Spinal muscular atrophy is a common autosomal recessive neuromuscular disorder caused by mutations in the survival motor neuron gene (SMN), which exists in 2 nearly identical copies (SMN1 and SMN2). Exon 7 of SMN1 is homozygously absent in about 95% of spinal muscular atrophy patients, whereas the loss of SMN2 does not cause spinal muscular atrophy. Small mutations are found in the other 5% of affected patients, and these mutations cluster in the 3′ end of SMN1, a region important for protein oligomerization. SMN1 dosage testing can be used to determine the SMN1 copy number and to detect spinal muscular atrophy carriers and affected compound heterozygotes.

Dosage testing is compromised by the presence of 2 SMN1 copies per chromosome, which occurs in about 2% of carriers. Finally, although SMN2 produces less full-length transcript than SMN1, the number of SMN2 copies modulates the phenotype.

**Keywords:** spinal muscular atrophy; SMN1 gene; gene dosage testing; SMN2 gene

The autosomal recessive disorder proximal spinal muscular atrophy is a severe neuromuscular disease characterized by degeneration of α motor neurons in the spinal cord, which results in progressive proximal muscle weakness and paralysis. Spinal muscular atrophy is the second most common fatal autosomal recessive disorder after cystic fibrosis, with an estimated incidence of 1 in 10,000 live births. Childhood spinal muscular atrophy is subdivided into 3 clinical groups on the basis of age of onset and clinical course. Type 1 spinal muscular atrophy (Werdnig-Hoffmann) is characterized by severe, generalization muscle weakness and hypotonia at birth or within the first 3 months. Death from respiratory failure usually occurs within the first 2 years. Children with type 2 survive beyond 4 years and are able to sit, although they cannot stand or walk unaided. Type 3 (Kugelberg-Welander) is a milder form, with onset during infancy or youth; these patients learn to walk unaided. Adult-onset spinal muscular atrophy, variably referred to as type 3b or type 4, is less common but has also been reported; however, in a large majority of patients with spinal muscular atrophy, symptoms appear in infancy or early childhood.

**Molecular Diagnosis**

The survival motor neuron gene (SMN) is composed of 9 exons, with a stop codon present near the end of exon 7; it has been shown to be the primary spinal muscular atrophy–determining gene. Two almost identical SMN genes are present on chromosome 5q13: the telomeric or SMN1 gene, which is the spinal muscular atrophy–determining gene, and the centromeric or SMN2 gene. The coding sequence of SMN2 differs from that of SMN1 by a single nucleotide (840C > T), which does not alter the amino acid. Both copies of the SMN1 exon 7 are absent in about 95% of affected patients, whereas small, more subtle mutations have been identified in the remaining affected patients. The molecular diagnosis of spinal muscular atrophy consists of the detection of the absence of exon 7 of SMN1. Although this is a highly repetitive region with the almost identical SMN2 copy, the exon 7 base pair difference alters a restriction enzyme site and allows one to easily distinguish SMN1 from SMN2 using a polymerase chain reaction–based assay (Figure 1). As shown in Figure 1, the restriction enzyme DraI cuts only the SMN2 exon 7 polymerase chain reaction products, and in spinal muscular atrophy patients, the uncut SMN1 exon 7 is absent. The absence of detectable SMN1 in spinal muscular atrophy patients is used as a reliable and powerful diagnostic test for most spinal muscular atrophy patients; however, the polymerase chain reaction restriction enzyme assay is not quantitative and cannot detect spinal muscular atrophy carriers who have heterozygous deletion of SMN1.

Although a number of potential therapies are currently in clinical trials (discussed in this issue), their success may depend on identifying individuals as early as possible in order to begin treatment before potentially irreversible neuronal loss. This could potentially be accomplished through a newborn screening program. Spinal muscular atrophy presents a unique challenge because...
testing requires DNA as the substrate, which differs from current practices; however, we have recently demonstrated that newborn screening for spinal muscular atrophy can be technically accomplished. In a series of blood spots, all 59 affected samples were correctly found to have the homozygous SMN1 deletion. A newborn screening program would not only allow patients to be enrolled in clinical trials at the earliest time period but would also benefit families by providing timely and accurate counseling.

