Linkage of Familial Amyotrophic Lateral Sclerosis With Frontotemporal Dementia to Chromosome 9q21-q22

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**Context** Occasionally, 2 or more major neurodegenerative diseases arise simultaneously. An understanding of the genetic bases of combined disorders, such as amyotrophic lateral sclerosis (ALS) with frontotemporal dementia (FTD), will likely provide insight into mechanisms of these and related neurodegenerative diseases.

**Objective** To identify loci that contain genes whose defects cause ALS.

**Design** A genome-wide linkage analysis of 2 data sets from an ongoing study begun in the mid-1980s at 4 university research centers.

**Subjects** An initial subset of 16 families (549 people) potentially informative for genetic analysis, in which 2 or more individuals were diagnosed as having ALS, identified from a Boston data set of 400 families and 4 families potentially informative (244 people) subsequently identified from a Chicago data set of more than 300 families to test a hypothesis based on findings from the Boston families.

**Main Outcome Measures** Linkage calculations assuming autosomal dominant inheritance with age-dependent penetrance (a parametric logarithm-of-odds [lod] score of 1.0 or greater required for further study of a potential locus); crossover analysis involving the ALS-FTD locus.

**Results** In a set of families in which persons develop both ALS and FTD or either ALS or FTD alone, a genetic locus that is linked to ALS with FTD located between markers D9S301 and D9S167 was identified on human chromosome 9q21-q22. Families with ALS alone did not show linkage to this locus. Crossover analysis indicates this region covers approximately 17 cM.

**Conclusion** These data suggest that a defective gene located in the chromosome 9q21-q22 region may be linked to ALS with FTD.

MYOTROPHIC LATERAL SCLEROSIS (ALS) is a progressive disease characterized by degeneration of both upper and lower motor neurons. Typically, it develops as a pure motor syndrome. However, in rare cases (probably <5%) ALS arises concurrently with other neurodegenerative phenotypes such as frontotemporal dementia (FTD) or other extrapyramidal or cortical and subcortical syndromes. While most cases of ALS occur in unrelated individuals (sporadic ALS), familial inheritance is observed in about 10% of cases (FALS). Analogously, cases of neurodegenerative overlap syndromes may also arise on a familial basis. Thus, pedigrees have been reported with ALS-FTD and with combinations of ALS, Parkinson disease (PD), and dementia. Recently, genetic analyses have documented that several pedigrees with FTD, extrapyramidal manifestations suggestive of PD, and amyotrophy (a combination designated here as FTD-PD) arise from mutations in the MAPT gene, which encodes the tau protein. The cause of most cases of ALS is unknown. About 25% of FALS cases are caused by defects in the gene encoding copper-zinc cytosolic superoxide dismutase (SOD1). To define the pathogenesis of other FALS cases, we conducted genome-wide linkage analysis involving 16 pedigrees. In the course of this screening, we identified a new locus for a subtype of ALS in which, in the same family and often in the same individuals, ALS is conjoined with FTD.
METHODS

Family Data

Families included in this study fulfilled the El Escorial criteria for the diagnosis of ALS, with the exception that we included rare pedigrees with other neurodegenerative features such as FTD. The combined Boston and Chicago data sets now include more than 700 collected families (each with at least 2 individuals diagnosed as having ALS). Families known to have mutations in SOD1 were excluded from this study. Simulated linkage analysis with the program SIMLINK was used to identify an initial screening subset of 16 families potentially informative for genetic analysis from the 400 families in the Boston collection. This subset of families includes 549 people, of whom 93 were affected with either ALS or ALS and FTD. DNA was available from 305 persons, including 36 affected individuals and 44 “married-in” spouses. Because of the late onset and short duration of this disease, many individuals were not available for study. Based on findings from the Boston families as described in the “Results” section, 4 families from the Chicago data set were subsequently analyzed. These Chicago families include 244 people, of whom 38 were affected with ALS or FTD or both. The institutional review boards of the involved institutions approved this study. Informed consent was received from all participating patients. To protect patient confidentiality, the haplotype data essential for assessing the limits of candidate gene location are summarized herein in tabular form. Full pedigree and haplotype information was provided to reviewers and is available to researchers from the authors on request as appropriate.

