

## Spinal muscular atrophy: untangling the knot?

Ivan Biros, Susan Forrest

### Abstract

**Spinal muscular atrophy (SMA), a clinically and genetically heterogeneous group of neuromuscular diseases, is a disorder of motor neurones characterised by degeneration of spinal cord anterior horn cells and muscular atrophy.**

**SMA is an autosomal recessive disorder with a carrier frequency of about 1/50. Three candidate genes, the survival motor neurone (SMN) gene, the neuronal inhibitory protein (NAIP) gene, and the p44 (subunit of basal transcription factor TFIID) gene, have been considered as genes involved in this condition. The region spanning these genes has a complex organisation including duplications, repetitive sequences, truncated genes, and pseudogenes, which makes molecular analysis of this condition difficult. Although deletions have been found in the majority of SMA patients, a few microrearrangements (like duplications, missense mutations, microdeletions, and gene conversions) localised in the telomeric form of the SMN gene have also been reported. The function of the protein encoded by the SMN gene is still not fully understood but recent studies have indicated that it is found intracellularly in gems, novel nuclear structures. Its interaction with other proteins suggests a role in mRNA processing and metabolism. Whether the NAIP gene protein and other apoptosis associated proteins are directly involved in the initial stages of neurone degeneration and apoptosis, or acting downstream on the pathological pathway, has been difficult to determine. Further studies will be required to elucidate possible functional interactions between these proteins.**

(J Med Genet 1999;36:1-8)

Keywords: SMA; SMN; NAIP

The term spinal muscular atrophy (SMA) comprises a clinically and genetically heterogeneous group of diseases characterised by degeneration and loss of the anterior proximal horn cells in the spinal cord, and sometimes also in the brainstem nuclei, resulting in muscle weakness and atrophy.<sup>1</sup> Most cases of SMA are inherited as an autosomal recessive trait, although dominant families are known. This

neuromuscular disease is the second most common lethal autosomal recessive disorder in white populations with an overall incidence of 1 in 10 000 live births and a carrier frequency of about 1/50.<sup>2</sup>

In 1891, Guido Werdnig (1844-1919), an Austrian neurologist from the University of Graz, described two brothers with proximal SMA.<sup>3</sup> Two years later, Johan Hoffmann (1857-1919), a German neurologist from the University of Heidelberg, described four cases belonging to two families where some of these children did not show the fatal course of the disease.<sup>4</sup> In the mid fifties, Kugelberg and Welander<sup>5-6</sup> described 12 patients belonging to eight sibships with an onset of the disease between 2 and 17 years of age. All were able to walk at least eight to nine years after the symptoms started.

This resulted in the classification of childhood onset SMA into three types, distinguished on the basis of clinical severity and age of onset. Type I (Werdnig-Hoffman disease) is the most severe form with onset within the first six months of life. Children are never able to sit without support and usually die within two years. Type II (Dubowitz disease) is the intermediate form of SMA and has an onset before the age of 18 months. Children are unable to stand or walk and death usually occurs after the age of 2 years. Type III (Wohlfart-Kugelberg-Welander disease) is a mild, chronic form with onset after the age of 18 months.

A new era of understanding the molecular basis of SMA began in 1990. Linkage studies with microsatellite markers using consanguineous families<sup>7</sup> and large numbers of small families segregating different forms of SMA<sup>8-10</sup> showed that the mutation in all three forms of SMA mapped to the same region of chromosome 5q.

Radiation hybrid mapping<sup>11</sup> and construction of a non-chimeric YAC contig<sup>12-13</sup> reduced the candidate region to approximately 400 kb. As the region of interest narrowed, the complexity, that is, repetitive sequences, pseudogenes, retrotransposable elements, deletions, and inverted duplications,<sup>14-16</sup> became clearly evident and explained the difficulties encountered in attempting to generate physical maps of the region.

In the January 1995 issue of *Cell*, two different SMA candidate genes (survival motor neu-

The Murdoch  
Institute, Royal  
Children's Hospital,  
Parkville 3052,  
Melbourne, Australia  
I Biros  
S Forrest

Correspondence to:  
Dr Biros.

rone gene (SMN)<sup>17</sup> and neuronal apoptosis inhibitory protein gene (NAIP)<sup>18</sup> were described and, interestingly, each was present in at least two copies. More recently, a third gene, encoding p44 (a subunit of the basal transcription factor IIH (BTF2p44)), has also been found to be associated with SMA type I.<sup>19, 20</sup>

### Characterisation of the genes

Both copies of the SMN gene contain nine exons. The two copies are named telomeric (SMN<sup>T</sup>) and centromeric (SMN<sup>C</sup>) or <sup>C</sup>BCD541. They differ only in eight nucleotides (five are intronic and three are exonic, located within exons 6, 7, and 8),<sup>17, 21, 22</sup> thus enabling them to be distinguished either by SSCP (single stranded conformation polymorphism) analysis<sup>17</sup> or by enzyme digestion of PCR products.<sup>23</sup>

Although both copies are ubiquitously expressed mainly as a 1.7 kb transcript,<sup>17</sup> alternative splicing generates minor isoforms of the SMN protein.<sup>17, 24</sup> The majority of transcripts (~90%) from the SMN<sup>T</sup> gene are full length and about 10% of transcripts lack exon 5. The SMN<sup>C</sup> gene produces a variety of different isoforms, including transcripts without exon 5 or 7, transcripts lacking both exons, as well as full length SMN transcripts. The full length transcript accounts, surprisingly, for 20–30% of the total transcripts from the SMN<sup>C</sup> gene.<sup>17, 24</sup>

The neuronal apoptosis inhibitory protein gene (NAIP) was so named because of high homology between exon 5 and 6 and a portion of a baculoviral gene product, inhibitor of apoptosis protein (IAP). This homology raised the intriguing possibility that SMA was a disorder of unrestrained apoptosis. Later, however, gene homology studies and expression studies showed many differences in gene structure and expression patterns and concluded that NAIP and IAP both restrain apoptosis, but in different fashions.<sup>25</sup> The NAIP gene contains 17 exons.<sup>22</sup> A telomeric functional copy (NAIP<sup>T</sup>),  $\Psi$  (pseudo) NAIP copy, and several truncated copies are present in the SMA region.<sup>18</sup> Exons 5 and 6 are exclusively present only in the NAIP<sup>T</sup> copy. A PCR based assay for these exons enables one to distinguish the presence or absence of an intact NAIP<sup>T</sup> copy.<sup>18</sup>

