EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 37, No. 3, 147-154, June 2005

Genotype-Phenotype correlation of *SMN* locus genes in spinal muscular atrophy patients from India

Akanchha Kesari[1], M Mohammed Idris[2], Giri Raj Chandak[2] and Balraj Mittal[1,3]

[1]Department of Genetics
Sanjay Gandhi Postgraduate
Institute of Medical Sciences
Raebareli Road, Lucknow-226 014-India
[2]Centre for Cellular and Molecular Biology, Uppal Road,
Hyderabad-500 007-India
[3]Corresponding author: Tel, 91-522-2668005;
Fax, 91-522-2668973; E-mail, balraj@sgpgi.ac.in

Accepted 25 March 2005

Abbreviations: *BTFp44*, basal transcription factor subunit *p44*; NAIP, neuronal apoptosis inhibitory protein; SMA, spinal muscular atrophy; *SMN*, survival of motor neuron gene

Abstract

Spinal muscular atrophy has been classified into four groups based on the age of onset and clinical severity of the disease. Homozygous deletion in *SMN1* gene causes the disease but the clinical severity may be modified by copy number of homologous gene *SMN2* as well as the extent of deletion at *SMN* locus. In the view of scarcity of genotype and phenotype correlation data from India, this study has been undertaken to determine that correlation in *SMA* patients by using the *SMN* and *NAIP* genes and two polymorphic markers C212 and C272 located in this region. Two to four alleles of the markers C212 and C272 were observed in normal individuals. However, majority of Type I patients showed only one allele from both markers whereas in Type II and III patients, 2-3 alleles were observed. The *SMN2* copy number in our type III patients showed that patients carry 3-5 copies of *SMN2* gene. Our results suggest that extent of deletions encompassing *H4F5*, *SMN1*, *NAIP* and copy number of *SMN2* gene can modify the *SMA* phenotype, thus accounting for the different clinical subtypes of the disease.

Keywords: C212; C272; *NAIP*; *SMN1*; *SMN2*

Introduction

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, characterized by destruction of α-motor neurons of anterior horn cells, which leads to symmetrical muscle weakness and atrophy. It has an estimated incidence of 1/10,000 live births (Panigrahi *et al.,* 2002), and a carrier frequency of 1/50. Clinically proximal SMA is further divided into four types based on the age of onset and severity of the clinical course. Childhood onset SMAs can be classified into three types (Type I-III) (Biros and Forrest, 1999). Type I is the most severe form having symptoms of SMA before 6 months. Type II is an intermediate form with onset before the age of 18 months. Type III is a relatively milder, chronic form with onset after the age of 18 months. Type IV SMA is an adult form with onset after 30 years of age with variable severity but normal life span.

By molecular analysis all these forms had been mapped to chromosome 5q11.2-q13.3, suggesting that they are allelic disorders. The region has duplications of a large segment consisting at least four genes, which are present in a telomeric (t) and a centromeric (c) copy; Survival motor neuron gene (*SMN1* or *SMNt* and *SMN2* or *SMNc*), neuronal apoptosis inhibitory protein gene (*NAIP* and *φNAIP*) basal transcription factor subunit p44 (*BTFp44t* and *BTFp44c*) and a novel protein of unknown function *H4F5*t and *H4F5*c (Wirth, 2000). The *SMN* locus in 5q region is particularly unstable and prone to large scale deletions (Melki *et al.,* 1994) (Figure 1).

Survival motor neuron is the most important gene associated with SMA which has two homologous copies, *SMN1* and *SMN2*. It has been observed that the *SMN1* gene is deleted in majority of SMA patients (Wirth, 2000), but *SMN2* is not directly associated with disease because 26% of normal individuals in a populations lack both copies. The failure of *SMN2* to fully compensate for loss of *SMN1* is due to sequence difference in exon 7 which causes alternative splicing of *SMN2* and as a result lower amount of full-length protein is produced. An increase in *SMN2* copy number could raise protein levels and modify phenotype. However efforts to demonstrate a correlation between *SMN2* copy number and disease severity have produced conflicting results (Velasco *et al.,* 1996; McAndrew *et al.,* 1997; Taylor *et al.,* 1998; Feldkotter *et al.,* 2002; Anhuf *et al.,* 2003)

NAIP, a gene located 16.5 Kb downstream of *SMN*, has also been suggested to be an SMA modifier due to its proximity to *SMN*, and found to be deleted in 45% of SMA type I patients and in 18% of type II and III SMA patients (Roy *et al.,* 1995).

