Perspectives and diagnostic considerations in spinal muscular atrophy

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Abstract: Spinal muscular atrophy is an autosomal recessive neurodegenerative disease and the most common genetic cause of infant mortality. The disease results in motor neuron loss and skeletal muscle atrophy. Spinal muscular atrophy is caused by mutations in the telomeric copy of the survival motor neuron 1 (*SMN1*) gene, but all patients retain a centromeric copy of the gene, *SMN2*. In the majority of cases, the disease severity correlates inversely with an increased *SMN2* gene copy number. Because spinal muscular atrophy is both a severe and common disorder, a direct carrier testing has been beneficial to many families. The survival motor neuron protein is ubiquitously expressed and performs a role in the assembly of the spliceosome. It is still not understood why mutations in the *SMN1* gene only seem to affect motor neurons. Progress has been made by developing therapeutic strategies based on understanding the pathogenesis of the disease. This review attempts to highlight some of the recent advances in the understanding of the disease with a focus on molecular diagnostics. ***Genet Med* 2010: 12(3):145–152.**

Key Words: *spinal muscular atrophy, survival motor neuron, SMN1, SMN2, genetic testing, carrier testing*

OVERVIEW

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the survival motor neuron (*SMN1*) gene. SMA is the second most common fatal autosomal recessive disorder after cystic fibrosis (CF), with an estimated incidence of ~1 in 10,000 live births.[1] The disease is characterized by progressive symmetrical muscle weakness resulting from the degeneration and loss of anterior horn cells in the spinal cord and brain stem nuclei. The disease is classified on the basis of age of onset and clinical course. Two almost identical *SMN* genes are present on 5q13: the *SMN1* gene, which is the SMA-determining gene, and the *SMN2* gene. The homozygous absence of the *SMN1* exon 7 has been observed on the majority of patients, whereas the remaining patients have intragenic *SMN1* mutations. Although *SMN2* produces less full-length transcript than *SMN1*, the number of *SMN2* copies modulates the phenotype. Carrier detection, in the deletion cases, relies on the accurate determination of the *SMN1* gene copies. Because SMA is one of the most common lethal genetic disorders, with a carrier frequency of 1/40–1/60, a direct carrier dosage testing has been beneficial to many families. The American College of Medical Genetics has recently recommended population carrier screening for SMA.[2] The management of SMA involves supportive and preventive strategies. New treatments based on increasing the expression of full-length SMN protein levels from the *SMN2* gene are being investigated.

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Clinical manifestations

The autosomal recessive disorder proximal SMA (Types I, II, and III [OMIM 253300, 253550, and 253400]) is a severe neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord, which results in progressive muscle weakness and paralysis. The predominant pathologic feature of autopsy studies of patients with SMA is loss of motor neurons in the ventral horn of the spinal cord and in brain stem motor nuclei. SMA is the second most common fatal autosomal recessive disorder after CF, with an estimated incidence of ~1 in 10,000 live births.[1] Childhood SMA is subdivided into three clinical groups on the basis of age of onset and clinical course.[3,4] Type I SMA (Werdnig-Hoffman disease) is characterized by severe, generalized muscle weakness, and hypotonia at birth or within the first 3 months. Death from respiratory failure usually occurs within the first 2 years. Approximately 60% to 70% of patients with SMA have the type I disease.[5] Type II children are able to sit, although they cannot stand or walk unaided, and typically survive beyond 4 years. The phenotypic variability exceeds that observed in type I patients, ranging from infants who sit transiently and demonstrate severe respiratory insufficiency to children who can sit, crawl, and even stand with support. Prognosis in this group is largely dependent on the degree of respiratory involvement. Type III SMA (Kugelberg-Welander disease) is a milder form, with onset during infancy or youth: patients learn to walk unaided and have prolonged survivals. They comprise a less fragile group than type II patients with regard to respiratory and nutritional vulnerability. Type III SMA is further subdivided into

two groups, type IIIa (onset before 3 years) and type IIIb (onset at age ≥3 years). Cases presenting with the first symptoms of the disease at the age of 20 to 30 years are classified as type IV or proximal adult-type SMA. The described classification is based on age of onset and clinical course, but it should be recognized that the disorder demonstrates a continuous range of severity. Finally, although the disease affects both sexes equally, there have been reports that the severe type I is more common in female subjects and that female subjects are less affected than male subjects in the milder SMA types.[6,7]

