Motor Neuron loss is characteristic of several neurodegenerative diseases, including spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Spinal muscular atrophy is a relatively common childhood disorder resulting from the loss of spinal motor neurons. Childhood SMA is subdivided into 3 groups according to clinical severity and age at onset; all forms are inherited recessively. An adult form of SMA exists with an age of onset of 17 to 55 years and either recessive or dominant modes of inheritance. Amyotrophic lateral sclerosis is characterized pathologically by the premature death of cortical, spinal, and brainstem motor neurons. In approximately 10% of ALS cases, an autosomal dominant mode of inheritance is observed; familial ALS (FALS) cases cannot be distinguished clinically or pathologically from sporadic ALS (SALS) cases. Clinically, the neuronal loss results in rapid progression of muscular weakness to death typically 2 to 5 years after disease onset in the fourth or fifth decade of life.

Spinal muscular atrophy was mapped to chromosome 5q11.2-13.3, and partial deletions of genes in this region are reported. In 67% of type 1 and 42% of types II and III SMA chromosomes, deletions were identified in the neuronal apoptosis inhibitory protein (NAIP) gene, while exons 7 and 8 of the telomeric survival motor neuron (SMN) gene are mutated in 98.6% of patients with autosomal recessive childhood-onset SMA and in some patients with adult-onset SMA. SMN is proposed to be the disease-determining gene. Clinical severity and age at onset appear to be correlated with SMN gene expression. SMN protein is detectable in patients with deletions in the telomeric SMN gene, indicating the presence of protein encoded by the highly homologous centromeric copy (BCD541). The loss of the NAIP gene and other as yet unknown genes in the duplicated SMA...
PATIENTS AND METHODS

PATIENT DATA

Two hundred sixty-two unrelated patients with ALS were analyzed for the SMA deletion. Sixty-eight of the patients had a family history of ALS. The diagnosis of ALS was established by at least one neurologist subspecialized in the assessment and treatment of ALS based on the El Escorial World Federation of Neurology criteria.14 All cases studied were negative for mutations in the SOD1 gene.

MUTATION ANALYSIS

DNA was extracted from whole blood specimens and used to amplify exon 6 of the NAIP gene by the polymerase chain reaction (PCR); coamplification of exon 13 was used as a control. Multiplex PCR was performed using the conditions and primers previously described.6 Electrophoresis was performed on the PCR products using a 1% agarose gel for 1½ hours at 100 V; the PCR products were then stained with ethidium bromide and visualized under UV light.

Exons 7 and 8 of the SMN gene were amplified via PCR sulfur 35–adenosine triphosphate incorporation.7 Electrophoresis was performed on the PCR products using a ×0.5 mutation detection enhancement acrylamide gel overnight at 4 W and 4°C. The gel was dried and exposed to x-ray film.

RESULTS

We analyzed 262 patients with ALS for the NAIP and SMN gene deletions found in patients with SMA. Exon 6 of the NAIP gene was successfully amplified via PCR, and electrophoresis was performed using a 1% agarose gel (coamplification of exon 13 provided an internal control). In all cases, ethidium bromide staining visualized under UV light revealed 2 bands of 434 base pairs (bp) (exon 6) and 242 bp (exon 13), indicating the absence of homozygous deletions seen in some patients with SMA.

Patients were also screened for deletions or other mutations in exons 7 and 8 of the SMN gene by single strand conformation analysis; the Table summarizes these results. In controls, 4 bands are apparent after PCR amplification and single strand conformation analysis, revealing the presence of the SMN gene and ‘BCD541 (Figure). Ninety-three percent of the patients in whom SMA is diagnosed are reported to have a deletion of exon 7 in the SMN gene.7 One patient, in whom ALS was clinically diagnosed, was identified to have an exon 7 deletion in the SMN gene as shown in lane 4 of the Figure; the centromeric copy remains. Review of this patient’s clinical history confirmed that symptoms were consistent with a diagnosis of SMA. A polymorphism, C to T transition in intron 7, was observed in 22 patients (9 with FALS and 13 with SALS; Figure, lane 7); however, in each case, the intact SMN gene and ‘BCD541 genes were also observed. Eighteen patients (2 with FALS and 16 with SALS) lack the centromeric ‘BCD541 copy as shown in the Figure, lane 1. Ninety-three patients were screened