Marker Analysis

DNA was isolated from whole blood or cultured lymphoblasts and analyzed by polymerase chain reaction (PCR) amplification using standard techniques. Markers spaced across the entire genome were selected from the Cooperative Human Linkage Center Human screening set, Weber version 8 and 8A (Center for Medical Genetics, Marshfield, Wis), and the ABI Prism screening set (Perkin Elmer, Foster City, Calif). Additional markers used to narrow the region of interest were chosen based on mapping information publicly available from the Whitehead Institute/MIT Center for Genome Research. Primers for PCR were obtained as Map Pairs from Research Genetics (Huntsville, Ala) or custom synthesized by Research Genetics or GIBCO (Gaithersburg, Md). For allele identification, the PCR products were separated and scored using automated DNA sequencing equipment from LiCor (Lincoln, Neb) or Perkin Elmer. Haplotype construction was done by hand and confirmed using SimWalk2 version 2.54.

Linkage Analysis

The MLINK and LINKMAP subprograms of the program LINKAGE (FASTLINK, version 4.0, National Center for Biotechnology Information) and the program VITESSE (Division of Statistical Genetics, University of Pittsburgh, Pittsburgh, Pa) were used to calculate pairwise and multipoint logarithm-of-odds (lod) scores for linkage between markers and disease locus. A parametric lod score of 1.0 or greater was required for further study of a potential locus. Allele frequencies for the markers were estimated using married-in members of the study families as a representative control population (n = 44). These frequencies were compared with data generated in other screening projects, and no significant differences were seen. Penetrance of the disease was assumed to be 90% by age 70 years, and intermediate risk classes based on age were assigned as previously described. Pedigrees (including haplotypes) were displayed using Cyrillic (version 2.1; Cherwell Scientific, Palo Alto, Calif).

RESULTS

Initial Marker Screening and Linkage Analysis

To screen the genome efficiently for new FALS loci, we instituted a 2-pronged approach, with screening of independent data sets at 2 sites (Boston and Chicago). In the Boston data set, the marker D9S1122 gave an overall lod score of 3.34 at θ = 0.10 (θ indicates recombination fraction) when examined in 16 families, thus reaching our criterion (lod score > 1.0) for further study. Two markers (D9S301 and D9S922) located about 7 cM to either side of D9S1122 were selected for further testing. These markers map to the chromosome 9q21-q22 region.

Family F222 had a lod score of 1.10 at θ = 0 for marker D9S922 and a score of 0.48 at θ = 0 for marker D9S1122, and family F17 had lod scores of 2.08, 0.07, and 3.15 with markers D9S301, D9S1122, and D9S922, respectively, at θ = 0. When we reviewed the records for these families, selected from our set solely on the basis of this analysis, we found that in each family several patients developed motor neuron disease concurrently with progressive dementia. In family F222, 1 patient was diagnosed with ALS and FTD while 2 showed only motor neuron symptoms. For 3 other persons, the clinical records and other available information confirmed a diagnosis of ALS accompanied by dementia symptoms but were inconclusive as to the type of dementia. In family F17, 2 patients were diagnosed with ALS and FTD while 2 had ALS alone. One patient had ALS accompanied by dementia symptoms. Information about possible dementia is unavailable for the affected person in the earliest generation. The mean (SD [range]) age of onset for affected individuals in these 2 families was 33.8 (8.2 [40-62]) years with an average (SD [range]) duration of 3.8 (4.0 [1.3-15]) years, with most persons surviving 4 years or less and 1 patient surviving 15 years. In the other 14 ALS families in the Boston linkage analysis subset, which showed no linkage to these markers, there were no individuals with both ALS and FTD.