Genetics

Linkage analysis mapped all clinical types of SMA to chromosome 5q11.2–13.3.[8,9] The *SMA* gene is within a complex region, containing multiple repetitive and inverted sequences.[10] The *SMN* gene (Entrez Gene ID number 6606) comprises nine exons with a stop codon present near the end of exon 7.[11] Two inverted *SMN* copies are present: the telomeric or *SMN1* gene, which is the SMA-determining gene, and the centromeric or *SMN2* gene. The two *SMN* genes are highly homologous, have equivalent promoters,[12,13] and only differ at five base pairs.[10] The base differences are used to differentiate *SMN1* from *SMN2*. The coding sequence of *SMN2* differs from that of *SMN1* by a single nucleotide (840C>T), which does not alter the amino acid sequence but has been shown to be important in splicing. Both copies of the *SMN1* exon 7 are absent in ~95% of affected patients. Although patients with SMA lack *SMN1*, they always carry at least one copy of *SMN2*, which is partially functional but unable to compensate for the absence of *SMN1*. The remaining 5% of affected cases are compound heterozygotes for *SMN1* exon 7 deletion and small intragenic mutations.

Genotype-phenotype association

Although mutations of the *SMN1* gene are observed in the majority of patients, no genotype-phenotype correlation was initially observed because *SMN1* exon 7 is absent in the majority of patients independent of the severity of SMA. This is due to the fact that routine diagnostic methods do not distinguish between a deletion of *SMN1* and a conversion event whereby *SMN1* is replaced by a copy of *SMN2*. Several studies have now shown that the *SMN2* copy number modifies the severity of the disease.[14–17] The copy number varies from zero to three copies in the normal population, with ~10% to 15% of controls having no *SMN2*. However, milder patients with type II or III SMA have been shown to have more copies of *SMN2* than type I patients. A majority of patients with the severe type I form have one or two copies of *SMN2*, most patients with type II have three *SMN2* copies, and most patients with type III have three or four *SMN2* copies. Three unaffected family members of patients with SMA, with confirmed *SMN1* homozygous deletions, were shown to have five copies of *SMN2*.[18] These cases not only support the role of *SMN2* modifying the phenotype but also demonstrate that expression levels consistent with five copies of the *SMN2* genes may be enough to compensate for the absence of the *SMN1* gene.

This inverse dose relationship between *SMN2* copy number and disease severity has also been supported by the SMA mouse model.[19,20] The SMA mouse models have not only confirmed the susceptibility of motor neuron degeneration to *SMN* deficiency but also verified that the degeneration can be prevented by increased *SMN2* dosage. Mice lacking the endogenous mouse *Smn* gene, but expressing two copies of the human *SMN2* gene develop severe SMA and die within 1 week of age, however, mice that express multiple copies of *SMN2* do not develop the disease.

In addition to the *SMN2* copy number, other modifying factors influence the phenotypic variability of SMA. There are very rare families reported in which markedly different degrees of disease severity are present in affected siblings with the same *SMN2* copy number. These discordant sib pairs, which share the same genetic background around the SMA locus, would indicate that there are other modifier genes outside the SMA region. Differences in splicing factors may allow more full-length expression from the *SMN2* gene and account for some of the variability observed between discordant sibs.[21] It was also found that in some rare families with unaffected SMN1-deleted females, the expression of plastin 3 (*PLS3*) was higher than in their SMA-affected counterparts.[22] *PLS3* was shown to be important for axonogenesis and therefore may act as a protective modifier. However, the exact role of *PLS3* in SMA needs further confirmation and *PLS3* will need to be expressed in the SMA mouse model to determine whether the lethality can be corrected.

Still, it remains unclear whether these are always fully intact copies of the *SMN2* gene in these patients. Because of the nature of the *SMN* loci, deletion break points have been difficult to identify. If the *SMN2* genes are truncated, they may not produce full-length transcripts and therefore be nonfunctional. The identification of gene modifiers not only provides important insight into pathogenesis of SMA but may also identify potential targets for therapy. Several drugs have been shown to increase *SMN2* expression in SMA patient-derived cell lines and are being studied in ongoing clinical trials (described "Therapy" section).

