SMN mRNA and protein levels in peripheral blood
Biomarkers for SMA clinical trials

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Abstract—Background: Clinical trials of drugs that increase SMN protein levels in vitro are currently under way in patients with spinal muscular atrophy. Objective: To develop and validate measures of SMN mRNA and protein in peripheral blood and to establish baseline SMN levels in a cohort of controls, carriers, and patients of known genotype, which could be used to follow response to treatment. Methods: SMN1 and SMN2 gene copy numbers were determined in blood samples collected from 86 subjects. Quantitative reverse transcription PCR was used to measure blood levels of SMN mRNA with and without exon 7. A cell immunoassay was used to measure blood levels of SMN protein. Results: Blood levels of SMN mRNA and protein were measured with high reliability. There was little variation in SMN levels in individual subjects over a 5-week period. Levels of exon 7-containing SMN mRNA and SMN protein correlated with SMN1 and SMN2 gene copy number. With the exception of type I SMA, there was no correlation between SMN levels and disease severity. Conclusion: SMN mRNA and protein levels can be reliably measured in the peripheral blood and used during clinical trials in spinal muscular atrophy, but these levels do not necessarily predict disease severity.

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Spinal muscular atrophy (SMA), an autosomal recessive motor neuron disease, is the leading inherited cause of infant and childhood mortality. There is a spectrum of SMA disease severity, and patients can be divided into three clinical groups based on the ability to achieve early motor milestones: type I patients never sit independently, type II patients sit, but never stand and walk unaided, and type III patients stand and walk independently at some point during their disease course.1

SMA is caused by mutation of both alleles of the survival of motor neurons 1 (SMN1) gene,2 however all patients retain at least one copy of the SMN2 gene. The SMN1 gene produces full-length SMN mRNA, whereas the SMN2 gene produces full-length mRNA and mRNA lacking exon 7 (SMNΔ7), as well as small amounts of mRNA lacking exon 5 or exon 3 or combinations thereof.3,4 SMNΔ7 mRNA encodes an unstable, truncated protein.5 SMA is postulated to be caused by insufficient expression levels of full-length SMN protein in motor neurons, perhaps during a critical stage of motor neuron development.6,7 Several studies have shown that SMN mRNA and protein are reduced in cell lines and tissues derived from patients with type I SMA compared to controls.8-11

SMN2 copy number has an important modifying effect on SMA disease severity.12 Most patients with SMA type I have one or two SMN2 copies; most patients with type II have three SMN2 copies; and most patients with SMA type III have three or four SMN2 copies.13 In addition, it has been demonstrated in transgenic mice lacking endogenous SMN that increasing SMN2 gene copy number from two to eight prevents the SMA disease phenotype.14 These observations suggest that increasing SMN expression levels may be an effective SMA treatment strat-
Drugs have been shown to increase SMN mRNA and protein levels in vitro,15-25 and several are being studied in ongoing and planned clinical trials in patients with SMA.24,25 In order to investigate whether there is adequate delivery, expected biologic activity, and efficacy of these compounds in vivo, methods are needed to measure SMN expression levels in patients with SMA.

Methods. Cell lines. Lymphoblastoid cell lines GM12497 (derived from a 7-month-old control patient) and GM10684 (derived from a 6-month-old type I SMA patient) were purchased from Coriell Cell Repositories (New Jersey) and maintained in RPMI media with 10% fetal bovine serum. RNA was isolated using Trizol reagent as previously described.26

Subjects and blood draws. SMA carriers and patients underwent a standard history, examination, and blood draw as part of an NIH and Children’s Hospital of Philadelphia Institutional Review Board (IRB)-approved protocol. Anonymous control subjects underwent a blood draw in the Department of Transfusion Medicine as part of an NIH IRB-approved protocol. At each blood draw, blood was collected into 1-2 Paxgene tubes (Qiagen) and 2-4 Cell Prep Preparation Tubes (BD Diagnostics/ Franklin Lakes, NJ). After rocking overnight at room temperature, RNA was isolated from the Paxgene tubes according to manufacturer instructions and stored at –80 °C. Peripheral blood mononuclear cells (PBMCs) were immediately isolated from the CPT tubes according to manufacturer instructions. The cells were counted and stored in aliquots at –80 °C.