The dementia specified as FTD in these families was characterized by socially inappropriate, impulsive behavior and a general deterioration in ability to perform routine daily tasks. These behavioral changes occurred months be...
the 5 ALS-FTD families, peak multipoint lod scores of 8.08 and 8.12 were obtained at markers D9S1867 and D9S922, respectively (for the marker set D9S1123, D9S1867, and D9S922) (Figure). Two-point lod scores for the markers are shown in Table 1 for all 16 families in the Boston data set and for the subset of ALS-FTD families.

### Crossover Analysis of the ALS-FTD Locus

For all families, haplotypes representing individual chromosome fragments were assembled. No single haplotype was consistently inherited with ALS in the non-FTD families, confirming that they are not linked to this chromosome 9 locus. The alleles comprising the linked haplotypes were dissimilar between the ALS-FTD families, suggesting independent origins for the disease-associated chromosomes. Although 3 of the 5 linked families show no evidence of crossovers occurring across this region, we can partially define the limits of the ALS-FTD locus based on crossover events observed in families F222 and F17. Genotype data are shown in Table 2 for affected individuals and an unaffected obligate carrier of the disease-associated chromosome.

A crossover observed in affected individual (individuals are identified as generation/individual throughout) II:3 in family F222 (Table 2) determines the centromeric border of the ALS-FTD region. The disease-associated allele for marker D9S301 in this family is allele 1. Individual II:3 has inherited allele 8 for this marker from affected parent I:1 and shares the family disease-associated haplotype for markers telomeric of D9S301. Thus, we concluded that the crossover seen in individual II:3 occurred between markers D9S301 and D9S927, establishing D9S301 as the centromeric limit of the disease locus.

The telomeric boundary of the region is less firmly established. Individual IV:4 in family F17 is affected. Thus, unaffected individual III:1, the parent of IV:4, is an obligate carrier of the disease-associated chromosome. Indi-
vidual III:1 shares the family disease haplotype for markers centromeric of marker D9S167 and carries alleles 10 and 11 for D9S167 (Table 2). While we could not determine whether allele 10 or 11 was physically associated with the disease chromosome (phase) in individual III:1, neither allele 10 nor allele 11 is the disease-associated allele 5 observed for D9S167 elsewhere in this family. This implies that a crossover has occurred at some point centromeric to this marker. Although DNA from individual IV:4 is not available, we can partially reconstruct this genotype using data available from other persons (spouse and 2 children). However, both children have inherited the same chromosome from their affected parent IV:4, and this is not present in III:1. It is most likely to be the chromosome that IV:4 inherited from the married-in parent (spouse of III:1). Thus, we cannot explicitly determine whether individual IV:4 inherited the modified disease-associated haplotype observed in III:1. If we assume that IV:4 did inherit it, the crossover centromeric of D9S167 would establish this marker as the telomeric border of the ALS-FTD locus.

We estimate the genetic distance between D9S301 and D9S167 as 17 cM, corresponding to approximately 14 cR. 

**COMMENT**

These data define a new locus for a variant of FALS that arises in conjunction with frontotemporal dementia. This locus was identified via systematic genomic screening of families ascertained only for ALS. The fact that the families with linkage in the Boston data share the unusual ALS-FTD phenotype strengthens our confidence in the validity of this result. Moreover, the initial linkage was independently verified in a second data set that included 3 ALS-FTD families. This 2-stage approach can be viewed as hypothesis generating and hypothesis testing. The lod scores in the first data set reach classical levels of significance without limiting the analysis to the ALS-FTD subset and without including the Chicago ALS-FTD families. Testing the ALS-FTD hypothesis in the second data set with inclusion of the Chicago families generated higher scores, greatly increasing our confidence in this result.