A single copy of the gene encoding p44, a subunit of the basal transcription factor TFIIH, was characterised and localised in 1994–1995.<sup>26, 27</sup> Very recently, new data about the p44 gene in relation to its potential involve-

ment in SMA were described by Carter *et al.*<sup>19</sup> and Burglen *et al.*<sup>20</sup> They showed that the p44 gene is present in the SMA region in two copies, as a telomeric and centromeric form, and both are transcribed. The full length gene contains 16 exons. Four nucleotide substitutions enable the telomeric p44<sup>T</sup> and the centromeric p44<sup>C</sup> genes to be distinguished. Burglen *et al.*<sup>20</sup> showed that p44 is also subject to alternative splicing. The probable alignment of genes in the SMA critical region is depicted in fig 1.

### Which gene(s) causes the disease?

It is important to determine whether one of these genes in particular plays a more significant role in the development of spinal muscular atrophy.

It was clearly documented by Lefebvre *et al.*,<sup>17</sup> Velasco *et al.*,<sup>28</sup> Rodrigues *et al.*,<sup>29, 30</sup> and Cobben *et al.*<sup>31</sup> that up to 95% of patients suffering from different forms of SMA are homozygously deleted for both telomeric copies of the SMN gene. In the detailed initial study by Lefebvre *et al.*,<sup>17</sup> the patients who did not have homozygous deletions were shown to have point mutations in the SMN gene. Further studies<sup>32–35</sup> also confirmed the involvement of missense mutations in the SMN gene as part of the mutation spectrum. Interestingly, Talbot *et al.*<sup>33</sup> illustrated a clustering of all known missense mutations within a small interval from codon 262 to codon 279 in the SMN gene. This region has been shown to be 100% conserved between humans, mouse, and rat<sup>33</sup> and therefore can be considered to be an important functional domain of the SMN<sup>T</sup> protein. In addition, further small SMN gene rearrangements like microdeletions<sup>17, 36–38</sup> and duplications<sup>39</sup> have also been detected in SMA patients. The topographic distribution of various microrearrangements and missense mutations identified in the SMN gene are listed in table 1 and depicted in fig 2.

In contrast, as documented in table 1, deletion of both copies of NAIP<sup>T</sup> was observed initially in 45% of SMA type I patients.<sup>18</sup> Slightly higher proportions (62–69%) were reported from the studies conducted by Velasco *et al.*,<sup>28</sup> Rodrigues *et al.*,<sup>29</sup> and Hahnen *et al.*<sup>40</sup> These groups and also the original description by Roy *et al.*<sup>18</sup> showed that fewer SMA type II and III patients (12–18 %) were deleted for NAIP. Homozygous deletion of the NAIP gene is the only mutational event so far detected in this gene. It was well documented by Somerville *et al.*<sup>41</sup> that when both the SMN and NAIP genes are homozygously deleted, there is a five-fold increased risk of type I SMA. Although in most cases if a NAIP<sup>T</sup> deletion is detected, the patient is also deleted for the SMN<sup>T</sup> gene, there are exceptions. A NAIP<sup>T</sup> deletion with retention of at least one copy of the SMN<sup>T</sup> gene was found in about 2% of SMA type I patients.<sup>28, 30</sup> These studies, however, did not analyse the sequence of the entire SMN gene to determine if point mutations or other small intragenic rearrangements were involved.

The role of the p44 gene in the clinical manifestation is still questionable. Both origi-

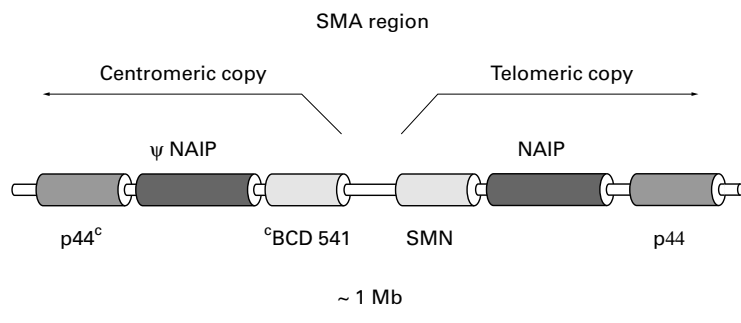


Figure 1 The probable alignment of genes in the SMA critical region (based on Burglen *et al.*<sup>20</sup>).

Table 1 Mutations involved in SMA

No	Gene	Type of mutation	Reference
93%	SMN <sup>T</sup>	Homozygous deletion of exons 7 & 8	17
5.6%	SMN <sup>T</sup>	Homozygous deletion of exon 7 only	17
1	SMN <sup>T</sup>	Missense mutation on codon 245 (P245L)	35
1	SMN <sup>T</sup>	Missense mutation on codon 262 (S262I)	34
1	SMN <sup>T</sup>	Missense mutation on codon 272 (Y272C)	17
1	SMN <sup>T</sup>	Missense mutation on codon 274 (T274I)	34
1	SMN <sup>T</sup>	Missense mutation on codon 275 (G275S)	32
1	SMN <sup>T</sup>	Missense mutation on codon 279 (G279V)	33
1	SMN <sup>T</sup>	4 bp microdeletion in 5' splice donor site of intron 7 (921+4del4)	17
1	SMN <sup>T</sup>	7 bp microdeletion in 3' splice acceptor site of exon 6 (868-10del7)	17
4	SMN <sup>T</sup>	4 bp microdeletion on exon 3 (430del4)	36
1	SMN <sup>T</sup>	Compound heterozygote 1st copy - 5 bp microdeletion on exon 3 (472del5) 2nd copy - deletion	37
1	SMN <sup>T</sup>	Compound heterozygote 1st copy - 2 bp microdeletion on exon 2 (542del2) 2nd copy - deletion or conversion	38
1	SMN <sup>T</sup>	11 bp duplication on exon 6 (800ins11)	39
45% of SMA I and 18% of SMA I,II,III	NAIP	Homozygous deletion	18
73% of SMA I	p44 <sup>T</sup>	Homozygous deletion	20
15% of SMA I,II,III	p44 <sup>T</sup>	Homozygous deletion	19

nal papers<sup>19,20</sup> showed that the telomeric version of the p44 gene is deleted in about 15% of all SMA types. A combined analysis of the entire SMA region, however, indicated that p44 gene deletions are part of a large scale deletion involving the SMN and NAIP genes as well. Carter *et al.*<sup>19</sup> did report two type III SMA sibs deleted for both copies of the p44<sup>T</sup> gene while retaining at least one of their functional copies of the SMN and NAIP genes. Further studies are needed to determine if p44 is critical for the development of SMA.