Pathogenesis

Because all individuals with SMA have at least one *SMN2* gene copy and there are no differences in the amino acid sequence between the two genes, the obvious question that arises is why do individuals with *SMN1* mutations have a SMA phenotype? It has been shown that the *SMN1* gene produces full-length transcript, whereas the *SMN2* gene produces predominantly an alternatively spliced transcript (exon 7 deleted) encoding a protein (SMNΔ7) that does not oligomerize efficiently and is unstable.[23,24] The inclusion of exon 7 in *SMN1* transcripts and exclusion of this exon in *SMN2* transcripts are caused by the single nucleotide difference at +6 in *SMN1* exon 7 (c.840C>T). Although the C to T change in *SMN2* exon 7 does not change an amino acid, it does disrupt an exonic splicing enhancer (ESE) or creates an exon silencer element (ESS) that results in the majority of transcripts lacking exon 7.[25,26] The ESEs and ESSs are *cis*-acting exonic sequences that influence the use of flanking splice sites. ESEs stimulate splicing and are often required for efficient intron removal, whereas ESSs inhibit splicing. Whether it will be the loss of an ESE or creation of an ESS, the result is a reduction of full-length transcripts generated from *SMN2*. A single *SMN2* gene produces less functional protein compared with a single *SMN1* gene.[27–29] Therefore, SMA arises because the *SMN2* gene cannot fully compensate for the lack of functional SMN when *SMN1* is mutated. However, small amounts of full-length transcripts generated by *SMN2* are able to produce a milder type II or III phenotype when the copy number of the *SMN2* gene is increased (Fig. 1). SMA is, therefore, caused by low levels of SMN protein, rather than a complete absence of the protein.

Finally, a recent report described three unrelated patients with SMA who possessed *SMN2* copy numbers that did not correlate with the observed mild clinical phenotypes.[30] A single base substitution in *SMN2*, c.859G>C, was identified in exon 7 in the patients DNA, and it was shown that the substitution

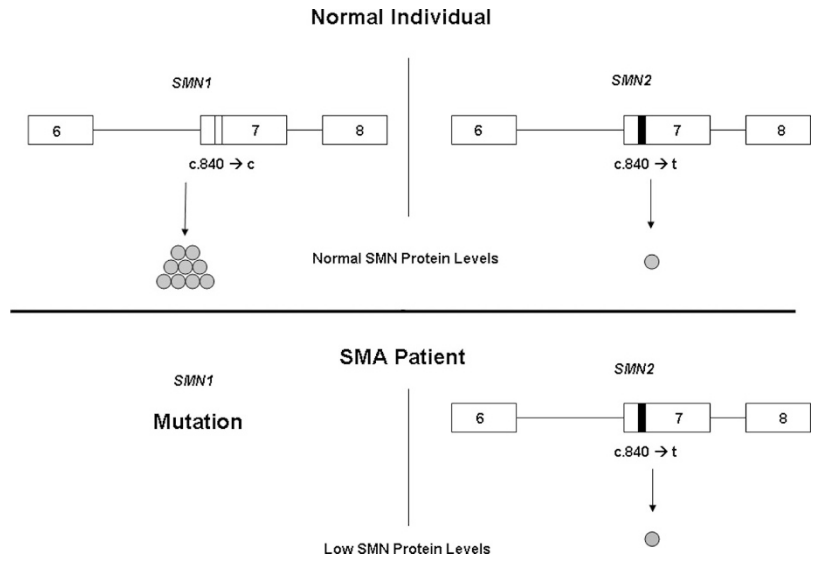

Fig. 1. In normal individuals, most full-length SMN transcript and protein are generated from the *SMN1* gene. Patients with SMA have homozygous mutations of *SMN1* but retain at least one copy of the *SMN2* gene. During transcription of *SMN2*, the *SMN2* gene produces predominantly an alternative transcript (exon 7 deleted) encoding a protein (SMNΔ7) that does not oligomerize efficiently and is unstable. Small amounts of full-length transcripts generated by *SMN2* are able to produce a milder Type II or III phenotype when the copy number of the *SMN2* gene is increased. SMA results from low levels of SMN protein, rather than a complete absence of the protein.

created a new ESE element. The new ESE increased the amount of exon 7 inclusion and full-length transcripts generated from *SMN2*, thus resulting in the less severe phenotypes. Therefore, the SMA phenotype may not only be modified by the number of *SMN2* genes but *SMN2* sequence variations can also affect the disease severity. It should, therefore, not be assumed that all *SMN2* genes are equivalent and sequence changes found within the *SMN2* gene must be further investigated for potential positive or negative effects on *SMN2* transcription.