Genotyping. A aliquot of PBMCs was sent to the University of Pennsylvania Molecular Diagnostics and Genotyping Center for determination of SMN1 and SMN2 copy number. Copy number was determined using a PCR restriction fragment length polymorphism assay previously described.26

Quantitative reverse transcriptase PCR. Total RNA was quantified by absorption at 260 nm on a spectrophotometer (Amer sham Biosciences), and 1,250 ng of each RNA sample was converted to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems). In a 384 well plate format, 20 µL quantitative PCR reactions were run in triplicate for each primer set on the ABI Prism 7900 sequence detection system (Applied Biosystems). Two endogenous control primer sets, β-glucuronidase (GUSB) and 18S (Applied Biosystems), and two SMN primer sets, SMN+ (designed to amplify SMN1 gene and two copies of SMN2 gene) and SMN− (designed to amplify SMN transcript lacking exon 7), were used on each sample. The sequences of the SMN− primer set (spanning exons 6, 7, and 8) were forward 5’-CAAAAAGAAGGAAGGTGCTCACATT-3’ and reverse 5’-GTGTCATTTAGTGCTGCTCTATGC-3’ and SMN+ (designed to amplify SMN1 gene and two copies of SMN2 gene) and SMN− (designed to amplify SMN transcript lacking exon 7), were used on each sample. The sequences of the SMN+ primer set (spanning exons 6, 7, and 8) were forward 5’- CAGATCCTATGTCTGCTATG-3’, reverse 5’-TGTCCTG TATGATTAGTCTGCTATG-3’, and probe 5’-FAM-CAGATCCTATGTCTGCTATG-TAMRA-3’ and the sequences of the SMN7 (spanning the exon 6-8 junction) were forward 5’-CATGATGCAGCTGTATACATG-3’, reverse 5’-TGTCCTGC ATTTAGTCTGCTATG-3’, and probe 5’-FAM-CACCAGCAT TCCATATAATAC-NGFQ-3’. The quantity of RNA for each primer set was calculated by comparison to the standard curve for each primer set. Standard curves were run on every plate. SMN+ or SMN+ mRNA levels were calculated by considering the raw, uncorrected SMN level or by dividing the SMN value by either of the two endogenous genes (GUSB or 18S). A single “calibrator” sample was run on every plate, and the SMN+ and SMN7 values for this sample were each set to 1. All samples were normalized to this value in order to standardize between plates. The calibrator RNA was derived from a large volume of blood isolated from a single blood draw from a control subject with two copies of SMN1 and one copy of SMN2. Three plates were run on each sample.

SMN protein cell immunoassay. SMN levels from peripheral blood samples were determined using a cell immunoassay as described elsewhere.27 1.5 × 10^5 cells/well were adhered to the well bottom of a 96-well plate by centrifugation (700 x g) for 5 minutes at room temperature. The cells were then fixed to the plate with 2.5% formaldehyde for 30 minutes and washed in PBS. All washes were performed with PBS using a Biotech ELX405 automatic plate washer. The plates were permeabilized with 0.1% Triton X-100 for 5 minutes and blocked with 20% FBS for 1 hour. The antibody used for the immunosorbent assay was monoclonal antibody 2B1 (1:500) against SMN. The plates were washed, and bound antibodies were detected using peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) and Supersignal ELISA Femto Maximum Sensitivity Substrate (Pierce). The luminescence intensity within each well was measured using a Perkin Elmer Victor2 plate reader. Signal background was determined for each sample by omitting the primary antibody. The background signal was less than 4% of the SMN signal.

Statistical analysis. The reliability of SMN measures was assessed using the coefficient of variation (CV) and intraclass correlation coefficient (ICC) statistics. The CV was calculated by dividing the SD of three repeated assays in a given subject by the average SMN level for that subject. The mean CV was then calculated by taking the average CV of all subjects. ICC was calculated using the following formula:

\[
ICC = \frac{\sigma_1^2}{\sigma_1^2 + \sigma_2^2 + \tau^2}
\]

where \(\sigma_1\) represents the estimated variability in three assays from an individual subject and \(\sigma_2\) represents the estimated variability in SMN between samples from different subjects. The ICC therefore represents the proportion of total variation that is due to subject-to-subject variability and the value of (1 – ICC) can be interpreted as the proportion of total variability attributable to technical assay variability.24 All three SMN values from an individual were defined as outliers when their mean value was more than 4 standard deviations away from the mean of all other samples. A single SMN value from an individual was defined as an outlier if inclusion of that value resulted in that individual’s within-person variability being more than 4 standard deviations greater than the average within-person variability.