From all these studies, one could conclude that deletions of this SMA determining region would invariably result in an SMA phenotype. However, Wang *et al.*<sup>12</sup> clearly documented SMN<sup>T</sup> homozygous null alleles in parents of SMA patients. Even more surprising observations were published by Hahnen *et al.*,<sup>40</sup> Burghes,<sup>43</sup> and Cobben *et al.*<sup>31</sup> Both groups identified a pair of haploidentical sibs, both homozygously deleted for SMN<sup>T</sup> with phenotypic variation, one fully expressing the SMA

phenotype and the other being asymptomatic. Similarly, Velasco *et al.*<sup>28</sup> and Rodrigues *et al.*<sup>30</sup> showed that about 1-2% of phenotypically normal SMA carriers are deleted for NAIP<sup>T</sup>. Carter *et al.*<sup>19</sup> also found a homozygous deletion of the telomeric copy of the p44 gene in one normal subject.

### Genotype versus phenotype

How, then, does the knowledge of the different genes involved explain the range of phenotypes observed in people affected with spinal muscular atrophy? Absence of exons 7 and 8 of SMN<sup>T</sup> detected by PCR was found in more than 95% of all types of SMA patients, but exact correlation between the extent of the apparent deletion and the clinical severity has not been established. What about the possible involvement of the centromeric counterpart of the SMN gene, <sup>C</sup>BCD541, in the pathophysiology of SMA?

Because homozygous deletion of both copies of <sup>C</sup>BCD541 in the presence of at least one copy of the SMN<sup>T</sup> gene does not cause the SMA phenotype, it was originally thought that the centromeric copy was non-functional. <sup>C</sup>BCD541 was shown, however, to produce 20-30% of its transcripts as full length functional protein. Lefebvre *et al.*<sup>17</sup> were the first to suggest a possible inverse relationship between the number of <sup>C</sup>BCD541 copies and the severity of the spinal muscular atrophy. This suggestion was later confirmed by Campbell *et al.*<sup>44</sup> who, using pulsed field gel electrophoresis, clearly showed a higher number of <sup>C</sup>BCD541 copies in type II and III SMA patients compared with type I patients. Velasco *et al.*<sup>28</sup> had performed densitometric analysis in parents of type II and III SMA patients and showed that they carried three or more copies of <sup>C</sup>BCD541. The mechanism(s) for the presence of extra copies of <sup>C</sup>BCD541 has not been fully elucidated but gene conversion or duplication have been hypothesised as likely possibilities.<sup>45</sup> These groups also concluded that type I SMA is, in the majority of cases, characterised by physical deletion of

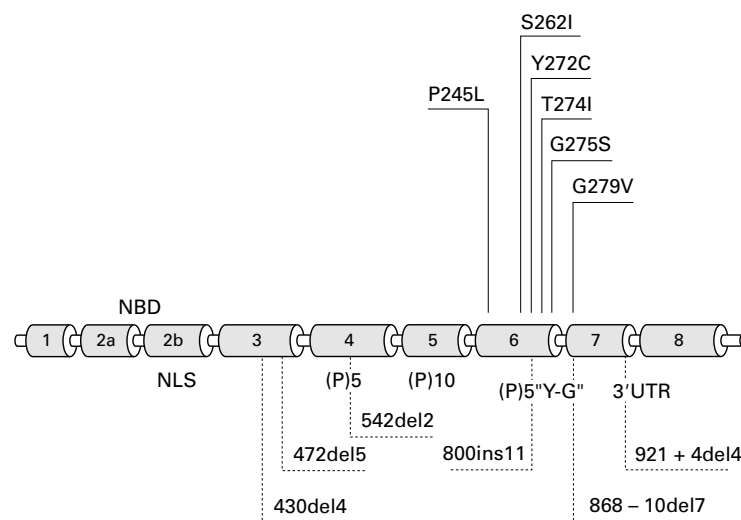
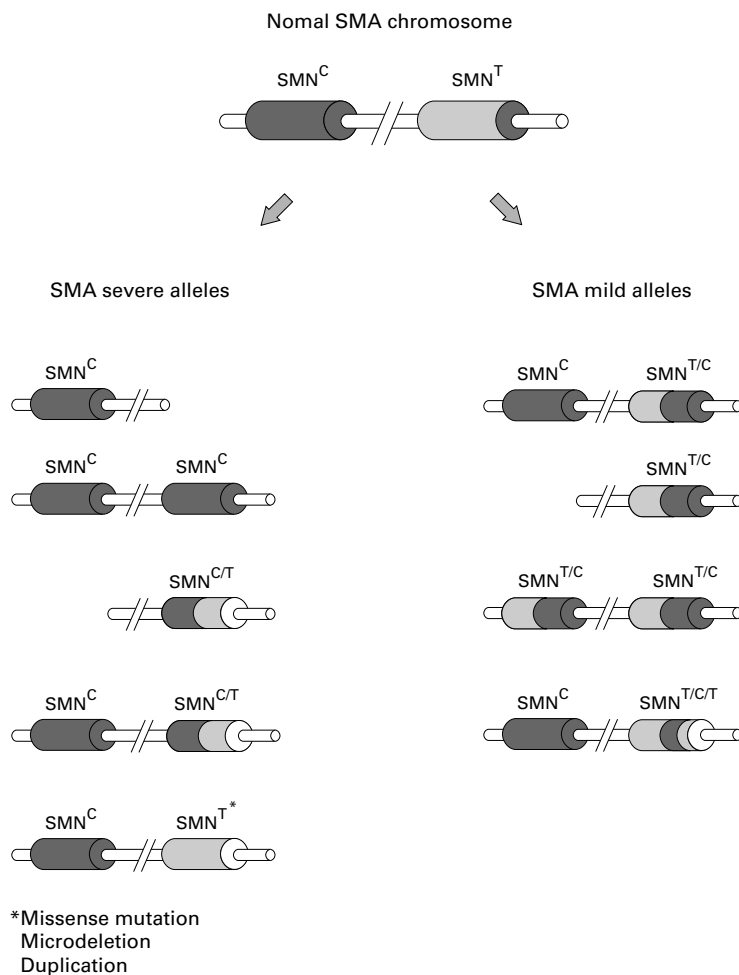