Carrier Testing

Because spinal muscular atrophy is one of the most common lethal genetic disorders, with a carrier frequency of 1 of 40 to 1 of 60, direct carrier testing has been beneficial to many families. Carrier detection for the heterozygous state has, however, proved to be more technically challenging because the spinal muscular atrophy region is characterized by the presence of many repeated elements. It has been observed that the SMN2 copy number fluctuates: approximately 10% to 15% of normal subjects lack the SMN2 copy, whereas many of the more mildly affected spinal muscular atrophy patients have more copies of it. Thus, a straightforward dose assay using SMN2 as the internal control would not be reliable. McAndrew et al developed the first highly accurate competitive dosage assays for determination of spinal muscular atrophy carrier status. In the competitive polymerase chain reaction method, a known number of copies of a synthetic mutated internal standard are introduced with the patient sample into the polymerase chain reaction mixture. The internal standards are designed to be amplified with the same primer pairs for the SMN1 copy, with efficiencies similar to those of the genomic DNA counterparts, and yield polymerase chain reaction products slightly smaller than the SMN polymerase chain reaction product. The copy number of SMN1 is determined by coamplification of SMN1, SMN2, the SMN internal standard, calibration factor, and the calibration factor internal standard and quantitated on an automated sequencer (Figure 2). The major advantage of this technique is that the internal standard is amplified with the same primers that amplify the target sequence. Thus, the efficiency of the amplification of the patient DNA and the internal standard DNA should be very similar and allow one to determine the gene copy number accurately. The assay can also be used for the identification of the 5% of spinal muscular atrophy–affected individuals who are compound heterozygotes. These individuals have 1 deletion and a second nondeletion type of mutation (described in the preceding section).

Studies of spinal muscular atrophy families have revealed 2 unusual occurrences. First, approximately 2% of spinal muscular atrophy cases arise as the result of de novo mutation events, which is high when compared with most autosomal recessive disorders. The high rate of de novo mutations in SMN1 may account for the high carrier frequency in the general population despite the genetic lethality of the disease. The large number of repeated sequences around the SMN1 and SMN2 locus likely predisposes this region to unequal crossovers and recombination events and results in the high de novo mutation rate. The de novo mutations have been shown to occur primarily during paternal meiosis. Second, the copy number of SMN1 can vary on a chromosome; we have observed that about 4% of the normal population possess 3 copies of SMN1. It is, therefore, possible for a carrier to possess 1 chromosome with 2 copies and a second chromosome with no copies. Using somatic cell hybrids, Mailman et al identified a parent of an affected child with a 2-copy chromosome.

The finding of 2 SMN1 genes on a single chromosome has serious genetic counseling implications because a carrier with 2 SMN1 genes on 1 chromosome would have the same dosage result as a noncarrier with 2 SMN1 genes on each chromosome. Thus, the finding of normal 2 SMN1 copy dosages significantly reduces the risk of being a carrier; however, there is still a small risk of recurrence among future offspring for individuals with 2 SMN1 gene copies. Risk assessment calculations using Bayesian analysis are essential for the proper genetic counseling of spinal muscular atrophy families.

Pathogenesis of Spinal Muscular Atrophy

Because virtually all individuals with spinal muscular atrophy have at least 1 SMN2 copy, the obvious question that arises is why do individuals with SMN1 mutations have a spinal muscular atrophy phenotype? It has now been shown that SMN1 produces predominately full-length transcript, whereas SMN2 produces predominately an alternatively transcribed (exon 7 deleted) product. The inclusion of
exon 7 in SMN1 transcripts and exclusion of this exon in SMN2 transcripts are caused by the single nucleotide in SMN exon 7. Although the C to T change in SMN2 exon 7 does not change an amino acid, it does disrupt an exonic splicing enhancer, which results in most transcripts lacking exon 7. The transcripts lacking exon 7 produce mostly an unstable protein that is degraded, and only a small amount of full-length transcript is generated by SMN2; therefore, spinal muscular atrophy arises because SMN2 cannot fully compensate for lack of SMN1 expression when SMN1 is mutated.

Genotype-Phenotype Association

Although mutations of SMN1 are observed in most patients, no phenotype-genotype correlation was initially observed because SMN1 exon 7 is absent in the majority of patients independent of the type of spinal muscular atrophy; however, several studies have now shown that the SMN2 copy number influences disease severity. The copy number varies from 0 to 3 in the normal population, with approximately 10% to 15% of normal individuals having no SMN2. In a previous study, it was shown that 95% of type 1 patients had 1 or 2 copies of SMN2, whereas 100% of type 3 patients had 3 or more copies. Thus, the small amount of full-length transcripts generated by SMN2 often produces a milder type 2 or type 3 phenotype when the copy number of SMN2 is increased.