Molecular diagnosis

The first diagnostic test for a patient suspected to have SMA should be the *SMN* gene deletion test. Both copies of the *SMN1* exon 7 are absent in ~95% of affected patients, whereas small more subtle mutations have been identified in the remaining affected patients. The molecular diagnosis of SMA consists of the detection of the absence of exon 7 of the *SMN1* gene. Although this is a highly repetitive region with the almost identical *SMN2* copy, the exon 7 base pair difference (840C>T) alters a restriction enzyme site (because of a mismatched primer) and allows one to easily distinguish *SMN1* from the *SMN2* using a polymerase chain reaction (PCR)–based assay (Fig. 2). As shown in Figure 2, the restriction enzyme *DraI* cuts only the *SMN2* exon 7 PCR products, and in patients with SMA, the uncut *SMN1* exon 7 is absent.[31] The absence of detectable *SMN1* in patients with SMA is being used as a reliable and powerful diagnostic test for the majority of patients with SMA. *SMN2* is homozygously absent in ~5% to 10% of unaffected individuals.[10,15] The SMA deletion test is currently being performed by several diagnostic laboratories and the result can easily be obtained in 2 weeks. Limitations of the diagnostic test are the failure to detect the nondeletion mutations of *SMN1* gene and the inability to determine carrier status.

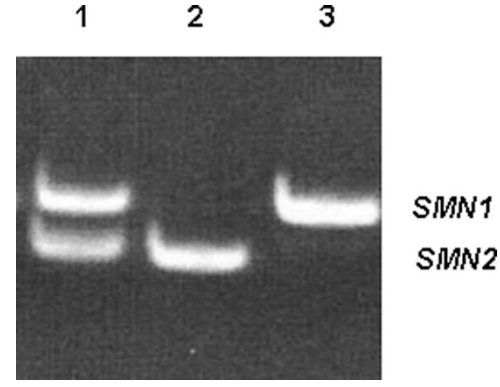

Fig. 2. Restriction enzyme digestion of PCR products distinguishes SMN1 from SMN2 exon 7. Lanes 1: unaffected patients; Lane 2: patient with SMA with SMN1 deletion; and Lane 3: unaffected patient with SMN2 deletion.

Newborn screening

Although a number of potential therapies are currently in clinical trials (see "Therapy" section), their success may depend on identifying individuals as early as possible to begin treatment before potentially irreversible neuronal loss. In infants with type I SMA, rapid loss of motor units occurs in the first 3 months and severe denervation with loss or >95% of units within 6 months.[32] Therefore, a very small window for beneficial therapeutic intervention exists in infants with type I SMA, and therapies will need to be administered within the newborn period for maximum benefit. This could potentially be accomplished through a newborn screening program for SMA. The identification of patients with SMA during the newborn period

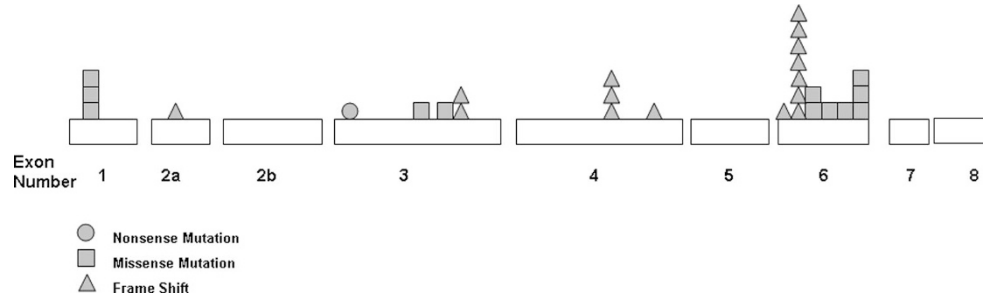

Fig. 3. Distribution of intragenic small mutations in the *SMN1* gene. The *SMN1* gene has nine exons and there is a hot spot for mutations in Exon 6. Table 1 specifies the actual nucleotide changes.