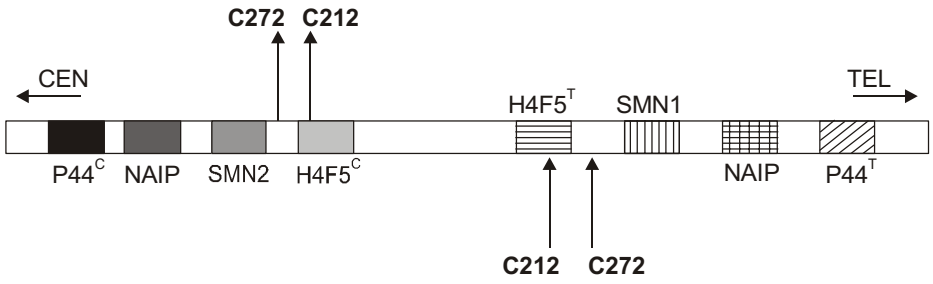

Figure 1. Schematic diagram showing telomeric and centromeric copies of genes and polymorphic loci at SMN locus on chromosome 5.

Deletions in *NAIP* appear to be associated with increased severity of the disease, although the association is not absolute (Roy *et al.,* 1995; Somerville *et al.,* 1997).

Using comparative genomics to screen for factor/gene Scharf *et al.* (1998) identified a novel transcript, *H4F5*, which lies closer to *SMN1* than any previously identified gene in the region. A multicopy microsatellite marker, C212 is located in the last intron of *H4F5* gene and is often present in two copies per chromosome. Due to the inherent instability of SMA locus, C212 copy number varies from 1-3 alleles per chromosome in the normal population. It has been reported that polymorphic locus C212 is deleted in more than 90% of type I SMA patients indicating that *H4F5* is also highly deleted in type I SMA, and thus is a candidate phenotypic modifier for SMA. The other dinucleotide repeat C272 (Ag1-CA) is located 463 bp upstream of the putative ATG translation initiation sites of both genes (Burglen *et al.,* 1996). Due to its close proximity, C272 is considered as a marker for integrity of the 5' end of the *SMN* gene. Analysis using C272 showed that 50% of type I patients had a heterozygosity deficiency of the marker compared with 6% and 10% of types II and III patients respectively (Burlet *et al.,* 1996).

Gene deletion studies of 5q region suggest involvement of multiple genes with SMA phenotype (Wirth *et al.,* 1995; Burlet *et al.,* 1996; Rodrigues *et al.,* 1996; Velasco *et al.,* 1996; Scharf *et al.,* 1998). Till date no data on such a correlation has been reported from India. So the main objective of our study was to establish the genotype-phenotype correlation in Indian patients using the *SMN*, *NAIP* genes and C212, C272 markers as well as to find out *SMN2* copy number in milder patients.

Materials and Methods

Subject

The study was carried out in patients belonging to all the four groups of proximal SMA. A total of 50 unrelated SMA patients were included in the study. The patients were selected according to the criteria of International SMA Consortium 1999. Seventeen had type I form of SMA, 7 had type II, 23 had type III, and 3 had type IV SMA.

One hundred healthy age, sex and religion matched individual without any history of neurological disease and normal in neurological examination were included as control to know the status of these genes and marker copies in normal individuals.

Methods

Five ml blood was collected in EDTA tubes from 50 patients and 100 controls. DNA was extracted from blood by the standard salting out method (Olernp and Zetterqnist, 1992).

Molecular analysis of SMN gene

Patients were tested for homozygous deletion of exons 7 and 8 of *SMN1* gene by a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method as described previously by Kesari *et al.,* 2003.

Molecular analysis of NAIP gene

All individuals were also tested for exon 5 deletion of *NAIP* gene. PCR conditions and primers used to amplify the exon 5 were identical to those of Somerville *et al.* (1997). The PCR was started with initial denaturation at 96°C for 7 min followed by amplification for 30 cycles (94°C, 60°C and 72°C for 1.30 min each) followed by a final extension for 10 min at 72°C. The PCR products were analysed on 2% agarose gel containing ethidium bromide.