The combination of FTD with ALS has been described. Such cases arise both on a sporadic basis and as autosomal dominantly inherited traits. However, genetic loci for ALS-FTD have not been reported. While it is possible that the families presented herein are phenotypic variants of the FTD-PD toa-

### Table 2. Pairwise Lod Scores for Familial Amyotrophic Lateral Sclerosis (FALS) and Chromosome 9 Markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Boston 16-Family Data Set, Age Corrected</th>
<th>Recombination Fraction, f</th>
<th>Subset of 5 FALS-FTD Families, Age Corrected</th>
<th>Un</th>
<th>0</th>
<th>0.05</th>
<th>0.10</th>
<th>0.15</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
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<tr>
<td>D9S301</td>
<td>-16.70</td>
<td>-3.25</td>
<td>0.45</td>
<td>0.85</td>
<td>1.40</td>
<td>1.29</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D9S927</td>
<td>-10.64</td>
<td>-0.19</td>
<td>0.88</td>
<td>1.73</td>
<td>1.99</td>
<td>1.57</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D9S1122</td>
<td>0.37</td>
<td>3.04</td>
<td>3.34</td>
<td>3.21</td>
<td>2.86</td>
<td>1.90</td>
<td>0.67</td>
<td></td>
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<td></td>
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<tr>
<td>D9S1123</td>
<td>-15.87</td>
<td>-4.61</td>
<td>-2.53</td>
<td>-1.35</td>
<td>-0.62</td>
<td>0.03</td>
<td>0.09</td>
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<tr>
<td>D9S1867</td>
<td>-26.10</td>
<td>-4.03</td>
<td>-0.66</td>
<td>0.88</td>
<td>1.56</td>
<td>1.54</td>
<td>0.65</td>
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<tr>
<td>D9S922</td>
<td>-23.53</td>
<td>-4.10</td>
<td>-0.68</td>
<td>0.96</td>
<td>1.70</td>
<td>1.67</td>
<td>0.70</td>
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<tr>
<td>D9S167</td>
<td>-30.98</td>
<td>-5.45</td>
<td>-1.43</td>
<td>0.50</td>
<td>1.41</td>
<td>1.58</td>
<td>0.66</td>
<td></td>
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</table>

*FTD indicates frontotemporal dementia; lod, logarithm of odds.

### Table 3. Haplotype Data Defining the Limits of the Chromosome 9 Locus

<table>
<thead>
<tr>
<th>Markers</th>
<th>Family F222†</th>
<th>Family F17†</th>
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<tbody>
<tr>
<td>D9S301</td>
<td>18</td>
<td>17</td>
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<tr>
<td>D9S927</td>
<td>35</td>
<td>35</td>
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<tr>
<td>D9S1122</td>
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<tr>
<td>D9S1123</td>
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<tr>
<td>D9S1867</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>D9S922</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>D9S167</td>
<td>311</td>
<td>316</td>
</tr>
</tbody>
</table>

*Indicates a reconstructed genotype (haplotypes inferred based on pedigree analysis).
†Individual with observed crossover determining centromeric border of the ALS-FTD region.
‡Individual family members are identified by generation/individual. Disease status is given in parentheses.
§Individual with proposed crossover determining possible telomeric border of the ALS-FTD region. It was not possible to determine whether allele 10 or 11 was physically associated with the disease chromosome.
pathy kindreds with amyotrophy, FTD, and parkinsonism, in our view, 3 findings refute that possibility. First, while amyotrophy is observed in some tauopathy cases, it is not usually the predominant, presenting clinical feature. Second, unlike the tauopathy cases that map to the tau-gene locus (MAPT) on chromosome 17q, the disease in these families maps to chromosome 9q. Moreover, genetic analysis in these families revealed recombinations between polymorphisms at the tau locus and the inherited ALS-FTD trait (B.A.H., P.C.S., and R.H.B., unpublished data, 1999). Finally, at autopsy, a striking deposition of neurofibrillary tangles is usually seen in FTD-PD cases. Such tangles were only rarely evident in multiple regions of the brains of persons from each of the 5 families described here. Although the data do not suggest involvement of tau gene mutations in these ALS-FTD families, we cannot exclude the possibility that the primary genetic defect in these families is related to a pathway involving a tau-like protein or the tau protein itself.