can be accomplished only by DNA testing because the disorder does not have a biochemical marker. Thus, SMA presents a unique challenge because the testing requires DNA as the substrate, which differs from current practices. However, direct DNA testing is the next innovation in newborn screening but currently is used primarily for reflex testing for first-tier-positive results. With its sizeable capacity for multiplexing, array technology has been touted as the application of choice for the first-tier analysis of DNA in newborn screening.[33,34] Using liquid microbead array for the detection of the homozygous *SMN1* exon 7 deletion, Pyatt et al.[35] demonstrated that newborn screening for SMA can be technically accomplished. In a series of blood spots, all 164 affected samples were correctly found to have the homozygous *SMN1* deletion, whereas 157 unaffected samples were excluded.[35]

There is a growing consensus that newborn screening can be extremely valuable for genetic conditions for which we do not have a specific effective treatment. A newborn screening program for SMA would not only allow patients to be enrolled in the clinical trials at the earliest time period but would enable patients to obtain proactive treatment earlier in the disease progression with regard to nutrition, physical therapy, and respiratory care. Furthermore, identifying SMA-afflicted individuals at birth eliminates the pain and cost of unnecessary testing that often takes place in attempting to diagnose an affected patient. The results from newborn screening are also important for the child's family because of the possibility for the prevention of additional cases through genetic counseling and carrier testing of at-risk family members.

Table 1 *SMN1* mutations

Exon	Mutation	Type	No. patients
1	c.5 C>G; p.A2G	Missense	3
2a	c.109insA	Frameshift	1
3	c.305 G>A; p.WIO2X	Nonsense	1
3	c.389A>G; p.YI3OC	Missense	1
3	c.4I8–432del15 (GATCTACTTTCCCCA)	Frameshift	2
3	c.419 A>T; p. DI4OV	Missense	1
4	c.509–510delGT	Frameshift	3
4	c.584delC	Frameshift	1
6	c.735insC	Frameshift	1
6	c.770–780dup11 (CTGATGCTTTG)	Frameshift	7
6	c.785G>T; p.S2621	Missense	2
6	c.796flC; p.S266P	Missense	1
6	c.818A>G; p.H273R	Missense	1
6	c.821C>T; p.T2741	Missense	3

The other SMA mutations

Although the absence of both copies of the *SMN1* gene is a very reliable and sensitive assay for the molecular diagnosis of SMA, ~5% of affected patients have other types of mutations in the *SMN1* gene 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 one *SMN1* allele being deleted and the other allele with a point mutation or other types of small mutations. If a patient with SMA possesses only a single copy of *SMN1*, it is likely that the remaining copy contains a more subtle mutation, including nonsense mutations, missense mutations, splice site mutation insertions, and small deletions. Figure 3 and Table 1 present the small intragenic mutations identified at The Ohio State University Molecular Pathology Laboratory. These intragenic types of mutations provided strong support that mutations in the *SMN1* gene alone can produce the disease. This was important because there was initial speculation that genes in close proximity, which were often also deleted with the *SMN1* gene, might modify the phenotype. These mutations have also been essential in defining important structural and functional domains of the SMN protein. As shown in Figure 3, although the mutations are distributed across the gene, there is a hot spot for missense mutations in Exon 6. Exon 6 corresponds to a domain in the protein, which has been shown to be important in protein oligomerization.[36] Many of the same intragenic mutations have now been reported in unrelated patients.[7,37,38] The most frequently reported mutations are the p.Tyr272Cys, c.399_402delAGAG, c.770–780dup11, and p.Thr274Ile. These four mutations were shown to account for 42% of cases with small changes in the *SMN1* gene.[7] We have observed the c.770–780dup11 in seven unrelated patients.

The characterization of these intragenic *SMN1* mutations has provided additional support regarding the role of *SMN2* in modifying the phenotype.[38] The severe type I SMA phenotype is often the result of frameshift mutations that can be ameliorated by an increase in the *SMN2* copy number. As a consequence of the *SMN1* gene 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. However, it is necessary to verify that the intragenic mutation has occurred in the *SMN1* gene and not in the *SMN2* gene. This requires either a long-range PCR protocol or a subcloning. Finally, 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 also have these smaller types of mutations. If the patient possesses two copies of *SMN1*, then other motor neuron disorders should be considered such as: SMA with respiratory distress, X-linked SMA, distal muscular atrophy, and juvenile amyotrophic lateral sclerosis.