Figure 2 The topographic distribution of the SMN protein domains encoded by the SMN gene and various microrearrangements and missense mutations identified in the SMN gene. NBD=nucleic acid binding domain, NLS=nuclear localisation signal, (P)5=stretch of five prolines, (P)10=stretch of 10 prolines, "Y-G"="Y-G box". See text and table 1 for details and references.



**Figure 3** Schematic overview of the possible SMN alleles causing severe or milder forms of SMA (modified from Burghes *et al*<sup>43</sup>). SMN<sup>C/T</sup>: allele retaining 5' end of SMN<sup>C</sup> with exons 7 and 8 originating from SMN<sup>T</sup>. SMN<sup>T/C</sup>: allele retaining 5' end of SMN<sup>T</sup> with exons 7 and 8 originating from SMN<sup>C</sup>. SMN<sup>T/C/T</sup>: rare conversion allele in which exon 7 of SMN<sup>T</sup> is converted to SMN<sup>C</sup>. SMN<sup>T\*</sup>: SMN<sup>T</sup> allele with small rearrangements like missense mutation, microdeletion, or duplication.

the SMN<sup>T</sup> gene while the mutations in type II and III SMA are replacements of the telomeric copy by the centromeric form.

A more detailed examination of the SMN<sup>C</sup> copy number conducted by McAndrew *et al*<sup>46</sup> indeed showed a shift in distribution with the milder phenotype having a higher number of SMN<sup>C</sup> copies. An unusual example was described by McAndrew *et al*<sup>46</sup> where one asymptomatic subject who had homozygous deletion of both copies of the SMN<sup>T</sup> gene had four copies of SMN<sup>C</sup>. Interestingly, this group reanalysed the haploidentical sibs described by Burghes.<sup>43</sup> These sibs varied clinically from expressing SMA to mildly affected/unaffected and both had three copies of SMN<sup>C</sup> and no copy of SMN<sup>T</sup>. Therefore, at a simplistic level, study of the copy numbers of SMN<sup>C</sup> and SMN<sup>T</sup> do not explain the phenotypic difference. Burghes,<sup>43</sup> however, proposed that two different functional SMN<sup>C</sup> alleles exist: one such allele, created by gene conversion (but not including the 5' end of SMN<sup>T</sup>), can modify the phenotype, whereas the other allele, SMN<sup>C</sup> in its original physical context or formed by extended conversion through the entire SMN<sup>T</sup> gene, cannot modify the phenotype.

Partial gene conversion events have been also shown to be the explanation for about 5% of SMA patients who have absence of exon 7 of SMN<sup>T</sup> on PCR but are positive for exon 8.<sup>43</sup> From the description of all these different types of alleles, Burghes<sup>43</sup> has classed them into mild and severe. He proposed that type I SMA is caused when two severe SMA alleles are present, type II SMA when one mild allele is present, and type III SMA when two mild alleles are present. A modified version of his model is presented schematically in fig 3.

### Diagnostic testing

Relatively simple DNA tests based either on SSCP analysis<sup>17</sup> or PCR followed by restriction enzyme digestion<sup>23</sup> enables confirmation of a suspected clinical diagnosis of SMA or prediction of the outcome of a pregnancy in families with a history of SMA (as reviewed by Zerres *et al*<sup>47</sup> and Brahe and Bertini<sup>48</sup>). The absence of both copies of the SMN<sup>T</sup> gene is a very reliable and powerful diagnostic assay for the majority of SMA patients, confirming their clinical SMA phenotype. Zerres *et al*<sup>47</sup> suggested that as all NAIP deleted patients are also deleted for SMN<sup>T</sup>, NAIP testing does not need to be part of routine diagnosis. However, there are about 2% of patients that are deleted for NAIP but not both copies of SMN<sup>T</sup>.<sup>28-30</sup> One could therefore test with NAIP if patients are not deleted for SMN<sup>T</sup>.

With respect to linkage analysis in prenatal diagnosis, opinions are varied. Many diagnostic laboratories still perform linkage analysis in addition to direct SMN<sup>T</sup> mutation analysis in families where the previously affected child lacks both copies of SMN<sup>T</sup>. The basis of this appears to stem from the fact that there are people in the normal population who lack both copies of SMN<sup>T</sup> but are not clinically affected. Therefore one of the carrier parents may possess two deleted alleles, but only one when passed to the next child will result in SMA. The only way to confirm that the same allele has been inherited from both parents, and therefore that the next pregnancy is at risk of SMA, is to include linkage analysis when the fetus lacks both copies of SMN<sup>T</sup>. Linkage analysis is the only option in the families where no deletion has been observed but the clinical findings are consistent with 5q SMA.

On the other hand, the PCR based assay for determining the presence or absence of SMN<sup>T</sup> is not quantitative and therefore it cannot detect subjects with heterozygous absence of the SMN<sup>T</sup> gene. Practically, this test therefore cannot identify SMA carriers, or distinguish between a non-5q SMA-like patient and a compound heterozygote 5q SMA patient with a point mutation in one allele and absence of the other. The development of a quantitative assay was therefore of high priority.