The variability of SMN level over time was assessed using a CV statistic (the SD of multiple samples assessed at different time points was divided by the average value of different time points). Differences in SMN levels between disease groups were assessed using a one-way analysis of variance (ANOVA). The statistical contribution of SMN1 and SMN2 copy number to SMN levels was assessed using a regression model of the following form:

\[
\text{Expression} = \alpha + \beta_1 \cdot (\text{SMN1 copy #} - 1) + \beta_2 \cdot (\text{SMN2 copy #} - 2) + \text{error term}
\]

This model was applied independently to the data obtained from each of the SMN measures. In this model, the \(\alpha\) term represents the estimated SMN expression level for an individual with one SMN1 gene and two SMN2 genes, the median SMN1 and SMN2 gene copy number in our study population. The \(\beta_1\) and \(\beta_2\) parameters indicate how a one unit change in the number of genes (SMN1 for \(\beta_1\) and SMN2 for \(\beta_2\)) from this median is estimated to effect expression level. The median numbers of SMN1 and SMN2 genes (one and two) are subtracted from each individual’s count of the SMN genes so that the SMN level for an individual with one copy of SMN1 and two copies of SMN2 would be equal simply to \(\alpha\), whereas the SMN level for an individual with zero copies of SMN1 and four copies of SMN2, for example, would be equal simply to \(\alpha + (\beta_1 \times 1) + (\beta_2 \times 2)\). For both the ANOVA and regression models involving copy number, age was considered as a possible covariate given the disparate age distributions among the patient groups, but was not found to be an important factor and was therefore omitted from the models.

Results. Assay development and validation. It has been shown in several studies that SMN mRNA and protein levels are reduced in cell lines derived from type I SMA patients compared to controls.8-10 In order to verify that the RT-PCR assay and cell immunoassay measure the expected SMN levels in standard, well-characterized samples, we investigated SMN+ mRNA, SMN7 mRNA, and SMN protein in lymphoblastoid cell lines derived from a control subject (two SMN1 copies and two SMN2 copies) and a type I SMA patient (zero SMN1 copies and three SMN2 copies). As expected, SMN+ mRNA and SMN protein levels were reduced in the type I SMA cell line compared to the control cell line, whereas there was little difference in SMN7 mRNA levels between the two cell lines.

Statistical analysis of the variability of SMN levels measured in all subjects showed that SMN measures derived from all subjects were reliable, with ICC values ranging from 0.69 to 0.84. Differences in SMN levels between disease groups were assessed using a one-way analysis of variance (ANOVA). The statistical contribution of SMN1 and SMN2 copy number to SMN levels was assessed using a regression model of the following form:
lines (figure 1). These results were evident whether considering the uncorrected SMN levels (data not shown) or when using GUSB or 18S as endogenous controls for the RNA measures. We found that the magnitude of difference in SMN protein levels between the control and the SMA type I cell lines measured by the cell immunoassay is comparable to that measured by western blot.27

The mRNA and protein assays were next used to measure SMN levels in peripheral blood samples derived from a cohort of control subjects, SMA carriers, and SMA patients. SMN+7 and SMNΔ7 were measured in 85 subjects, and SMN protein was measured in 57 subjects. The patient characteristics are shown in table 1. In order to assess reliability, three replicates of each assay were done on each sample. For the RNA measures, the three replicates represented values obtained from three separate plates (the value from a plate was the average of the replicates on that plate). From a total of 255 mRNA measures, outliers that were excluded from further analysis were two individuals’ three SMN+7 (relative to 18S) values, one individual’s three SMNΔ7 (relative to 18S) values, three single SMN+7 (relative to GUSB) values, two single SMNΔ7 (relative to GUSB) values, and one single SMNΔ7 (relative to 18S) value. For protein measures, because of limited sample quantity, the three replicates represented three runs of the sample on a single plate. Plate-to-plate variability in the cell immunoassay was corrected using a regression analysis in which assay level was the dependent variable and plate number was an explanatory factor. Because the distribution of patient types varied by plate a factor indicating patient group was also included. The estimated plate effects obtained from the regression were then subtracted from the initial assay level to obtain adjusted values. Please see figure E-1 for data before and after statistical correction (go to the Neurology Web site at www.neurology.org).