Marker analysis (C212 and C272)

PCR was performed according to Wirth *et al.* (1995) and Di Donato *et al.* (1994) respectively with some modifications. The samples were denatured initially at 96°C for 7 min, followed by amplification for 30 cycles (94°C for 1 min, 62°C for 30 s, 65°C for 2 min) and a final extension for 10 min at 72°C. For sizing of C212 and C272 alleles, a sequencing ladder of pUC18 plasmid DNA was prepared using γ^{32}-P dATP end labelled primer by di-deoxy thermosequenase cycle sequencing kit (USB-Amersham Life Sciences, Cleveland, OH) (Mukherjee *et al.,* 2002). The PCR products of C212/C272 markers along with pUC18 ladder (sequencing ladder) were run on 6% denaturing polyacrylamide sequencing gel to separate the polymorphic alleles. A nomenclature based on the

size of PCR product was used for C212 so that alleles 40 and 49 correspond to 214 and 232 bp respectively (Wirth *et al.,* 1995). The alleles for C272 correspond to the exact size of PCR product (Wirth *et al.,* 1995). The χ^2 statistics for 2×2 tables were calculated using standard procedures and Cervus (Version 1.0) programme was used to calculate the heterozygosity.

SMN2 Copy number analysis

SMN2 gene copy number analysis was carried out by comparing the levels of exon 7 *SMN2* amplified products to those of a two-copy control gene following PCR amplification using *MTHHR* gene as an internal control. After PCR, densitometry analysis was done to calculate the exact copies of *SMN2* gene in individuals.

Results

Our result showed that *SMN1* gene was absent in 11/17 (65%) in type I, 5/7 (71%) in type II, 18/23 (78%) in type III SMA patients. Deletion in exon 5 of *NAIP* gene was observed in 7/17 (41%) in type I, 1/7(14%) in type II, 6/23 (26%) in type III SMA patients, while it was present in all type IV and controls (Table 1). *NAIP* gene deletion was observed only in those individuals who had *SMN1* gene deletion. None of type IV SMA patients showed absence of *SMN1*/*SMN2* or *NAIP* genes.

Fifteen different alleles were observed in the normal individual for C212 marker. At an average controls had 2-3 alleles, and in few individual maximum of 4 alleles were detected, with the observed heterozygosity was 0.871 in 100 unrelated controls (Figure 2). Allele frequencies were similar to those reported in French, Spanish and Italian population (Melki *et al.,* 1994; Capon *et al.,* 1996). Alleles 48 (230 bp), 49 (232 bp) and 50 (234 bp) and 53 (240 bp) were frequent in our population (Figure 3). Alleles 45 (224 bp), 48 (230 bp), 49 (232 bp) were frequent in patient group. Alleles 45 and 48 showed significant association ($P < 0.002$ and $P < 0.010$ respectively) with the disease. Type I patients showed significant heterozygosity deficiency (defined as the reduction of PCR products) by marker C212 ($P < 0.001$) as majority of (91%) patients had one allele of C212 marker, whereas their parents had 2-3 alleles. Heterozygosity deficiency was not significant in type II ($P < 0.06$) and in type III ($P < 0.09$) in our patient group.

Thirteen different types of alleles with sizes of 106-130 bp were observed in C272 marker. One to three alleles were present with the observed heterozygosity of 0.901 in 100 unrelated controls (Figure 4). Alleles corresponding to 110 bp, 118 bp and 120 bp were

Table 1. Combined analysis of *SMN*, *NAIP* and microsatellite markers in deletion-positive SMA patients at 5q13 locus.

Proximal SMA (N=50)	Type I	Type II	Type III	Type IV	Control
Absence of *SMN1*	11/17 (65%)	5/7 (72%)	18/23 (78%)	0	0
Absence of *NAIP*	7/17 (41%)	1/7 (14%)	6/23 (26%)	0	0
No of alleles for C212 marker					
1	10 (91%)	2 (40%)	6 (33%)	0	12 (12%)
2	1 (9%)	1 (20%)	10 (56%)	2 (75%)	67 (67%)
3	0	2 (40%)	2 (11%)	1 (25%)	18 (18%)
4	0	0	0	0	3 (3%)
No of alleles for C272 marker					
1	8 (73%)	1 (20%)	3 (16%)	1 (25%)	10 (10%)
2	3 (27%)	4 (80%)	14 (78%)	2 (75%)	72 (72%)
3	0	0	1 (6%)	0	18 (18%)