### DOSAGE ANALYSIS

The first attempts to estimate the copy number used the densitometric assessment of the SMN<sup>T</sup>/SMN<sup>C</sup> ratio<sup>28-49</sup> or solid phase minisequencing.<sup>50</sup> The drawback of these methods was that they were not suitable for



SMA carrier analysis or detection of compound heterozygotes because they relied on the ratio of  $SMN^T/SMN^C$  without the use of an external standard. Incorporation of two external controls became an essential part of a novel dosage analysis designed by McAndrew *et al.*<sup>46</sup> which improved the diagnostic power to detect absence of one copy of the  $SMN^T$  gene.

An example of the successful application of the technique to confirm the diagnosis of a possible case of severe SMA was given by Parsons *et al.*<sup>38</sup> The patient was directly tested and found not to have the homozygous deletion of  $SMN^T$  found in about 95% of patients. The quantitative PCR assay was performed and the patient was found to have lost one copy of  $SMN^T$ . Subsequent sequencing resulted in the identification of a 2 bp deletion in exon 4. This illustrates the value of the methodology, not only for carrier detection but also for confirmation of diagnosis in patients who are compound heterozygotes with loss of one  $SMN^T$  allele and a mutation in the second.

Another strategy for determining the copy number of the  $SMN^C$  and  $SMN^T$  genes was designed by Campbell *et al.*<sup>44</sup> They used pulsed field gel electrophoresis and interpretation of the hybridisation pattern of  $SMN$  and NAIP probes for their analysis. Although an elegant technique, it is not likely to be routinely used in a DNA diagnostic laboratory.

Although carrier detection studies have focused on the analysis of the  $SMN^T$  copy number,  $SMN^C$  copy number is also determined in the procedure. The exact interpretation of the  $SMN^C$  analysis with respect to carrier status prediction is not clear at present.

### From genes to proteins

#### $SMN^T$ PROTEIN CHARACTERISATION

When the  $SMN$  gene was initially identified in 1995 by Lefebvre *et al.*<sup>17</sup> it had no known homology to existing proteins, and the function and subcellular localisation were unknown. Lefebvre *et al.*<sup>7</sup> did identify a stretch of five prolines in exons 4 and 6 and 10 prolines in exon 5 which might have an important role in the folding of the  $SMN$  protein, thus initiating the elucidation of several functional domains. DiDonato *et al.*<sup>51</sup> showed a putative nuclear localisation signal (NLS) encoded in exon 2b. Finally, Talbot *et al.*<sup>33</sup> described the clustering of missense mutations within exons 6 and 7 (the "Y-G box"), which is part of an evolutionarily highly conserved region, and is an important functional domain involved in mRNA metabolism. Further insight into functional characterisation of this domain was elucidated by Lorson *et al.*<sup>52</sup> They identified a small, 30 amino acid region which directly mediates  $SMN$  self-oligomerisation. The authors also compared wild type to mutant  $SMN$  proteins of type I, II, and III SMA patients and found a direct correlation between oligomerisation and clinical type. Recently, Lorson *et al.*<sup>53</sup> identified a potent non-sequence specific nucleic acid binding domain (NBD) encoded by exon 2 of the  $SMN$  gene. Interestingly, they tested the previously reported missense mutations S262I<sup>34</sup> and Y272C<sup>17</sup> and found that RNA binding activity

dropped two to three-fold. The topographic distribution of the above mentioned  $SMN$  protein domains are depicted in fig 2.

#### SUBCELLULAR LOCALISATION

The first information about the subcellular localisation of the  $SMN$  protein was described by Liu and Dreyfuss in 1996.<sup>54</sup> In searching for  $SMN$  interacting proteins using a yeast two hybrid screen, they found that the  $SMN$  protein interacts with the RGG box region of hnRNP U protein, with itself, with fibrillarin, the small nucleolar RNA binding protein, and with several novel proteins as well.

Using antibodies raised against the  $SMN$  protein, Liu and Dreyfuss<sup>54</sup> localised it into discrete dot-like novel nuclear structures named gems (gemini of coiled bodies), as well as finding it in the cytoplasm. Gems are located in close proximity to coiled bodies which are conserved subnuclear structures. Both are disassembled and reassembled during the cell cycle in a similar fashion. Although the specific functions of coiled bodies are as yet unknown, their components suggest their possible role in the metabolism of snRNPs (small nuclear ribonucleoprotein particles) and in pre-mRNA processing. As documented by Covert *et al.*<sup>55</sup> the number of gems is significantly reduced in type I SMA patients (100-fold reduction compared to unaffected controls) and this also correlates with the clinical severity of SMA.

Further detailed studies from Liu *et al.*<sup>6</sup> showed that the N-terminal region of the  $SMN$  gene product is tightly associated with a novel protein SIP1 ( $SMN$  interacting protein). The  $SMN$ -SIP1 complexes are large and contain several U snRNP proteins. One subclass of these proteins is known as the common proteins as they associate with all U snRNAs and contain a related Sm domain. Most of the common proteins bind recombinant  $SMN$  protein *in vitro*.<sup>56</sup> Antibodies directed against the  $SMN$ -SIP1 complex strongly interfere with the cytoplasmic assembly of the common snRNP proteins, with spliceosomal snRNAs, and with the import of the snRNP complex into the nucleus.<sup>57</sup> Based on these findings, Fischer *et al.*<sup>57</sup> therefore suggested a role for  $SMN$  and SIP1 in spliceosomal snRNP biogenesis and a functional defect in this assembly may, therefore, be the cause of SMA.

#### EXPRESSION STUDIES

$SMN$  gene expression studies<sup>58 59</sup> showed that both forms of the gene were expressed in various non-CNS tissues (including heart, liver, muscle, lung, thymus, pancreas, kidney, and also lymphoblastoid cell lines) as well as CNS derived tissues. Lefebvre *et al.*<sup>68</sup> showed that the  $SMN$  protein was widely but unevenly present in all parts of the brain and even within different motor neurone cells. An increased level of the protein found in the spinal cord inversely correlated with the severity of the disorder.

Lastly, it should be asked why such a devastating effect is observed only in the motor neurone cell, when the  $SMN$  protein is expressed in various non-CNS derived tissues. One possibility which was named "the threshold

effect theory” was suggested by Battaglia *et al.*<sup>59</sup> They proposed that motor neurones are exquisitely dependent upon SMN expression, requiring high amounts of the SMN protein to survive. When the required amount of the SMN protein drops below the threshold critical for the survival of motor neurones, this might lead to their degeneration and to dysregulation of apoptosis. However, reduced levels might still be compatible with the survival of other less susceptible cell types in the CNS or other tissues.

