A Locus for Primary Lateral Sclerosis on Chromosome 4pter-4p16.1

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Background: Primary lateral sclerosis (PLS) is an adult-onset upper motor neuron disease resulting in spinal and bulbar spasticity. A family with 8 individuals diagnosed with PLS was previously reported.

Objective: To identify a locus for a large family with PLS.

Methods: A 550-marker whole-genome scan was performed on this family followed by fine mapping with sequence-tagged site markers to identify a candidate region.

Results: A locus was identified for this family between the telomere of chromosome 4 and marker D4S2928 (4pter-4p16.1). A maximum lod score of 3.01 was obtained for marker D4S2936. The region spans 23.17 cM (10.2 megabase pairs) and encompasses 130 genes.

Conclusions: PLS1 does not map near any other identified loci for upper or lower motor neuron diseases and thus represents a novel locus for PLS.

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Primary lateral sclerosis (PLS) is characterized by spinal and bulbar spasticity due to degeneration of upper motor neurons. Criteria for the diagnosis of PLS include disease onset as an adult and gradually progressing spastic paresis with symmetrical distribution, usually commencing in the lower limbs, though upper limb and bulbar onset does occur. Few families segregating this disorder have been reported. Mutations have been identified in ALS2 in juvenile PLS, but no genes have been identified for patients with adult-onset PLS. A related family of diseases affecting upper motor neurons are the hereditary spastic parapareses (HSPs). Hereditary spastic paraparesis and PLS are generally distinguished by the absence or limited involvement of the bulbar and upper limb muscles in the former. The HSPs comprise a large group of inherited neurological disorders, with at least 14 genes and 33 loci identified to date. This genetic diversity has not been previously observed in adult-onset PLS, primarily because 1 of the initial diagnostic criterion was the absence of family history. However, we recently identified and described a large French Canadian family with progressive involvement of upper motor neurons consistent with PLS. A genome-wide scan was performed on this family and yielded a unique locus for PLS on chromosome 4.

Methods

Blood samples were collected from 18 individuals (8 affected individuals) who all signed a consent form, which was approved by the ethics review board of the Centre Hospitalier Affilié Universitaire de Québec. Phenotypic details of these family members have been described previously. A 550-marker, 8-cM whole-genome scan was performed on DNA samples from 10 individuals by deCODE Genetics (Reykjavik, Iceland). Genome scan results were analyzed using Genehunter, version 2.1 (Whitehead Institute, Cambridge, Massachusetts), with an autosomal dominant mode of inheritance, a disease frequency of 1 in 10,000, 90% disease penetrance, equal allele frequencies, and equal male-to-female recombination rates. Subsequent 2-point analysis was performed using the MLINK program from the LINKMAP software package (GSF Software, Coral Gables, Florida). Additional markers were genotyped by polymerase chain reaction using radiolabeled α-sulfur-35-2′-deoxyadenosine 5′-triphosphate and were loaded on denaturing polyacrylamide gels, 6%. Polymerase chain reactions were performed using 50 ng of DNA and amplified on Perkin
Elmer 9600 thermocyclers (Global Medical Instrumentation, Ramsey, Minnesota) using the following protocol: DNA melting at 94°C for 5 minutes followed by 30 replication cycles (30 seconds at 72°C, 40 seconds at 55°C, and 40 seconds at 72°C) and a 10-minute final extension step at 72°C. Polymerase chain reaction products were sequenced at the Genome Quebec Centre for Innovation.

**RESULTS**

Loci for ALS1, ALS2, SPG17, and SAX1 were previously excluded by genotyping markers surrounding the loci.5 SPG3A, SPG4, and ALS1 were excluded as well by genetic testing. An X-linked inheritance pattern was excluded based on the evidence of male-to-male disease transmission. A whole-genome scan performed on 8 affected and 2 unaffected family members yielded a maximum lod score of 2.65 at marker D4S412. Two other regions had lod scores greater than 1 but less than 1.5 (Figure 1). One region on chromosome 16p12.3 was excluded when reconstructing haplotypes by hand. Another region on chromosome 10p15.3 through 10p15.1 was excluded after fine mapping with additional markers revealed that the haplotype did not overlap for all affected individuals. This was confirmed by genotyping the

**Figure 1.** Genehunter, version 2.1 (Whitehead Institute, Cambridge, Massachusetts), multipoint results for all autosomal chromosomes performed in a genome-wide scan in the PL17 family. The 3 chromosomes with lod scores above 1 (4, 10, and 16) were followed up with fine mapping to define a candidate region on chromosome 4p.
markers D10S1745, D10S1142, D10S1706, D10S552, D10S1729, and D10S1713. On chromosome 4p, 9 additional markers that surrounded the positive markers from the genome scan were selected to delineate the locus boundaries. All affected individuals share 6 markers, which span 10.2 megabase pairs, or 23.17 cM, from the telomere of chromosome 4 to marker D4S2928 (Figure 2). The maximum lod score obtained when genotyping all individuals in this family was 3.01 at marker D4S2936 (Table). This segregating disease haplotype contains 130 known and predicted genes based on the University of California–Santa Cruz genome browser March 2006 update. The myosin regulatory light chain 5 gene (MYL5) was selected for immediate sequencing based on its expression in the brain and its relation to the KIF5A molecular motor gene responsible for SPG10.7 No mutations were identified in the coding region of this gene or in any intronic regions at a minimum of 50 base pairs from exons.

Analysis of the genome scan and subsequent fine mapping show that 1 clear disease locus is present for this family, the first such locus described for PLS. The rarity of families with diagnosed PLS makes it difficult to narrow the search for the causative gene on chromosome 4p. However, the overlap between various motor neuron diseases is often extensive, so it is quite plausible that the gene on
chromosome 4p that causes disease in this family may also predispose other families with diagnosed HSP or amyotrophic lateral sclerosis. In this regard, no previous locus responsible for a motor neuron disease has been identified on the p arm of chromosome 4, including HSP, amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal and bulbar muscular atrophy.

Individuals with diagnosed PLS typically have a sporadic form of the disease (ie, no family history). The disease may still have a monogenic basis in some of these individuals, and thus studied patients with sporadic PLS will be used to aid in the gene-identification process. Nonetheless, the region contains a considerable number of genes that prevent full-fledged candidate gene sequencing at present. The identification of a gene on chromosome 4p that causes PLS would aid in the understanding of this disease and its context with respect to other motor neuron diseases.

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REFERENCES

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