spina bifida. In studies of births in Great Britain prior to 1979, the prevalence of spina bifida at birth was within the range of 1.5–4.1 per 1,000 births, and, in studies in the early 1980s, the prevalence was within the range of 0.7–1.9 per 1,000 births (Little and Elwood 1992). Some of these infants would have been stillborn. For example, in Northern Ireland during the period 1974–79, 15.5% of 569 cases of spina bifida or encephalacoele were stillborn (Little and Nevin 1989). In Glasgow and Liverpool during the period 1980–92, when fetuses from terminated pregnancies were excluded, 16% of 262 cases of spina bifida were recorded to have resulted in fetal deaths (EUROCAT Working Group 1995). Therefore, it appears that the proportion of cases of childhood cancer with neural tube defects is similar to what would be expected on the basis of data on the prevalence of these defects at birth, in Great Britain.

In the study by Narod et al. (1997), eight of the children with tumors of the brain or of the spinal cord were recorded as having spina bifida, compared with the 5.6 expected on the basis of the frequency of spina bifida among children with other types of cancer in Great Britain and with the 2.4 expected on the basis of the data for British Columbia. Again, the proportion of children with tumors of the brain or of the spinal cord who were recorded as having spina bifida (1.7 per 1,000 births) would appear to be within the range of prevalences at birth reported for Great Britain during the period in which the children included in the study by Narod et al. would have been born. Thus, the study by Narod et al. does not appear to support the hypothesis of a common maternal factor for brain tumors and spina bifida.

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Reply to Little

To the Editor:

My colleagues and I thank Dr. Little (1997 [in this issue]) for his important data. We saw an excess of neural tube defects in children with cancer, in the United Kingdom, compared with healthy controls from British Columbia. It is unclear to what extent the control group from British Columbia was comparable to the children from Britain, and our approach is inadequate when the baseline rates of disease differ for the two countries. Unlike the rates of other malformations, the rate of spina bifida was not significantly greater in children with solid tumors than in those with leukemia. We agree that our data do not allow us to conclude that there is an excess of cancer among children with neural tube defects.

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Reference

Little J (1997) Childhood cancer and neural tube defects. Am J Hum Genet 61:1204–1205 (in this issue)

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Lafora Progressive Myoclonus Epilepsy: Narrowing the Chromosome 6q24 Locus by Recombinations and Homozygosities

To the Editor:

Lafora disease (LD) is an autosomal recessive and rare but fatal epilepsy syndrome characterized by stimuli-sensitive myoclonus, absence and grand mal seizures, progressive intellectual and neurological deterioration, and periodic acid Schiff (PAS) stain–positive intracellular inclusion bodies. Eighty-four years after Gonzalo Lafora

(1911*a,* 1911*b*) first described such PAS-positive "intracellular amyloid bodies" in the CNS of a young adult who died from a progressive myoclonus epilepsy, we encountered extended areas of homozygosities in chromosome 6q23-25 in nine LD patients who were products of consanguineous marriages (families LD1, LD4, LD5, and LD9). We also detected significant linkage to chromosome 6q23-25 microsatellites in one large inbred family, LD9, and thus localized the LD gene to a 17-cM interval on chromosome 6q23-25, between D6S292 and D6S420 (Serratosa et al. 1995).

To reduce the size of the 17-cM candidate region, we have studied an expanded series of 39 biopsy-proved LD patients who belong to 26 unrelated families (12 inbred) from Spain, Canada, France, the United States, Palestine, Iran, Ecuador, and Saudi Arabia. We provide further proof for significant linkage of LD to chromosome 6q24 in a second and new large inbred family (LD33). Homozygosities and recombinations in six new informative families reduce the size of the previously reported 17-cM LD interval to 2.7 cM flanked centromerically by D6S1003 and telomerically by D6S311.

The clinical diagnosis of LD was initially established by the referring physician and was corroborated by the senior epileptologist in this study. PAS-positive inclusion bodies were demonstrated in skin and/or muscle and in liver and/or brain biopsies of all affected family members, including affected individuals carrying recombinant chromosomes. High-molecular-weight DNA was extracted either from 10 ml of venous blood from living family members, by use of phenol/chloroform followed by isopropanol precipitation (Sambrook et al. 1989), or from 200 μl of peripheral blood by use of the QUIAamp blood kit (Qiagen). DNA from deceased family members (LD9-10, LD9-12, LD9-16, LD18-3, and LD19-3) was extracted from paraffin-embedded archived autopsy specimens of liver, brain, and muscle (Jackson et al. 1990; Greer et al. 1991). All primers for amplification were obtained from Research Genetics. The method of Weber and May (1989) was used to type highly polymorphic short tandem repeats or microsatellites (heterozygosity >.7) in 50 parents and in 39 affected and 56 unaffected individuals.