An alternative explanation was offered by Mattaj<sup>60</sup> based on the observation of Liu *et al.*<sup>60</sup> that SMN protein binds to the Sm domain in most common proteins in the U snRNP family proteins. Two of these Sm proteins are replaced in neurones by a neurone specific Sm protein (named SmN protein). Mattaj<sup>60</sup> suggested that this form may have a different interaction with SMN thus accounting for the tissue specificity of the phenotype.

A third possibility comes from the study by Iwahashi *et al.*<sup>61</sup> which helps shed some light on how lack of SMN results in the degeneration of the anterior horn cells of the spinal cord. In investigating the anti-apoptotic function of Bcl-2 (anti-apoptotic protein), a yeast two hybrid screen yielded SMN as an interactive protein. SMN on its own was shown to have a weak anti-apoptotic effect, but when coexpressed with Bcl-2 a synergistic preventative effect was observed. Furthermore, they also showed that SMN protein carrying a missense mutation Y272C<sup>17</sup> exerts no synergism with Bcl-2. Bcl-2 coupled with SMN was shown to inhibit Bax (pro-apoptotic protein) induced apoptosis. This is particularly interesting as Bax is necessary for the death of sympathetic neurones and motor neurones.

#### WHAT DOES THE SMN<sup>C</sup> PROTEIN DO?

Both forms of the SMN gene encode protein with no altered amino acid sequence.<sup>17</sup> Covert *et al.*<sup>65</sup> indicated that the SMN<sup>C</sup> protein level and number of gems differed in SMA type I from SMA type II patients, even if they possessed apparently the same number of SMN<sup>C</sup> copies. These data favour the view that the SMN<sup>C</sup> protein is not functionally equivalent. Covert *et al.*<sup>65</sup> suggested that the SMN<sup>C</sup> loci produced by conversion of the SMN<sup>T</sup> gene generates reduced but still sufficient levels of protein for gem formation (SMA type II, III), while the SMN<sup>C</sup> gene remaining in the SMA type I patient is not capable of forming gems.

Lefebvre *et al.*<sup>68</sup> postulated that the hyperexpression of the SMN<sup>C</sup> gene(s), therefore, can be considered as an attractive strategy for a therapeutic approach to SMA treatment. Attempts to upregulate alternative forms of a gene (for example, the  $\gamma$  globin gene) to overcome a genetic defect have been considered for  $\beta$  thalassaemia, where continued production of the fetal haemoglobin may overcome the lack of the  $\beta$  globin chain. The same strategy has also been proposed for Duchenne muscular dystrophy, where utrophin can substitute for dystrophin and, therefore, upregulation of

the utrophin may ameliorate the clinical phenotype.<sup>62</sup> Further studies focusing on the characterisation of the SMN promoter region<sup>63</sup> would be essential to pursue this strategy.

#### THE ROLE OF NAIP

In 1996, Liston *et al.*<sup>65</sup> proved that NAIP, and possibly other members of the IAP (inhibitor of apoptosis proteins) family of proteins, have anti-apoptotic effects in mammalian cells. They also showed that NAIP is expressed in motor but not in sensory neurones. This finding is in accordance with the fact that the protein is acting as a negative regulator of motor neurone apoptosis and once it is deficient or absent, it contributes to the development of the SMA phenotype. Whether NAIP is acting solely downstream on the pathological pathway of SMA or is interacting with the SMN protein, as was indicated from the two hybrid study carried out by Lu *et al.*,<sup>64</sup> is too early to say. Further studies are required to elucidate possible functional interactions between these proteins.

Xu *et al.*<sup>65</sup> showed that overexpression of NAIP reduces the effect of transient ischaemia in rat hippocampal neurones. This opens up the exciting possibility that upregulation of NAIP expression may be used in therapy of stroke. Thus, research into a relatively uncommon disease such as SMA may offer new insights for therapy in more common disorders.

#### Conclusion

In conclusion, we have reviewed the currently available publications in order to develop a better understanding of the molecular basis of SMA. The clinical heterogeneity of this disorder (varying age of onset and severity of manifestation) undoubtedly mirrors the intrinsic complexity of the genes involved in its aetiology. The identification of two major genes (the survival motor neurone gene and neuronal apoptosis inhibitory protein gene) causing the SMA phenotype have provided new insight into the mechanism(s) involved in the development of this deleterious condition but highlights that the interactions between these proteins are complex and not fully resolved.

The message is therefore clear that even disorders that appear to follow simple Mendelian genetics can produce many novel and fascinating findings that continually challenge the minds of the researchers trying to untangle the knot.

#### Note added in proof

Scharf *et al.*<sup>66</sup> have recently identified a candidate phenotypic modifier for SMA called H4F5.

We wish to thank Dr Stephen Kahler for helpful discussions and for critical reading of the manuscript.

- 1 Pearn J. Classification of spinal muscular atrophies. *Lancet* 1980;i:919-22.
- 2 Emery AEH. The nosology of the spinal muscular atrophies. *J Med Genet* 1971;8:481-95.
- 3 Werdnig G. Zwei frühinfantile hereditäre Falle von progressiver Muskeldystrophie unter dem Bilde der Dystrophie, aber auf neurotischer Grundlage. *Arch Psychiatr* 1891;22:437-