Carrier testing

Because SMA is one of the most common lethal genetic disorders, with a carrier frequency of 1/40–1/60, a direct carrier testing has been beneficial to many families. Carrier detection for the heterozygous state was initially shown to be more technically challenging because the SMA region is characterized by the presence of many repeated elements. It has been observed that the *SMN2* copy number fluctuates: ~10% to 15% of controls lack the *SMN2* copy, whereas many of the more mildly affected patients with SMA have more copies of it. Thus, a straightforward dosage assay using the *SMN2* gene as the internal control would not be reliable. McAndrew et al.[15] developed the first highly accurate competitive dosage assays for determination of SMA carrier status. In the competitive PCR method, a known number of copies of a synthetic mutated internal standard are introduced with the patient sample into the PCR 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 PCR products slightly smaller than the *SMN* PCR product. The copy number of *SMN1* is determined by coamplification of *SMN1*, *SMN2*, the *SMN* internal standard, CF, and the CF internal standard and quantitated on an automated sequencer (Fig. 4). 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 accurately determine the gene copy number. The assay can also be used for the identification of the 5% of SMA-affected individuals who are compound heterozygotes. There have now been a number of techniques developed for the detection of SMA carriers including: real-time PCR,[6] competitive PCR with primer extension,[39] TaqMan technology,[40] denaturing high-performance liquid chromatography,[41] and multiple ligation-dependent probe amplification.[42]

There are two limitations of the carrier test. First, ~2% of cases with SMA arise as the result of de novo mutation events,[43] 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 type I 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.[43] Second, the copy number of *SMN1* can vary on a chromosome; we have observed that ~5% of the normal population possess three copies of *SMN1*.[15] It is, therefore, possible for a carrier to possess one chromosome with two copies and a second chromosome with zero copies.[2,15,44] Using haploid conversion technique, which allows for single chromosome analysis, Mailman et al.[45] identified a parent of an affected child with a two-copy chromosome. The finding of two *SMN1* genes on a single chromosome has serious genetic counseling implications because a carrier with two *SMN1* genes on one

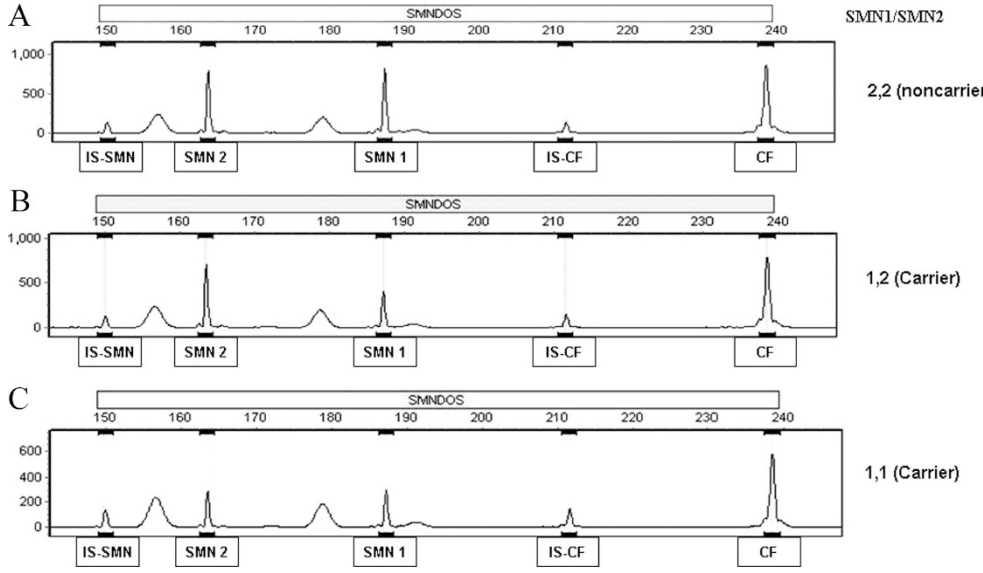

Fig. 4. *SMN1* dosage analysis of a normal control and two SMA carriers. The *SMN1*/CF ratio for the carrier is half the ratio of the normal control. The quantitative PCR assay uses an exon of the cystic fibrosis (CF) gene as a standard to determine the copy number of the *SMN1* and *SMN2* genes. The assay also incorporates the use of two internal standards (IS-CF and IS-SMN) to monitor the efficiency of the PCR reaction and to ensure that equal amounts of target genomic DNA are added to each tube. A, normal control with two *SMN1* copies and two *SMN2* copies. B, SMA carrier with one *SMN1* copy and two *SMN2* copies. C, SMA carrier with one *SMN1* copy and one *SMN2* copy.