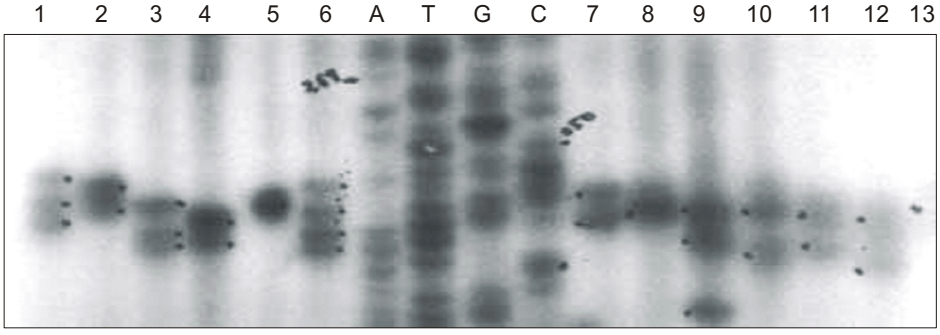

Figure 2. Autoradiograph showing electrophoresis pattern of C212 marker and number of alleles in normal individuals with pUC18 sequencing ladder (ATGC) in the center used for sizing of alleles. One to four alleles were observed in normal individuals.

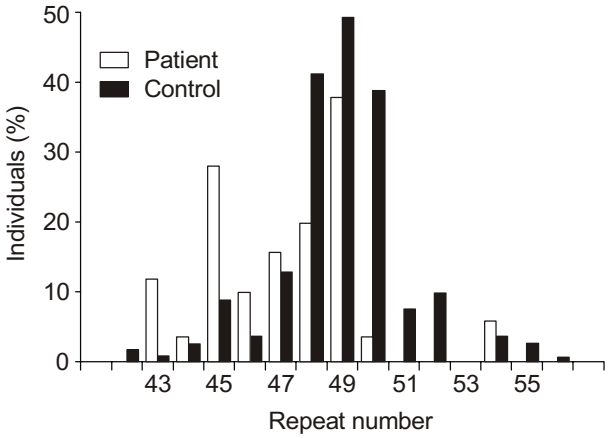

Figure 3. Allelic distribution of C212 marker in patients and controls.

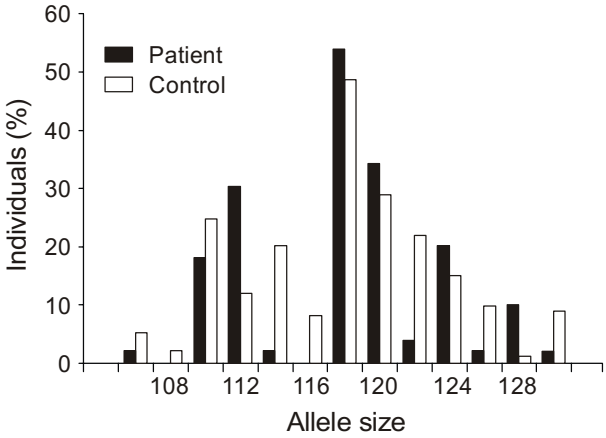

Figure 5. Allelic distribution of C272 marker in patients and controls.

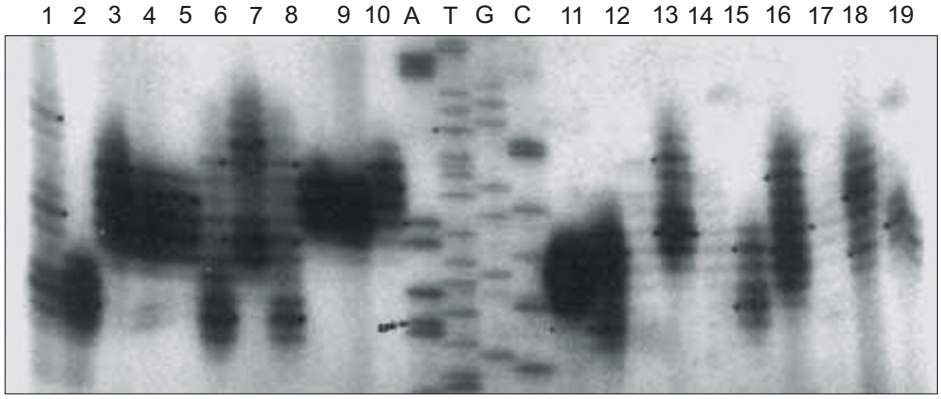

Figure 4. Autoradiograph showing electrophoresis pattern of C272 marker and number of alleles in normal individual along with pUC18 sequencing ladder (ATGC) in the center used for sizing of alleles. One to three alleles were observed in normal individuals in our population.

common (Figure 5). Eleven alleles of different sizes were observed in the patient group. In this group, alleles 110 bp, 112 bp, 118 bp and 120 bp were common. The marker C272 analysis also showed a significant heterozygosity deficiency ($P < 0.001$) in type I SMA patients as compared with their parents (Figure 6). No discordance was observed between SMA III and normal chromosome from both STR markers. The heterozygosity deficiency has been previously ascribed to the deletion of one or two loci spanned by the polymorphic markers (Melki *et al.,* 1994).