- 81, cited in Rimoin DL, Connor JM, Pyeritz RE. *Emery and Rimoin's principles and practice of Medical Genetics* 1996: 2387.
- 4 Hoffmann J. Über chrinische spinale Muskelatrophie im Kindersalter, auf familiarer Basis. *Dtsche Z Nervenheilkd* 1893;3:427-70, cited in Rimoin DL, Connor JM, Pyeritz RE. *Emery and Rimoin's principles and practice of medical genetics* 1996:2387.
  - 5 Kugelberg E, Welander L. Familial neurogenic (spinal?) muscular atrophy simulating ordinary proximal dystrophy. *Acta Psychiatr Scand* 1954;29:42-43, cited in Rimoin DL, Connor JM, Pyeritz RE. *Emery and Rimoin's principles and practice of medical genetics* 1996:2388.
  - 6 Kugelberg E, Welander L. Heredofamilial juvenile muscular atrophy simulating muscular dystrophy. *Arch Neurol Psychiatry* 1956;75:500-9, cited in Rimoin DL, Connor JM, Pyeritz RE. *Emery and Rimoin's principles and practice of medical genetics* 1996:2388.
  - 7 Gilliam TC, Brzustowicz LM, Castilla LH, et al. Genetic homogeneity between acute and chronic forms of spinal muscular atrophy. *Nature* 1990;345:823-5.
  - 8 Brzustowicz LM, Lehner T, Castilla LH, et al. Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. *Nature* 1990;344:540-1.
  - 9 Melki J, Abdelhak S, Sheth P, et al. Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q. *Nature* 1990;344:767-8.
  - 10 Melki J, Sheth P, Abdelhak S, et al. Mapping of acute (type I) spinal muscular atrophy to chromosome 5q12-q14. The French Spinal Muscular Atrophy Investigators. *Lancet* 1990;336:271-3.
  - 11 Thomson TG, Morrison KE, Klein P, et al. Refinement of the spinal muscular atrophy locus by genetic and physical mapping. *Hum Mol Genet* 1993;2:1169-76.
  - 12 Francis MJ, Morrison KE, Campbell L, et al. A contig of non-chimaeric YACs containing the spinal muscular atrophy gene in 5q13. *Hum Mol Genet* 1993;2:1161-7.
  - 13 Kleyn PW, Wang CH, Lien LL, et al. Construction of a yeast artificial chromosome contig spanning the spinal muscular atrophy disease gene region. *Proc Natl Acad Sci USA* 1993; 90:6801-5.
  - 14 Francis MJ, Nesbit MA, Theodosiou AM, et al. Mapping of retrotransposon sequences in the unstable region surrounding the spinal muscular atrophy locus in 5q13. *Genomics* 1995;27:366-9.
  - 15 Sargent CA, Chalmers IJ, Leversha M, Affara NA. A rearrangement on chromosome 5 of an expressed human beta-glucuronidase pseudogene. *Mamm Genome* 1994;5: 791-6.
  - 16 Selig S, Bruno S, Scharf JM, et al. Expressed cadherin pseudogenes are localized to the critical region of the spinal muscular atrophy gene. *Proc Natl Acad Sci USA* 1995;92: 3702-6.
  - 17 Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995;80:155-65.
  - 18 Roy N, McLean MD, Besner-Johnston A, et al. Refined physical map of the spinal muscular atrophy gene (SMA) region at 5q13 based on YAC and cosmid contiguous arrays. *Genomics* 1995;26:451-60.
  - 19 Carter TA, Bonnemant CG, Wang CH, et al. A multicopy transcription-repair gene, BTF2p44, maps to the SMA region and demonstrates SMA associated deletions. *Hum Mol Genet* 1997;6:229-36.
  - 20 Burglen L, Seroz T, Miniou P, et al. The gene encoding p44, a subunit of the transcription factor TFIIF, is involved in large-scale deletions associated with Werdnig-Hoffmann disease. *Am J Hum Genet* 1997;60:72-9.
  - 21 Burglen L, Lefebvre S, Clermont O, et al. Structure and organization of the human survival motor neurone (SMN) gene. *Genomics* 1996;32:479-82.
  - 22 Chen Q, Baird SD, Mahadevan M, et al. Sequence of a 131-kb region of 5q13.1 containing the spinal muscular atrophy candidate genes SMN and NAIP. *Genomics* 1998;48:121-7.
  - 23 van der Steege G, Grootcholten PM, van der Vlies P, et al. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. *Lancet* 1995;345: 985-6.
  - 24 Gennarelli M, Lucarelli M, Capon F, et al. Survival motor neuron gene transcript analysis in muscles from spinal muscular atrophy patients. *Biochem Biophys Res Commun* 1995;213:342-8.
  - 25 Liston P, Roy N, Tamai K, et al. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature* 1996;379:349-53.
  - 26 Humbert S, van Vuuren H, Lutz Y, Hoeijmakers JH, Egly JM, Moncollin V. p44 and p34 subunits of the Btf2/Tfiif transcription factor have homologies with Ssl1, a yeast protein involved in DNA repair. *EMBO J* 1994;13:2393-8.
  - 27 van der Steege G, Draaijers TG, Grootcholten PM, et al. A provisional transcript map of the spinal muscular atrophy (SMA) critical region. *Eur J Hum Genet* 1995;3:87-95.
  - 28 Velasco E, Valero C, Valero A, Moreno F, Hernandez-Chico C. Molecular analysis of the SMN and NAIP genes in Spanish spinal muscular atrophy (SMA) families and correlation between number of copies of cBCD541 and SMA phenotype. *Hum Mol Genet* 1996;5:257-63.
  - 29 Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Hum Mol Genet* 1995;4:631-4.
  - 30 Rodrigues NR, Owen N, Talbot K, et al. Gene deletions in spinal muscular atrophy. *J Med Genet* 1996;33:93-6.
  - 31 Cobben JM, van der Steege G, Grootcholten P, de Visser M, Scheffer H, Buys CH. Deletions of the survival motor neuron gene in unaffected siblings of patients with spinal muscular atrophy. *Am J Hum Genet* 1995;57:805-8.
  - 32 Burglen L. A novel point mutation in the SMN gene in a patient with type III spinal muscular atrophy. First Congress of the World Muscle Society. London: Elsevier, 1996.
  - 33 Talbot K, Ponting CP, Theodosiou AM, et al. Missense mutation clustering in the survival motor neuron gene: a role for a conserved tyrosine and glycine rich region of the protein in RNA metabolism? *Hum Mol Genet* 1997;6:497-500.
  - 34 Hahnen E, Schonling J, Rudnik-Schoneborn S, Raschke H, Zerres K, Wirth B. Missense mutations in exon 6 of the survival motor neuron gene in patients with spinal muscular atrophy (SMA). *Hum Mol Genet* 1997;6:821-5.
  - 35 Rochette C, Surh LC, Ray PN, et al. Molecular diagnosis of non-deletion SMA patients using quantitative PCR of exon 7. *Neurogenetics* 1997;1:141-7.
  - 36 Bussaglia E, Clermont O, Tizzano E, et al. A frame-shift deletion in the survival motor neuron gene in Spanish spinal muscular atrophy patients. *Nat Genet* 1995;11:335-7.
  - 37 Brahe C, Clermont O, Zappata S, Tiziano F, Melki J, Neri G. Frameshift mutation in the survival motor neuron gene in a severe case of SMA type I. *Hum Mol Genet* 1996;5:1971-6.
  - 38 Parsons DW, McAndrew PE, Allinson PS, Parker WD Jr, Burghes AHM, Prior TW. Diagnosis of spinal muscular atrophy in an SMN non-deletion patient using a quantitative PCR screen and mutation analysis. *J Med Genet* 1998; 35:674-6.
  - 39 Parsons DW, McAndrew PE, Monani UR, Mendell JR, Burghes AH, Prior TW. An 11 base pair duplication in exon 6 of the SMN gene produces a type I spinal muscular atrophy (SMA) phenotype: further evidence for SMN as the primary SMA-determining gene. *Hum Mol Genet* 1996; 5:1727-32.
  - 40 Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. *Hum Mol Genet* 1995;4:1927-33.
  - 41 Somerville MJ, Hunter AG, Aubry HL, Korneluk RG, MacKenzie AE, Surh LC. Clinical application of the molecular diagnosis of spinal muscular atrophy: deletions of neuronal apoptosis inhibitor protein and survival motor neuron genes. *Am J Med Genet* 1997;69:159-65.
  - 42 Wang CH, Xu J, Carter TA, et al. Characterization of survival motor neuron (SMNT) gene deletions in asymptomatic carriers of spinal muscular atrophy. *Hum Mol Genet* 1996;5:359-65.
  - 43 Burghes AHM. When is a deletion not a deletion? When it is converted. *Am J Hum Genet* 1997;61:9-15.
  - 44 Campbell L, Potter A, Ignatius J, Dubowitz V, Davies K. Genomic variation and gene conversion in spinal muscular atrophy: implications for disease process and clinical phenotype. *Am J Hum Genet* 1997;61:40-50.
  - 45 Melki J. Spinal muscular atrophy. *Curr Opin Neurol* 1997;10:381-5.
  - 46 McAndrew PE, Parsons DW, Simard LR, et al. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. *Am J Hum Genet* 1997;60:1411-22.
  - 47 Zerres K, Wirth B, Rudnik-Schoneborn S. Spinal muscular atrophy-clinical and genetic correlations. *Neuromuscul Disord* 1997;7:202-7.
  - 48 Brahe C, Bertini E. Spinal muscular atrophies: recent insights and impact on molecular diagnosis. *J Mol Med* 1996;74:555-62.
  - 49 Matthijs G, Schollen E, Legius E, et al. Unusual molecular findings in autosomal recessive spinal muscular atrophy. *J Med Genet* 1996;33:469-74.
  - 50 Schwartz M, Sorensen N, Hansen FJ, et al. Quantification, by solid-phase minisequencing, of the telomeric and centromeric copies of the survival motor neuron gene in families with spinal muscular atrophy. *Hum Mol Genet* 1997;6:99-104.
  - 51 DiDonato CJ, Chen XN, Noya D, Korenberg JR, Nadeau JH, Simard LR. Cloning, characterization, and copy number of the murine survival motor neuron gene: homolog of the spinal muscular atrophy-determining gene. *Genome Res* 1997;7:339-52.
  - 52 Lorson CL, Strasswimmer J, Yao JM, et al. SMN oligomerization defect correlates with spinal muscular atrophy severity. *Nat Genet* 1998;19:63-6.
  - 53 Lorson CL, Androphy EJ. The domain encoded by exon 2 of the survival motor neuron protein mediates nucleic acid binding. *Hum Mol Genet* 1998;7:1269-75.
  - 54 Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. *EMBO J* 1996;15:3555-65.
  - 55 Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet* 1997;6:1205-14.
  - 56 Liu Q, Fischer U, Wang F, Dreyfuss G. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell* 1997;90:1013-21.
  - 57 Fischer U, Liu Q, Dreyfuss G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* 1997;90:1023-9.
  - 58 Lefebvre S, Burlet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet* 1997;16:265-9.