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient disease group</th>
<th>Genotype, (SMN1#/SMN2#) (#subjects)</th>
<th>Average age (range)</th>
<th>Gender (M/F)</th>
<th>Clinical features at time of examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, n = 28</td>
<td>2/2 (12)</td>
<td>38 y (8–55 y)</td>
<td>11/16</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2/1 (12)</td>
<td>1 unknown</td>
<td>1 unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/0 (2)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3/1 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/2 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carriers, n = 29</td>
<td>1/4 (3)</td>
<td>41 y (6–78 y)</td>
<td>13/16</td>
<td>Normal examination</td>
</tr>
<tr>
<td></td>
<td>1/3 (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/2 (11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/1 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA III, n = 14</td>
<td>0/5 (1)</td>
<td>32 y (2.5–56 y)</td>
<td>7/7</td>
<td>10 ambulatory</td>
</tr>
<tr>
<td></td>
<td>0/4 (6)</td>
<td></td>
<td></td>
<td>4 wheelchair bound</td>
</tr>
<tr>
<td></td>
<td>0/3 (6)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0/2 (1)</td>
<td></td>
<td></td>
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<tr>
<td>SMA II, n = 9</td>
<td>0/4 (2)</td>
<td>12 y (2–47 y)</td>
<td>3/6</td>
<td>All able to sit</td>
</tr>
<tr>
<td></td>
<td>0/3 (7)</td>
<td></td>
<td></td>
<td>None able to stand</td>
</tr>
<tr>
<td>SMA I, n = 6</td>
<td>0/2 (5)</td>
<td>21 mo (4–53 mo)</td>
<td>4/2</td>
<td>3 unable to sit, no BiPAP or G-tube,</td>
</tr>
<tr>
<td></td>
<td>Unknown (1)</td>
<td></td>
<td></td>
<td>3 minimal movement with BiPAP and G-tube,</td>
</tr>
</tbody>
</table>

ND = not done; BiPAP = bilevel positive airway pressure; G-tube = gastric feeding tube.
The reliability of all assays as measured by ICC and CV (excluding outlier values) is reported in table 2. The ICC was in excess of 0.90 for the mRNA and protein assays, indicating that less than 10% of total variation was attributable to assay technical variation (i.e., problems with reproducibility). When outlier values were not excluded, the ICC of all assays remained greater than 0.90 (data not shown). The reliability of the SMN mRNA measurements was slightly better when expressed relative to GUSB compared to 18S. The ICCs of uncorrected SMN values were also greater than or equal to 0.90 (data not shown). Because of space constraints, SMN+7 and SMNΔ7 data for the rest of this report are shown relative to GUSB only, although correlations of SMN levels with disease group and genotype were comparable whether considering GUSB or 18S corrected mRNA values or uncorrected SMN values (figure E-2).

**SMN levels are stable over time.** In order to examine the variability of SMN levels in the peripheral blood over time, we drew blood samples from five subjects (four controls and one carrier) weekly over 5 weeks. The mean CV in all subjects over time was 10% for SMN+7, 10% for SMNΔ7, and 13% for SMN protein. An additional one control, six carriers, four type II SMA patients, and one type I SMA patient underwent two or three blood draws separated in time (between 0.5 and 7 months). Mean CV over time in these subjects was 11% for SMN+7 and 15% for SMNΔ7.

**SMN levels and disease group.** ANOVA was performed to assess differences in SMN levels between patient disease groups (figure 2). A one-factor model using patient group indicates there are significant differences in SMN+7 patterns between groups (F4,80 = 5.25, p < 0.001) with the type I SMA group largely responsible for this difference (unadjusted pairwise comparisons of the carriers and disease populations with the control group were only significant for the type I SMA group (p < 0.001) (figure 2A). Omitting the type I SMA group resulted in a p value of 0.0846 (F3,76 = 2.29) indicating a suggestion of differences among the remaining groups. The differences among groups for SMNΔ7 showed a different pattern, with type II and type III SMA patient groups producing increased levels of truncated product compared to controls, carriers, and type I SMA patients (F4,80 = 8.31, p < 0.001) (figure 2B). The SMN protein assay showed results similar to those found for SMN+7 (figure 2C). The F-test showed that overall differences were present (F3,50 = 11.89, p < 0.001), however no overall difference exists if the SMA type I group is omitted (F2,49 = 2.85, p = 0.067). When age was included as a covariate to control for the different age distributions of the patient groups, the results were qualitatively unchanged. Sex was also not found to be a significant factor in determining SMN level.