To determine whether *SMN1* deletions in SMA patients extended to the flanking region, *SMN1* gene deletion results were combined with those obtained for the multicopy markers and *NAIP* gene. The combined analysis of all markers showed that a large proportion of type I patients (41%) carried deletions of both *SMN1* and *NAIP* gene. The markers analysis in these patients showed loss of heterozygosity of markers, as compared with type II (14%) and type III (26%) patients. No such loss was observed in type IV patients. These results support the idea that heterozygosity deficiency in type I SMA is mainly due to loss of alleles at these loci.

SMN2 copy number analyses were carried out in eleven patients of whom five patients had type I/II SMA, no homozygous deletion of *SMN1* gene was observed in them. *SMN2* analysis showed 2 copies of *SMN2* gene in each of them. *SMN2* copy number analyses were also carried out in six type III patients, and we found that 3-5 copies of *SMN2* gene were present in these patients. This increase in copy number of *SMN2* gene may be responsible for the less severe form of SMA with late onset of symptoms (Table 2).

Discussion

Utilizing genotype data obtained from *SMN1*, *SMN2*, and polymorphic markers, we postulate that the extent of deletions in SMA region may play an important role in determining the disease severity in patients. However, the exact molecular nature of these mutant alleles remains unclear (Wirth *et al.,* 1995). Our results provide evidence indicating that deletion in *SMN1*

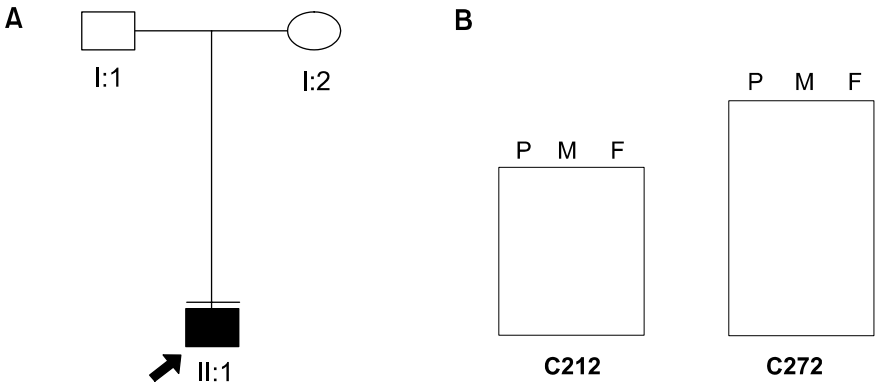

Figure 6. Heterozygosity deficiency in type I patients by marker C212 and C272. (A) Pedigree of type I family. Mother (I:2) has two and one allele and father (I:1) has one and two alleles for C212 and C272 markers respectively. The proband (II:1) has only one allele for both the markers. (B) Electrophoretic pattern of C212 and C272 as shown in autoradiograph. P, Proband; M, Mother; F, Father.

Table 2. Number of *SMN2* and *SMN1* copies in deletion-positive SMA patients along with *NAIP* gene and markers status.

Lab I.D	Age and sex	*SMN2*	*SMN1*	*NAIP*	C272	C212
SMA type I & II						
S-18	Since birth/F	2	2	Present	1	1
S-70	3/M	2	1	Present	1	2
S-84	Since birth/M	2	2	Present	2	2
S-85	Since birth/M	2	2	Present	2	3
S-86	Since birth/F	2	2	Present	1	2
SMA type III						
S-12	17/M	4	0	Absent	2	3
SB-30	10/M	4	0	Present	1	1
S-40	15/M	3	0	Absent	2	2
S-61	17/M	5	0	Present	1	2
S-75	10/F	4	0	Absent	1	2
S-94	19/M	4	0	Present	2	2

is important to cause SMA and *SMN2* to modify the disease phenotype. Deletion of polymorphic markers at 5q region may modify the clinical presentation of the disease. We have identified two categories of SMA alleles. 'Severe' SMA alleles have large-scale deletions, which remove the entire coding region of *SMN1* gene as well as copies of polymorphic markers (C212/C272). In contrast 'mild' allele harbours apparent deletions of exons 7 and 8 of *SMN1* gene while 5' end of *SMN1* appears to be intact. Although *NAIP* has been implicated as a modulator of severity of disease but our results suggests that *NAIP* may not have a direct role in SMA severity.