Parametric or model-dependent two-point linkage analyses using LINKAGE 5.1 (Ott 1974) were performed in eight multiplex families (LD3, LD4, LD6, LD9, LD12, LD27, LD28, and LD33) and in five simplex consanguineous families (LD1, LD5, LD7, LD22, and LD25). We estimated the frequency of the disease allele to be .001, and penetrance was set at 100%, assuming an autosomal recessive model. The gene mutation rate was set at 0. We calculated LOD scores at recombination fractions ($\theta_{m=f}$). We performed multipoint linkage analyses in family LD33, using a new software package, GENEHUNTER (Kruglyak et al. 1996).

We first looked for recombinations and homozygosities (Lander and Botstein 1987) in families LD9 and LD33, because they provided independent proof for linkage to chromosome 6q24. We had previously published significant LOD scores obtained during two-point analyses in family LD9 (Serratosa et al. 1995). We used the new generation of microsatellites in family LD9 but did not reduce the size of the 17-cM LD region, flanked centromerically by D6S292 and telomerically by D6S420, that we had reported in 1995.

In family LD33, the LOD score for D6S1703 was 3.24 ($\theta_{m=f} = 0$) during two-point analyses (Ott 1974), exceeding the threshold for significance. We also computed 10-point LOD scores (Kruglyak et al. 1996) in family LD33, against a fixed genetic map with nine markers (D6S308, D6S409, D6S1003, D6S1010, D6S1703, D6S1042, D6S311, D6S978, and D6S420) in an 11-cM region surrounding the LD gene. During multipoint analysis, we obtained maximum location scores of 4.03 for markers D6S1010, D6S1703, and D6S1042, which are situated between D6S1003 and D6S311.

Recombinations and homozygosities in LD33 were consistent with results of two-point and multipoint analyses and reduced the size of the LD-gene region to the interval flanked by D6S1003 and D6S1687 (fig. 1). Homozygosities in all three living affected members (see haplotypes of LD33-3, LD33-5, and LD33-6; fig. 1) involved 20–27 microsatellites, covering 13–17 cM. These homozygosities indicated that the three affected individuals inherited two copies of the same mutation from a common ancestor—in this case, a grandmother—six generations earlier. A recombination between D6S1553 and D6S1687 in LD33-6 determined that the telomeric border of the LD region is D6S1687. In addition, a recombination centromeric to the LD locus, between D6S1003 and D6S1010, in individual LD33-3 further identified the centromeric border of the LD region, as being D6S1003. These two recombinations (see fig. 1, *arrows*) effectively reduced the critical LD interval, to ~7 cM flanked centromerically by D6S1003 and telomerically by D6S1687.

Our second level of analyses looked at families whose extended regions of homozygosities strongly supported the presence of an LD locus in chromosome 6q24, even though the small sizes of their families precluded LOD scores from reaching significance. Homozygosities in families LD20 and LD22 show the centromeric flanking marker to be D6S308 and D6S403, respectively (see fig. 1). Data on LD22 are not shown. These observations verify the general vicinity of the centromeric border of the LD region, since D6S403 and D6S308 are <2 cM from D6S1003. They lend support to the observation, in family LD33, of D6S1003 as the centromeric flanking marker.

