had a crossover in the maternal affected chromosome such that the child presumably did not inherit her abnormal gene (fig 2, I.2). This recombination event narrows the critical region for the Lafora disease gene to the region flanked by markers D6S308 and D6S311.

Two point linkage analysis using the LINKAGE program version 5.2 was performed with

the results from the informative markers D6S403, D6S310, D6S314, D6S453, D6S311, and D6S420.⁷ The Lafora disease gene frequency, assuming full penetrance, was varied from 10^{-3} to 10^{-6} having a minor effect on the lod score results. Each locus was assumed to have three alleles occurring with equal frequency allowing present data to be combined with those previously reported. Lod score results showed the highest degree of linkage in this family between the disease locus and locus D6S311 (lod=0.852 at $\theta=0.0$). Lod score results in this family may indicate that the Lafora disease gene lies closer to chromosome 6q distal marker D6S311 than to D6S308. Combining scores from nine informative families previously reported⁶ with the present results gives a lod score of 11.392 ($\theta=0.0$) for marker D6S311.

Subjects II.2 and II.4 were not diagnosed with typical Lafora disease; however, they did inherit one disease associated haplotype. Consanguinity was denied, although it could not be ruled out considering the two affected members of this family (II.1 and II.3) showed homozygosity for D6S308 to D6S420. In the present study, an additional family affected by Lafora disease has narrowed the 17 cM critical region for the Lafora disease gene on chromosome 6q23-25 to a 4 cM region flanked by markers D6S308 and D6S311.

- 1 Lehesjoki AE, Koskineniemi M, Pandolfo M, et al. Linkage studies in progressive myoclonus epilepsy: Unverricht-Lundborg and Lafora's diseases. *Neurology* 1992;42:1545-50.
- 2 Busard HLSM, Gabreëls-Festen AA, Renier WO, Gabreëls FJM, Stadhouders AM. Axilla skin biopsy: a reliable test for the diagnosis of Lafora's disease. *Ann Neurol* 1987;21:599-601.
- 3 Carpenter S, Karpati G. Sweat gland duct cells in Lafora disease: diagnosis by skin biopsy. *Neurology* 1981;31:1564-8.
- 4 Sakai M, Austin J, Witmer F, Trueb L. Studies in myoclonus epilepsy (Lafora body form). II. Polyglucosans in the systemic deposits of myoclonus epilepsy and in corpora amylacea. *Neurology* 1970;20:160-76.
- 5 Yokoi S, Austin J, Witmer F, Sakai M. Studies in myoclonus epilepsy (Lafora body form). I. Isolation and preliminary characterization of Lafora bodies in two cases. *Arch Neurol* 1968;19:15-33.
- 6 Serratos JM, Delgado-Escueta AV, Posada I, et al. The gene for progressive myoclonus epilepsy of the Lafora type maps to chromosome 6q. *Hum Mol Genet* 1995;4:1657-63.
- 7 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1994;81:3443-6.
- 8 Gyapay G, Morissette J, Vignal A, et al. The 1993-1994 Génethon human genetic linkage map. *Nat Genet* 1994;7:246-339.