A reduction of C212 alleles was found in type I patients with *SMN1* deletion, implying a deletion of one or two copies of C212 in these individuals. Although a small proportion of the C212 allele reductions could be due to homozygosity at the locus rather than true deletion. C212 allele homozygosity is low as reported by Scharf *et al.* (1998); we are also getting only 12% of homozygosity in normal chromosomes as compared to 91% of homozygosity observed in type I SMA patient. Therefore, we believe that C212 allele reduction in type I patients is due to deletion rather than homozygosity of the marker. In comparison with the high rate of allelic loss in type I SMA, the allelic loss in type II and III SMA is between that of type I and normal chromosomes. The allelic loss of C212 in type I patients indirectly implies a deletion of at least a part of *H4F5* gene, as C212 polymorphic locus is located in exon 3b of the gene (Scharf *et al.,* 1998). Therefore, it appears that *H4F5* may be deleted at a higher frequency and nearly as high as *SMN1* in type I SMA patients. It has been proposed that *H4F5* protein function in snRNP biogenesis, as the gene shows homology to a snRNP-interacting protein that colocalizes with the same proteins as *SMN*. Such function would be appropriate for an SMA modifier gene and *H4F5* deletion may be considered important for modulating disease severity.

Due to its location C272 may serve as a marker for the integrity of 5' end of *SMN* gene. Most acute type I SMA patients harbour loss of alleles detected by this marker. Although small proportion of allelic

reduction may be due to homozygosity at the locus but chances are very rare (Capon *et al.,* 1996). In our population, we observed 9% of homozygosity in normal chromosome as compared to 73% in type I patient. Therefore, we believe that allelic reduction just as C212 in type I patient may also be due to deletion rather than homozygosity of the marker. In comparison with high rate of allelic loss in type I SMA, the allelic loss in type II (20%) and III (16%) SMA is low. Higher percentage of loss of alleles associated with *SMN* suggests that type I SMA patients may not produce even a partially functional *SMN* protein. However, type II/III and IV patients may produce some amount of functional protein, which may lead to milder phenotype (Simard *et al.,* 1997).

SMN protein interacts directly or indirectly with other proteins, including Bcl-2 and p53, the proteins involved in apoptotic processes, suggesting that *SMN* may play a role in apoptosis (Iwahashi *et al.,* 1997; Young *et al.,* 2002). *NAIP* has been mapped in close proximity to the *SMN* gene and shows homology with the baculoviral IAPs (Inhibitor of apoptosis protein family), which functions as an inhibitor of apoptosis (Mclean *et al.,* 1996). This characteristic is wholly compatible with the pathology of SMA.

In our Indian patients only 28% of the patients with proximal SMA were found to have deletion of *NAIP* gene. In type I SMA higher percentage (41%) of *NAIP* deletion has been observed as compared with type II and III SMA (Table 1). *NAIP* gene deletion frequencies in our patients are in concordance with the reported frequency from other populations (Rodrigues *et al.,* 1995; Roy *et al.,* 1995; Velsco *et al.,* 1996). From the data, it is clear that *NAIP* deleted individuals are a subset of those who also have the *SMN1* gene deletion. *NAIP* gene deletion was not present in all type I patients and neither was it absent in all types II and III patients, as 14 and 26% of type II and III patients had deletion of *NAIP* gene but disease severity was not changed. *NAIP* was not deleted in many of type I patients despite a severe course and early onset of disease. Our data thus substantiates the view that majority of type I SMA cases result from large deletions encompassing both *SMN* and *NAIP* gene but it dose not support the direct role of *NAIP* in disease severity. Taylor *et al.* (1998) have shown that small mutation within the *SMN1* gene do not disrupt *NAIP* but may still give rise to severe phenotype. These results suggest that *NAIP* may not have a direct role in SMA severity but simply delimit the extent of deletions within the SMA region (Simard *et al.,* 1997). We agree with other studies (Campbell *et al.,* 1997) that *NAIP* may be coincidentally deleted with *SMN* gene.