Furthermore, we have also observed 3 rare cases of asymptomatic persons with homozygous SMN1 deletions. In these patients, 5 copies of SMN2 were present in each. These cases support the potentially therapeutic benefit in increasing SMN2 expression to decrease the disorder’s severity. It has been shown that extra SMN2 copies in more mildly affected patients arise through gene conversions, whereby SMN2 is copied either partially or totally into the telomeric locus. Thus, most type 1 patients carry real homozygous deletions of SMN1, whereas most patients with type 2 or type 3 have an absence of SMN1 as a result of gene conversion of SMN1 into SMN2, causing an increase in SMN2 copy number. These results are also in agreement with studies in the mouse model, in which elevated SMN2 copies have been shown to rescue the disease phenotype; however, SMN2 cannot be the sole modifier, as we have observed rare type 3 patients with 5 SMN2 copies. Differences in the amount of splicing factors may allow more full-length expression from SMN2 and account for some of the phenotypic variability.

Figure 2. SMN1 dosage analysis of a normal control and a spinal muscular atrophy carrier. The SMN1/calibration factor ratio for the carrier is half the ratio of the normal control. The quantitative polymerase chain reaction assay uses an exon of the cystic fibrosis gene as a standard to determine the copy number of the SMN1 and SMN2 genes. The assay also incorporates the use of 2 internal standards (IS-CF and IS-SMN) to monitor the efficiency of the polymerase chain reaction and to ensure that equal amounts of target genomic DNA are added to each tube.
Other Spinal Muscular Atrophy Mutations

Although the absence of both copies of SMN1 is a reliable and sensitive assay for most patients with spinal muscular atrophy, about 5% have other types of SMN1 mutations that will not be detected by homozygous deletion testing. Because of the high deletion frequency and according to the Hardy-Weinberg equilibrium, most of these patients will be compound heterozygotes, with deletion of 1 SMN1 allele and the occurrence of a point mutation or other types of small mutations on the other allele. Small intragenic mutations that we have identified at The Ohio State University Molecular Pathology Laboratory are shown in Figure 3 and Table 1. These intragenic types of mutations provided strong support that mutations in SMN1 alone can produce the disease. This is important because there was initial speculation that genes in close proximity, which were often also deleted with SMN1, might modify the phenotype. Both missense mutations and frame-shifting nonsense mutations have been found. As shown in Figure 3, although the mutations are distributed across the gene, there is a hotspot for missense mutations in exon 6. Exon 6 corresponds to a domain in the protein shown to be important in protein oligomerization. Although only a relatively small number of intragenic SMN1 mutations have been identified, many of them have been found in more than 1 patient. We have observed the exon 6 11-base pair duplication in 5 unrelated patients (Table 1).

Characterization of these intragenic SMN1 mutations has provided additional support regarding the role of SMN2 in modifying the phenotype.18 We have observed that missense mutations are most often associated with milder disease in our patients and that the severe type 1 spinal muscular atrophy phenotype is the result of frameshift mutations that can be ameliorated by an increase in SMN2 copy number. As a consequence of SMN1 being relatively small and given the uniform spectrum of mutations, it is a relatively straightforward procedure to sequence the gene and identify mutations in patients who are negative for the diagnostic deletion test. Furthermore, the carrier test can be used as an initial and sensitive screen to identify patients heterozygous for the deletion of SMN1 who are likely to have these smaller types of mutations.

Conclusion

As a result of the discovery of SMN and elucidation of the mutational spectrum, clinical diagnostic testing for spinal muscular atrophy has significantly improved. In most cases, it is no longer necessary to perform an invasive muscle biopsy. Until an effective treatment is found to cure or arrest progression of the disease, prevention of new cases through accurate diagnosis and carrier and prenatal diagnosis is of the utmost importance.

Acknowledgments

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Table 1. Summary of Small Mutations in the SMA Gene

<table>
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<tr>
<th>Exon</th>
<th>Mutation Type</th>
<th>Type</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>c.5 C&gt;G; p.A2G</td>
<td>Missense</td>
</tr>
<tr>
<td>2a</td>
<td>c.109 ins A</td>
<td>Frameshift</td>
</tr>
<tr>
<td>3</td>
<td>c.305 G&gt;A; p.W102X</td>
<td>Nonsense</td>
</tr>
<tr>
<td>3</td>
<td>c.389 A&gt;G; p.Y130C</td>
<td>Missense</td>
</tr>
<tr>
<td>4</td>
<td>c.509-510 del GT</td>
<td>Frameshift</td>
</tr>
<tr>
<td>6</td>
<td>c.770-780 dup 11 (CTGATGCTTTG)</td>
<td>Frameshift</td>
</tr>
<tr>
<td>6</td>
<td>c.785G&gt;T; p.S262I</td>
<td>Missense</td>
</tr>
<tr>
<td>6</td>
<td>c.796T&gt;G; p.S266P</td>
<td>Missense</td>
</tr>
<tr>
<td>6</td>
<td>c.818A&gt;G; p.H273R</td>
<td>Missense</td>
</tr>
<tr>
<td>6</td>
<td>c.821C&gt;T; p.T274I</td>
<td>Missense</td>
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References