<table>
<thead>
<tr>
<th>Exon</th>
<th>SMN</th>
<th>‘BCD541</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>1/262</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>221/262</td>
</tr>
<tr>
<td></td>
<td>+/p</td>
<td>+</td>
<td>22/262</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>–</td>
<td>18/262</td>
</tr>
<tr>
<td></td>
<td>+/SSCP variants</td>
<td>+</td>
<td>3/93</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>–</td>
<td>6/93</td>
</tr>
</tbody>
</table>

*Plus sign indicates present; minus sign, absent; p, C/T intron 7 polymorphism; SMN, survival motor neuron; and ‘BCD541, protein encoded by the highly homologous centromeric copy.
for mutations in exon 8. Deletions in exon 8 of \( \text{BCD541} \) were observed in 6 cases. In 3 more cases, 2 cases of FALS and 1 of SALS, aberrant migration patterns were observed; however, in each case the SMN gene remained intact. These variants were due to sequence variations in the 3’ untranslated region: a 3-bp deletion, a C to T substitution in \( \text{BCD541} \), and an A to T substitution.

We found a deletion in exon 7 of the SMN gene in 1 patient with a clinical diagnosis of ALS. Symptoms exhibited by this patient were consistent with a diagnosis of SMA. No evidence was found of homozygous deletions in exon 6 of the NAIP gene or in exons 7 or 8 of the telomeric SMN gene in the remaining 261 patients with FALS, confirming a previous report of the absence of deletions in 10 unigeniculal patients with FALS and 54 patients with SALS. Furthermore, microdeletions or missense mutations were not identified in the SMN gene in either population. In cases in which SMN gene variants were identified, there was invariably an intact copy. The effect of mutation or deletion of \( \text{BCD541} \) is at present unknown. Although in conjunction with homozygous deletion of the SMN gene, a single copy of \( \text{BCD541} \) results in the development of a severe, congenital form of SMA with widespread neurodegeneration. Parents of patients with less severe SMA (types II and III) are reported to have more copies of \( \text{BCD541} \) compared with parents of patients with type I SMA and, thus, transmit to their affected offspring a chromosome 5 with multiple copies of \( \text{BCD541} \). \( \text{BCD541} \) expression partially compensates for SMN loss, thereby modulating disease progression. It would be interesting to investigate whether NAIP, SMN, or \( \text{BCD541} \) polymorphisms modify the phenotypic expression of ALS.

Phenotypic heterogeneity in motor neuron degenerative diseases invariably results in misdiagnosis of some cases. Mutation screening will, however, avoid misdiagnosis. Although ALS and SMA share some clinical and pathological features, the mutations causing SMA do not cause ALS. These data confirm the absence of a common genetic basis between ALS and SMA, strongly suggesting that the pathogenic molecular and cellular processes of ALS and SMA differ. This study provides evidence that mutations in the SMN gene are restricted to SMA. Thus, while molecular detection of the SMN deletion will be useful and accurate in diagnosing SMA, in more than 95% of ALS cases (non-SOD1), the diagnosis will continue to be based on clinical criteria and the exclusion of other motor neuron disorders.

Accepted for publication September 17, 1998.

We thank Alexander MacKenzie, MD, PhD, for providing us with the NAIP primers. We also thank the many patients with ALS, without whose support these studies could not be done. We also acknowledge support from the Muscular Dystrophy Association, Tucson, Ariz, and from the Medical Research Council of Canada.

Corresponding author: Guy A. Rouleau, MD, Centre for Research in Neuroscience, Montreal General Hospital Research Institute, 1650 Cedar Ave, Montreal, Quebec, Canada H3G 1A4.

REFERENCES