Our results extend our understanding of the genetic bases of both ALS and dementia. To date, loci for ALS have been confirmed or suggested on chromosomes 21q (SOD1 gene), 11 (neurofilament heavy subunit gene), 2 the X chromosome, 35 distal 9q, 36 2q (childhood onset, recessive ALS), 35 and 15q15.1-q21.1 (juvenile, recessive ALS). 36 The previously defined ALS locus on chromosome 9q 34 is at the extreme telomere of the chromosome and thus is genetically distinct from that defined by our linkage. Moreover, the earlier locus was identified in kindreds with juvenile-onset ALS with a slow course and no dementia, clearly clinically distinct from the characteristics of the ALS-FTD families discussed herein. Loci encoding genes or factors related to dementia have previously been identified on chromosomes 21q (amyloid protein), 37 14 (presenilin 1), 38 1 (presenilin 2), 39.40 19 (apolipoprotein E), 41 12 (a-macroglubulin) 42,43 and 3. 44 It thus appears that our new loci for ALS-FTD also defines a new dementia-related chromosomal address.

We have examined existing databases for possible candidate disease genes in our new ALS-FTD locus. One gene that maps near this region is the tyrosine kinase receptor trkB, a receptor for the neurotrophin family of proteins. Analysis of a known polymorphism within this gene has shown that it lies telomeric of the ALS-FTD locus (B.A.H., P.C.S., and R.H.B., unpublished data, 1999). Other known genes mapping within the candidate region include those for cytosolic aldehyde dehydrogenase (ALDH1), annexin 1 (ANX1), CDC28 protein kinase 2 (CKS2), glucosaminyl transferase 1 (GCNT1), and heterogeneous nuclear ribonucleoprotein K (HNRPK). 45 Also, a locus for chorea-athetosis has been mapped to this region by linkage studies, with a maximum lod score at marker D9S1867. 37 The 2 diseases affect both motor functions and classes of cortical neurons; further study will show whether choreoathetosis and ALS-FTD are genetically related.

Finally, we note that most of our families with inherited ALS still are not genetically associated with any of the identified ALS loci (B.A.H., T.S., J.L.H., and R.H.B., unpublished data, 2000). It thus appears that inherited ALS will show a high degree of genetic heterogeneity, as well as clinical diversity. This has implications for genetic linkage analysis strategies, rendering the conventional approach of establishing linkage with multiple large families exceedingly difficult. It remains to be determined whether the multiple genes involved in inherited ALS define sets of functionally significant, interacting genes. If this is the case, an understanding of the functions of the associated proteins and pathways may provide further insight into the pathogenesis of these diseases and ultimately lead to new approaches to treatment.

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Financial Disclosures: Dr Haines consulted for and received research grants from Glaxo Wellcome, PLC. Dr Horvitz was chair of the scientific advisory board and consulted for, and owned stock and stock options in, Iden Pharmaceuticals, and was a member of the scientific and oncology advisory boards and consulted for, and owned stock in, Avex Pharmaceuticals. Dr Brown consults for Dianarin.

Funding/Support: This research was supported by the Muscular Dystrophy Association (Drs Brown, Siddique, and Pericak-Vance and Ms McKenna-Yasek); the Amyotrophic Lateral Sclerosis Association (Dr Brown); the Pierre de Bourgknecht ALS Research Foundation (Drs Brown and Siddique); the Myrtle May MacLellan ALS Research Foundation (Dr Brown); the Cecil B. Day Investment Company (Drs Brown and Hosler, Ms McKenna-Yasek and Sailor, and Messrs Sapp and Huang); the Les Turner ALS Foundation (Dr Siddique); National Institutes of Health grants 1PO1NS31248-02 (Dr Brown), PO1NS21442 (Dr Siddique), and ROI5NS37912 (Drs Brown, Haines, Pericak-Vance, and Siddique); National Research Service Award Fellowship 1F32NS0064 (Dr Hosler); and the Howard Hughes Medical Institute (Dr Horvitz, Messrs Sapp and Huang, and Ms Sailor). Dr Horvitz is an investigator of the Howard Hughes Medical Institute. Dr Hung is a Muriel Heller Fellow.

Acknowledgment: We are indebted to the patients and their families for their assistance with this project.

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