SMN gene analysis of the spinal form of Charcot-Marie-Tooth disease

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Abstract
The spinal form of Charcot-Marie-Tooth disease (spinal CMT) is a rare genetic disorder of the peripheral nervous system, the genetic basis of which remains unknown. To test the hypothesis that a defect of survival motor neuron (SMN), the determining gene for spinal muscular atrophy (SMA), would result in spinal CMT, 18 unrelated spinal CMT patients were studied. Nine of them were sporadic cases and the other nine belonged to unrelated autosomal dominant pedigrees. None of the 18 patients showed deletions involving SMN exons 7 or 8, the most frequent gene alteration found in SMA. In addition, haplotype analysis in two large autosomal dominant pedigrees showed that the 5q13 locus was not segregating with the spinal CMT locus. Therefore, neither the sporadic nor the familial cases of spinal CMT are associated with a SMN gene deletion, nor are the familial cases linked to the 5q13 region, indicating that this neuropathy is genetically different from SMA.

Keywords: survival motor neurone gene; Charcot-Marie-Tooth disease; spinal muscular atrophy

The spinal form of Charcot-Marie-Tooth disease (spinal CMT), also known as distal hereditary motor neuropathy (distal HMN) or distal spinal muscular atrophy (distal SMA), is a rare inherited peripheral neuropathy. The disease is characterised by peroneal muscular atrophy, but distal upper extremities may also be affected. The disease shows similarities to Charcot-Marie-Tooth disease type II (CMT2), the major differences being that spinal CMT does not include distal sensory loss, decreased sensory evoked potentials, or axonal loss in sural nerves. A variable age of onset (both juvenile and adult cases have been noted), a variable mode of inheritance (autosomal dominant or recessive), and sporadic cases have been described suggesting genetic heterogeneity of the disease. So far, two loci have been identified, one mapping on chromosome 7p in an autosomal dominant pedigree with distal CMT of benign course affecting the upper limbs, and the other mapping on chromosome 12q24. The underlying biochemical defect(s) remains unknown.

Experiments described in this study were designed to determine whether spinal CMT could be associated with a defect of SMN, the determining gene for the 5q13 autosomal recessive form of spinal muscular atrophy. SMN gene deletions have been found in more than 90% of SMA patients, making possible a highly efficient test for both typical and atypical forms of SMA. Eighteen unrelated probands exhibiting symptoms of peroneal muscular atrophy associated with normal motor and sensory nerve conduction velocities as well as normal sensory evoked potentials were selected for the present study. Nine of them were sporadic cases, while nine belonged to unrelated families in which the disease locus was segregating as an autosomal dominant trait. Six sporadic and four familial cases showed distal muscle wasting and weakness affecting the four limbs with lower limb predominance. Three sporadic and three familial cases showed distal muscle weakness of the lower limbs only. Finally, two familial cases showed distal muscle weakness of the upper limbs only.

Analysis of exons 7 and 8, the nucleotide discrepancies of these allowing the SMN and the homologous copy to be distinguished, was conducted. Amplification created restriction site (ACRS) of exon 7 using the primers R111 and X7-Dra, as well as single strand conformation polymorphism (SSCP) analysis of exons 7 and 8 PCR amplified products, were performed using primers R111-541C261 (exon 7) and 541C960-541C1120 (exon 8). None of the 18 patients showed homozygous deletions involving exons 7 or 8 of the SMN gene or of the homologous copy (fig 1).

To exclude further the SMA region in probands belonging to two large autosomal dominant pedigrees and showing no SMN gene deletion, haplotype analysis of the 5q13...
region was performed. The polymorphic microsatellite markers flanking the SMN gene (cVS19 (D5S435) and 1105 (D5S351)) were mapping centromeric to the SMN gene (C212 (DF149S1) and C272 (DF150S1)) used to establish the most likely haplotypes. The PCR amplified products were separated by gel electrophoresis on a 6% denaturing polyacrylamide gel, transferred to a nylon membrane, then hybridised with a labelled (CA)₉ repeat, as previously described. The 5q13 region has been excluded by haplotype analysis of these families (fig 2). Indeed, in one case both affected and unaffected children inherited the same haplotype from an affected parent, and in the other case both affected children have inherited different haplotypes from an affected parent (fig 2).

The 5q13 locus has also been excluded previously in two other pedigrees with autosomal dominant inheritance of spinal CMT. The recent identification of SMN enabled the gene analysis of sporadic cases of spinal CMT. Our data show that neither the sporadic nor the familial cases of spinal CMT are associated with a SMN gene deletion, nor are the familial cases linked to the 5q13 region. The analysis of the spinal CMT loci recently reported will help to determine whether our familial cases are linked to either the 7p or 12q24 loci. Thus, these results strongly suggest that the molecular bases of degeneration of motor neurons resulting in either distal or proximal muscular atrophy are of separate origin.

This work was supported by the Association Francaise contre les Myopathies (AFM) and the Actions Concertees Coordonnees-Sciences du Vivant.