Deletions Causing Spinal Muscular Atrophy Do Not Predispose to Amyotrophic Lateral Sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, invariably lethal disease resulting from the premature death of motor neurons of the motor cortex, brainstem, and spinal cord. In approximately 15% of familial ALS cases, the copper/zinc superoxide dismutase gene is mutated; a juvenile form of familial ALS has been linked to chromosome 2. No cause has been identified in the remaining familial ALS cases or in sporadic cases and the selective neurodegenerative mechanism remains unknown. Deletions in 2 genes on chromosome 5q, \( \text{SMN} \) (survival motor neuron gene) and \( \text{NAIP} \) (neuronal apoptosis inhibitory protein gene), have been identified in spinal muscular atrophy, a disease also characterized by the loss of motor neurons. These genes are implicated in the regulation of apoptosis, a mechanism that may explain the cell loss found in the brains and spinal cords of patients with ALS.

Objective: To determine whether the mutations causing neurodegeneration in spinal muscular atrophy are present in patients with ALS in whom the copper/zinc superoxide dismutase gene is not mutated.

Patients and Methods: Patients in whom ALS was diagnosed were screened for mutations in the \( \text{SMN} \) and \( \text{NAIP} \) genes by single strand conformation analysis.

Results: We found 1 patient with an exon 7 deletion in the \( \text{SMN} \) gene; review of clinical status confirmed the molecular diagnosis of spinal muscular atrophy. No mutations were found in the remaining patients.

Conclusion: The \( \text{SMN} \) and \( \text{NAIP} \) gene mutations are specific for spinal muscular atrophy and do not predispose individuals to ALS.

Arch Neurol. 1999;56:710-712

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. 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. Electrophoresis was performed on the PCR products using a 1% agarose gel for 11/2 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. 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.

regions may also play a role in the variable clinical expression of the disease.

The mechanism of neuronal death in ALS is unknown, but abnormal apoptosis is considered a possibility. Mutations in the copper/zinc superoxide dismutase (SOD1) gene have been identified in approximately 15% of FALS cases, although the mechanism by which these mutations lead to motor neuron death is unknown. Although mutations in the SOD1 gene have been identified in some patients with ALS, in most cases the cause, genetic or otherwise, remains a mystery.

Reports of the coexistence of SMA and ALS within families suggest the possibility of a common genetic basis. Therefore, we elected to explore a possible role for the NAIP and SMN genes in the pathogenesis of FALS and SALS. Genomic DNA specimens from 69 patients with non–SOD1-mutated FALS and 194 patients with SALS were analyzed for the absence of exon 6 in the NAIP gene and for deletions or aberrant single strand conformational polymorphism bands in exons 7 and 8 of the SMN gene.

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. 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...
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 unigenicual 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 BCDD541 is at present unknown. Although in conjunction with homozygous deletion of the SMN gene, a single copy of BCDD541 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 BCDD541 compared with parents of patients with type I SMA and, thus, transmit to their affected offspring a chromosome 5 with multiple copies of BCDD541. BCDD541 expression partially compensates for SMN loss, thereby modulating disease progression. It would be interesting to investigate whether NAIP, SMN, or BCDD541 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.

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