chromosome and a *SMN1* deletion on the other chromosome will have the same dosage result as a noncarrier with one *SMN1* gene on each Chromosome 5. Thus, the finding of normal two *SMN1* copy dosage significantly reduces the risk of being a carrier; however, there is still a residual risk of being a carrier, and subsequently a small recurrence risk of future affected offspring for individuals with two *SMN1* gene copies. Risk assessment calculations using Bayesian analysis are essential for the proper genetic counseling of SMA families.[46] A recent report has shown that there are significant differences in carrier frequencies and the two-copy chromosome genotypes among different ethnic groups.[47] The results from this study provide adjusted detection rates based on ethnicity, and thus allow for more accurate Bayesian risk estimates.

Population carrier screening

In current practice, patients with a family history of SMA are most often tested for carrier status. However, the American College of Medical Genetics recently recommended population carrier screening for SMA.[2] Population carrier screening is currently recommended for a number of other genetic disorders with similar carrier frequencies. The prototype for heterozygote screening was testing for Tay-Sachs disease in the Ashkenazi Jewish population in whom carrier testing has been offered since 1969. Carrier screening, followed by prenatal diagnosis when indicated, has resulted in a dramatic decrease in the incidence of Tay-Sachs disease in the Jewish population.[48] It is generally accepted that the following criteria should be met in order for a screening program to be successful: (1) disorder is clinically severe, (2) high frequency of carriers in the screened population, (3) availability of a reliable test with a high specificity and sensitivity, (4) availability of prenatal diagnosis, and (5) access to genetic counseling. SMA does meet the criteria cited. However, the choice to have a SMA carrier test should be made by an informed decision. Educational brochures are available and provide information about SMA and the inheritance patterns.[49] It is important that individuals undergoing carrier testing recognize that the test does not provide genotype/phenotype information. Type I SMA occurs in ~60% to 70% of the cases, whereas the milder types II and III account for the remaining cases.[5] Formal genetic counseling services must be made available to anyone requesting this testing. It is important that all individuals undergoing testing understand that a carrier is a healthy individual who is not at risk of developing the disease but has a risk of passing the gene mutation to his/her offspring. Family planning options are available to parents and include: egg or sperm donation, adoption, preimplantation genetic testing, and termination of pregnancy. It is imperative that individuals understand the limitations of the molecular testing: two *SMN1* genes in *cis* on the one Chromosome 5, presence or rare de novo mutations, and nondeletion mutations. As is true for all carrier screening programs, the testing must be voluntary.

Protein

SMA results from an insufficient amount of the SMN protein and there is a strong correlation between the disease severity and SMN protein levels.[27,28] The SMN protein is a ubiquitously expressed, highly conserved 294-amino acid polypeptide. The protein is found in both the cytoplasm and nucleus and is concentrated in punctate structures call "gems" in the nucleus.[50] High levels of the protein have been found to exist in the spinal motor neurons, the affected cells in patients with SMA. The protein self-associates into a multimeric structures. Biochemically, SMN does not appear to exist within cells in isolation but instead forms part of a large protein complex, the SMN complex.[51] Many of these SMN-interacting proteins are components of various ribonucleoprotein (RNP) complexes that are involved in distinct aspects of RNA metabolism. The best characterized function of the SMN complex is regulating the assembly of a specific class of RNA-protein complexes, the small nuclear RNPs (snRNPs).[52] The snRNPs are a critical component of the spliceosome; a large RNA-protein that catalyzes pre-mRNA splicing.