Three proofs support D6S311 as the telomeric border

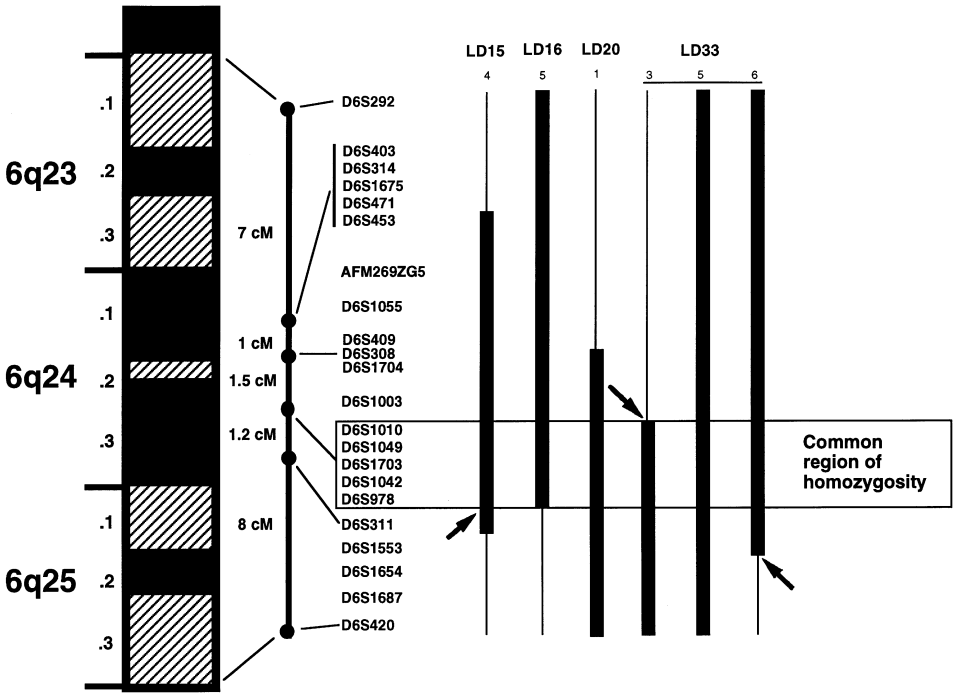

Figure 1 Map of extended regions of homozygosity and recombinations (*arrows*) in six LD-affected subjects. Blackened bars denote chromosome 6q23-25 regions of homozygosities in member LD15-4, member LD16-5, member LD20-1, and members LD33-3, LD33-5, and LD33-6. Common regions of homozygosities locate the LD gene between D6S1003 and D6S311. The borders of homozygosities in members LD33-3 and LD16-5 provide the centromeric (D6S1003) and telomeric (D6S311) flanking markers. To obtain the order of microsatellites in chromosome 6q23-25, we used 27 YAC clones to construct an 8-cM YAC contig that oriented the microsatellites located in the interval between D6S292 and D6S409. To determine the order of markers telomeric to D6S409, we analyzed phases and recombinations in members of the LD families. Our results with regard to the order of microsatellites in the interval spanned by D6S292 and D6S420 were consistent with the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (1997) (http://www-genome.wi.mit.edu/) and Stanford Human Genome Center (1997) (http://shgc-www.stanford.edu) physical maps. D6S409 was not represented in the Whitehead Institute map but was illustrated in the Généthon map (Chumakov et al. 1995; Dib et al. 1996). The Whitehead Institute map, on the other hand, placed D6S1003, D6S1010, D6S1049, D6S1703, and D6S1042 (markers that were not included in the Généthon map) centromeric to D6S311. The correct location of D6S409 was determined by our YAC contig and was consistent with the order of markers in the Généthon and CHLC-Marshfield maps. According to our YAC contig, D6S1003 is telomeric to D6S409, and, according to the Whitehead Institute and Stanford physical maps, the marker D6S1003 is centromeric to D6S311. On the basis of these maps and our YAC contig, we inferred that the location of D6S1003 is between D6S409 and D6S311. These latter two markers, D6S409 and D6S311, and the markers between them—namely, D6S1003, D6S1010, D6S1049, D6S1703, and D6S1042—define an interval of 2.7 cM, according to the Généthon map (Chumakov et al. 1995; Dib et al. 1996) and the CHLC-Marshfield map.

of the LD gene. First, homozygosities in family LD15 identify the telomeric border as D6S1553, and results for family LD16 cut the LD region further and identify D6S311 as the telomeric flanking marker (see fig. 1). Second, another family, LD17, has loss of homozygosity at the telomeric end in D6S311, but we were unable to genotype for the new generation of markers in the interval spanned by D6S1003 and D6S311, because of the minute amounts of DNA obtained from archived paraffin-embedded tissues. Although the genotypes for these new microsatellites are missing, the existing data support D6S311 as the telomeric flanking marker in family LD17 (data not shown). Third, a recombination between D6S311 and D6S978 in family LD15 (see fig. 1, *arrows*) provides further proof that D6S311 is the telomeric border of the disease gene.

In summary, we reduced the size of the LD interval to 2.7 cM flanked by D6S1003 and D6S311, by (*a*) correlations between recombinations and homozygosities in a new large family (LD33), which, by itself, independently proved linkage to chromosome 6q24 microsatellites, (*b*) extended area of homozygosities in affected members of smaller families (LD15, LD16, LD17, LD20, and LD22), and (*c*) a recombination in family LD15.

What kind of gene might be responsible for Lafora progressive myoclonus epilepsy? If the gene responsible for LD is involved in the degradation pathways of glycoprotein metabolism (Lafora 1955; Schwarz and Yanoff 1965*a*, 1965*b*; Yokoi et al. 1968; Sakai et al. 1970; Gambetti et al. 1971; Schwarz 1977; Federico et al. 1980), the alpha fucosidase-2 gene (FUCA2), located on

chromosome 6q24, would be a candidate gene. FUCA2 is tightly linked to the protein marker, plasminogen (Eiberg et al. 1984), which, in turn, is genetically linked to chromosome 6q (Murray et al. 1987).

A second candidate gene that maps to chromosome 6q22.3-q24 is that for L-isoaspartyl/D-aspartyl protein methyltransferase, or protein carboxyl methyltransferase 1 (PCMT1) (MacLaren et al. 1992), which is involved in repair of proteins (Ota et al. 1988). PCMT1 catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to the free carboxyl groups of D-aspartyl and L-isoaspartyl residues, which represent sites of covalent damage to aging proteins. LD may represent a disorder of protein repair, and the "intracellular amyloid inclusion bodies" could be evidence of impaired protein repair (Tsai and Clarke 1994).

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