In our study, type I patients showed large deletions, encompassing genomic regions upstream and downstream of the *SMN* gene. Large deletion in 5q13 region have been found to be associated with type I SMA, as 41% of patients showed a loss of alleles detected by markers C212 and C272 along with deletion of *SMN1* and *NAIP* genes, whereas most of type II and III showed deletions of *SMN1* only. This finding is in accordance with the observation that these types of defects are specific to the severe form of SMA (Wirth *et al.,* 1995; Burlet *et al.,* 1996). Extended gene deletions were low in our patients having a milder phenotype. An individual who has SMA and is homozygous for both *NAIP* and *SMN1* deletions is more likely to have type I SMA than type II or III SMA. Although extent of deletion apparently explains some of the phenotypic variation in SMA, there must be other disease modifying factors, as well in SMA pathogenesis.

SMN2 is another disease modifying gene for SMA and has been proposed to decrease severity of SMA in a dose dependent manner (McAndrew *et al.,* 1997; Fedkotter *et al.,* 2002). To determine whether mild SMA patients have more *SMN2* copies than severe patients, we performed a comparison of *SMN2* copy number in SMA patients with different severities. Our results demonstrated that there are more copies of *SMN2* in mild SMA patients compared with severe cases. Interestingly, 67% of type III patients had 4 copies of *SMN2* and 1 patient had 5 copies and 1 had 3 copies of *SMN2* gene. No type III patients were identified with 1 or 2 copies of *SMN2* gene. These data indicate that the presence of three or more copies of *SMN2* is clearly correlated with milder phenotype. The increase in copy numbers of *SMN2* may correspond to conversion of *SMN1* to *SMN2* gene rather than deletions of *SMN1* gene in these patients. However, the number of affected individuals of known SMA type in our study was small due to the limitation of sample for quantitative PCR. Previous studies with larger numbers of subjects indicate that individuals with milder SMA have more copies of *SMN2* than individuals with more severe SMA (Taylor *et al.,* 1998; Mailman *et al.,* 2002).

Combined analyses of *SMN1*/*SMN2*, together with *NAIP* and markers C212/C272 revealed an association between the copy number of gene and the SMA phenotype (Lefebvre *et al.,* 1995; Wirth *et al.,* 1995; Velasco *et al.,* 1996; Burglen *et al.,* 1997). As discussed earlier, type I SMA patients showed a large deletion at 5q locus. In type II and III patients, extended gene deletions were low. These observations favour two different mechanisms that would account for the lack of *SMN1* gene in SMA patients: a gene deletion of *SMN1* in type I SMA and a gene conversion (*SMN1* to *SMN2*) event in type II/III SMA (Lefebvre *et al.,* 1995; Wirth *et al.,* 1995; Velasco *et al.,* 1996; Campbell *et al.,* 1997; McAndrew *et al.,* 1997). Therefore, in the majority of cases an increased number of *SMN2* copies correlates with milder SMA phenotype. It also suggests that the *SMN2* gene is translated into an at least partially functional protein and could be regarded as a true modifying gene in SMA.

In conclusion, it is tempting to state that extent of deletions encompassing *H4F5*, *SMN1*, and *NAIP* along with copy number of *SMN2* may modify the SMA phenotype, thus accounting for the different clinical subtypes of the disease. Molecular dissection of *SMN*

locus will further aid in elucidating the phenotypic variability in SMA.

References

Anhuf D, Eggermann T, Rudnik-Schoneborn S, Zerres K. Determination of *SMN1* and *SMN2* copy number using Taq Man technology. Hum Mut 2003;22:74-8

Biros I, Forrest S. Spinal muscular atrophy: Untangling the knot. J Med Genet 1999;36:1-8

Burglen L, Lefebvre S, Clermont O, Burlet P, Viollet L, Cruaud C, Munnich A, Melki J. Structure and organization of the human survival motor neurone (*SMN*) gene. Genomics 1996;32:479-82

Burlet P, Burglen L, Clermont O, Lefebvre S, Viollet L, Munnich A, Melki J. Large scale deletions of the 5q13 region are specific to Werdnig-Hoffmann disease. J Med Genet 1996;33:281-3

Campbell L, Potter A, Ignatius J, Dubowitz V, Davies KE. Genomic variation and gene conversion in spinal muscular atrophy: implications for disease process and clinical phenotype. Am J Hum Genet 1997;61:40-50

Capon F, Levato C, Bussaglia E, Lo Cicero S, Tizzano EF, Baiget M, Silan V, Pizzuti A, Novelli G, Dallapiccola B. Deletion analysis of the simple tandem repeat loci physically linked to the spinal muscular atrophy locus. Hum Mutat 1996; 7:98-201