Because the SMN protein is ubiquitously expressed, it remains unknown how a loss of a general housekeeping function (snRNP assembly) causes a selective loss of motor neurons in SMA.[53] The high expression of SMN protein in motor neurons may suggest that the neuronal population is more sensitive to decreases in the SMN protein level. Possibly, the altered splicing of a unique set of pre-mRNAs results in deficient proteins that are necessary for motor neuron growth and survival. However, specific alterations in mRNAs have not been identified in patients with SMA. In addition to its role in spliceosomal RNP assembly, SMN may have other functions in motor neurons. A subset of SMN complexes are located in axons and growth cones of motor neurons and may be involved in some aspects of axonal transport and localized translation of specific mRNAs.[54,55]

Therapy

Management depends on treating or preventing complications associated with weakness and maintaining the quality of life. Pulmonary disease is the major cause of morbidity and mortality in type I SMA. Pulmonary compromise is caused by a combination of inspiratory and expiratory muscle weakness. Respiratory muscle weakness results in impaired cough and ability to clear lower airway secretions, lung and chest wall underdevelopment, and hypoventilation. Respiratory care of patients with SMA is essential to their survival and quality of life.[56] Chronic respiratory management includes providing methods for airway clearance and noninvasive ventilatory support. In SMA type I patients with increasingly frequent acute pulmonary infections, tracheotomy and ventilation can be considered but may not improve the quality of life or reduce hospitalizations. Discussion with the family about the management of respiratory failure should occur early in the disease and continue in an ongoing dialogue. Attention to nutritional status and to orthopedic complications, such as corrective spinal surgery for scoliosis, is also important.

Strategies are being actively investigated to increase the expression of full-length SMN protein levels from the *SMN2* gene. Several compounds have now been shown to up-regulate SMN expression by inhibiting histone deacetylase (HDAC). Control of the acetylation state of histones is an important epigenetic mechanism regulating gene expression. When the NH-2 terminus of core histones is acetylated in a region of chromatin, this region takes on a more relaxed chromatin structure that is more transcriptionally active due to increased accessibility of DNA to the transcriptional machinery. The level of histone acetylation is controlled by both the histone acetyltransferases, which acetylate the histones, and the HDACs, which deacetylate histones. Chang et al.[57] demonstrated that sodium butyrate, one of the earliest discovered HDAC inhibitors, increased full-length *SMN2* transcript levels and protein levels in lymphoblastoid cell lines derived from type I patients. Subsequent studies have shown that the *SMN2* promoter can be activated and full-length SMN RNA and protein levels increased by several other HDAC inhibitors including phenylbutyrate and valproic acid (VPA). Both of these drugs have been in clinical use for many years for other indications and have

well-established safety profiles and consequently are now being used in SMA clinical trials.[58,59] A pilot study of phenylbutyrate showed that the drug was well tolerated.[59] Two small open-label trials of VPA have reported modest strength or functional benefit in a subset of patients with SMA.[60,61] A phase II open-label study of VPA indicated that the drug could be used safely in patients with SMA older than 2 years, and there was some evidence in support of improvement in gross motor function in younger nonambulatory type II children.[62] The authors concluded that the study underscores the need for additional controlled clinical trial with VPA.[62]

A drug that has been proposed to work by activating *SMN2* expression is hydroxyurea (HU), another HDAC inhibitor in clinical use for many years. HU has previously shown to activate the fetal hemoglobin gene and has been used to treat patients with sickle cell disease and thalassemias. HU was shown to increase the amount of full-length transcript and protein in patient-derived lymphoblastoid cell lines and is currently being used in clinical trials in Taiwan and in the United States.[63]

Another possible treatment strategy would be to enhance *SMN2* exon 7 inclusion by using synthetic antisense oligonucleotides that bind *SMN2* transcripts and promote exon 7 inclusion during splicing.[64,65] Although such strategies have been successful in vitro, it will be a greater challenge to achieve efficient delivery of the oligonucleotides to the motor neuron in patients with SMA.

CONCLUSIONS

As a result of the discovery of the *SMN* gene and elucidation of the mutational spectrum, clinical diagnostic testing for SMA has significantly improved. It is no longer necessary to perform the invasive muscle biopsy in most cases. Until an effective treatment is found to cure or arrest the progression of the disease, prevention of new cases through accurate diagnosis and carrier and prenatal diagnosis is of the utmost importance. The goal of population-based SMA carrier screening is to identify couples at risk for having a child with SMA, thus allowing carriers to make informed reproductive choices. In the future, newborn screening for SMA may become a reality and allow for the implementation of more proactive treatments. The correlation between the SMA phenotype and the *SMN2* copy number in patients with SMA and the demonstration that sufficient SMN protein from *SMN2* in transgenic mice can ameliorate the disease has made the *SMN2* gene an obvious target that can be modulated in therapeutic strategies. Several compounds have now been shown to up-regulate the SMN protein from the *SMN2* gene and are being used in clinical trials.

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