- 59 Battaglia G, Princivalle A, Forti F, Lizier C, Zeviani M. Expression of the SMN gene, the spinal muscular atrophy determining gene, in the mammalian central nervous system. *Hum Mol Genet* 1997;6:1961-71.
- 60 Mattaj W. Ribonucleoprotein assembly: clues from spinal muscular atrophy. *Curr Biol* 1998;8:R93-5.
- 61 Iwahashi H, Eguchi Y, Yasuhara N, Hanafusa T, Matsuzawa Y, Tsujimoto Y. Synergistic anti-apoptotic activity between Bcl-2 and SMN implicated in spinal muscular atrophy. *Nature* 1997;390:413-17.
- 62 Deconinck AE, Rafael JA, Skinner JA, et al. Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell* 1997;90:717-27.
- 63 Monani U, Kathuria S, Offenbaker J, Burghes AHM, McPerson J. Sequence differences and promotor analysis of the survival motor neuron (SMN<sup>T</sup> and SMN<sup>C</sup>) genes. *Am J Hum Genet* 1997;61:U1394.
- 64 Lu Y, Chen Q, Gendron N, Tamai K, Toji S, MacKenzie A. Assessment for NAIP/IAP interaction with SMN in yeast two-hybrid system. *Am J Hum Genet* 1997;61:A1017.
- 65 Xu DG, Crocker SJ, Doucet JP, et al. Elevation of neuronal expression of NAIP reduces ischemic damage in the rat hippocampus. *Nat Med* 1997;3:997-1004.
- 66 Scharf JM, Endrizzi MG, Wetter A, et al. Identification of a candidate modifying gene for spinal muscular atrophy by comparative genomics. *Nat Genet* 1998;20:83.