Di Donato CJ, Morgan K, Carpten JD, Fuerst P, Ingraham SE, Prescott G,McPherson JD, Wirth B, Zerres K, Hurko O, Wasmuth JJ, Mendell JR, Burghes AHM, Simard LR. Association between AG-1-CA alleles and severity of autosomal recessive proximal spinal muscular atrophy. Am J Hum Genet 1994;55:1218-1229

Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of *SMN1* and *SMN2* based on real-time light Cycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am J Hum Genet 2002;70:358-68

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;390:413-7

Kesari A, Mukherjee M, Mittal B. Mutation analysis in spinal muscular atrophy using allele-specific polymerase chain reaction. Ind J of Biochemistry and Biophysics 2003;40:439-441

Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benechou B, Cruaud D, Millasseau P, Zeviani M, Le Paslier D, Frezal F, Cohen D, Weissenbach J, Munnich A, Meiki J. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 1995;80:155-165

Mailman MD, Heinz JW, Papp AC, Snyder PJ, Sedra MS, Wirth B, Burghes AH, Prior TW. Molecular analysis of spinal muscular atrophy and modification of the phenotype by *SMN2*. Genet Med 2002;4:20-6

McAndrew PE, Parsons DW, Simard LR, Rochette C, Ray PN, Mendell JR, Prior TW, Burghes AH. 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

McLean M, Ikeda JE, MacKenzie A, Korneluk RG. Suppression of apoptosis in mammalian cells by *NAIP* and a related family of IAP genes. Nature 1996;379:349-53

Melki J, Lefebvre S, Burglen L, Burlet P, Clermont O, Millasseau P, Reboullet S, Zeviani M, Le Paslier D,Cohen D. De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. Science 1994;264:1474-1477

Mukherjee M,. Vaish M, Mittal RD, B Mittal. Allelic variation of BAT-26 and BAT-40 poly-adenine repeat loci in North Indians. Int J Mol Med 2002;1:91-94

Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992;39:225-235

Panigrahi I, Kesari A, Phadke SR, Mittal B. Clinical and Molecular Diagnosis of spinal Muscular Atrophy. Neurol India 2002;50:117-22

Rodrigues NR, Owen N, Talbot K, Patel S, Muntoni F, Ignatius J, Dubowitz V. and Davies KE. Gene deletions in spinal muscular atrophy. J Med Genet 1996;33:93-6

Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Besner-Johnston A, Lefebvre C, Kang X. The gene for neuronal apoptosis inhibitor protein is partially deleted in individuals with spinal muscular atrophy. Cell 1995;80:167-78

Scharf JM, Endrizzi MG, Wetter A, Huang S, Thompson TG, Zerres K, Dietrich WF, Wirth B, Kunkel LM. Identification of a candidate-modifying gene for spinal muscular atrophy by comparative genomics. Nat Genet 1998;20:83-6

Simard LR, Rochette C, Semionov A, Morgan K, Vanasse M. *SMN*(T) and *NAIP* mutations in Canadian families with spinal muscular atrophy (SMA): genotype/phenotype correlations with disease severity. Am J Med Genet 1997;72:51-8

Somerville MJ, Hunter AGW, Aubry HL, Korneluk RG, Mac Kenzie AE, Surh LC. Clinical application of the molecular diagnosis of spinal muscular atrophy. Am J Med Genet 1997;69:159-165

Taylor JE, Thomas NH, Lewis CM, Abbs SJ, Rodrigues NR, Davies KE, Mathew CG. Correlation of *SMNt* and *SMNc* gene copy number with age of onset and survival in spinal muscular atrophy. Eur J Hum Genet 1998;6:467-74

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. Erratum in: Hum Mol Genet 1996;5:710

Wirth B, Hahnen E, Morgan K, DiDonato CJ, Dadze A, Rudnik-Schoneborn S, Simard LR, Zerres K, Burghes AH. Allelic association and deletions in autosomal recessive proximal spinal muscular atrophy: association of marker genotype with disease severity and candidate cDNAs. Hum Mol

Genet 1995;4:1273-84

Wirth B. An update of the mutation spectrum of the survival motor neuron gene (*SMN1*) in autosomal recessive spinal muscular atrophy (SMA). Hum Mut 2000;15:228-37

Young PJ, Day PM, Zhou J, Androphy EJ, Morris GE, Lorson CL. A direct interaction between the survival motor neuron protein and p53 and its relationship to spinal muscular atrophy. J Biol Chem 2002;277:2852-9