**SMN levels relative to SMN1 and SMN2 gene copy number.** Figure 3 shows SMN levels stratified by genotype. These data indicate that the copy numbers of both SMN1 and SMN2 determine the levels of SMN+7 and SMN protein production (figure 3, A and C). These impressions were examined with a regression model (detailed in Methods). The results in table 3 confirm the graphical impression of the importance of both types of genes, as the small p values for the β1 and β2 parameters reject the hypothesis that copy number has no effect. Here an increase by one in the number of SMN1 genes results in an increase of 0.26 units in SMN+7. Similarly, an increase by one in the number of SMN2 genes is associated with an increase of 0.18

![Figure 2. SMN+7 RNA (A), SMNΔ7 RNA (B), and SMN protein (C) expression levels in blood samples collected in controls, carriers, and types III, II, and I SMA patients.](image-url)
The results for protein expression are qualitatively similar although the scales are different. Again, the effect of an increase in SMN1 genes is larger than the effect of an increase in SMN2. When SMN1 is examined, the pattern is different. A change in the copy number of SMN1 has no significant effect on the amount of SMN, but the copy number of SMN2 has a strong effect.

Use of a relative standard curve method to quantify SMN mRNA precluded directly comparing SMN to SMN levels in individual patients. In order to investigate this relationship, we recalculated the mRNA data using the \( \Delta \Delta CT \) method. Pearson correlation coefficients for data calculated using the \( \Delta \Delta CT \) vs standard curve methods were as follows: \( R^2 = 0.96 \) for SMN+7 and \( R^2 = 0.93 \) for SMN7. When quantified in this way, SMN+7 values remained approximately the same and SMN7 values were approximately half those quantified by the standard curve method (data not shown). When comparing SMN+7 levels directly to SMN7 levels in SMA patients, 50% of transcript arising from each copy of the SMN2 was SMN+7 and 50% was SMN7 (the ratio of SMN+7/SMN7 in SMA patients was approximately 1.0, regardless of SMN2 copy number). In contrast, the average SMN+7/SMN7 ratio was 1.9 in carriers and 2.4 in the control group (excluding the subjects with three copies of SMN1 and 0 copies of SMN2). The variability in ratio among individual subjects was much greater in these groups than in the SMA patients.

**Discussion.** We have developed and validated measures of SMN mRNA and protein in the peripheral blood. Although SMN mRNA was measured in blood by quantitative RT-PCR in a small number of patients in one previous study, the reliability of this assay was not established.24 We demonstrate that our assays have low technical variability (less than 10% of total variability). We also show that in individual subjects studied repeatedly SMN levels remain relatively stable over weeks to months (CVs of 10 to 15%).

The SMN mRNA measures require small volumes of blood (2.5 mL) and may be more sensitive and reliable than the protein measure. In order to control for variation in RNA loading, this assay is done with an endogenous control, which can vary between individuals and in response to drug treatment. In order
to avoid this potential bias, we quantified each RNA sample and loaded equal amounts of RNA into each reaction. After investigating four different potential endogenous controls, we found that 18S and GUSB were the least variable in different subjects (unpublished observations). At the completion of our study, we examined the SMN mRNA data expressed as uncorrected values or as values corrected for GUSB or 18S and found similar reliabilities and patterns of change when stratified by disease group or genotype. We recommend that similar precautions be taken to minimize bias when using these measures in clinical trials. Before a particular endogenous control is used in a drug trial, it should be verified that the study drug does not alter its expression level. In addition, as was done in this study and has been recommended by others, more than one endogenous control should be used to confirm patterns of change.

The SMN protein measure is more biologically relevant than mRNA, as increasing the amount of functional SMN protein in cells is the goal of SMN-inducing drugs. The cell immunoassay compares expression in an equivalent number of cells and does not depend on a stable endogenous control. Moreover, this method does not require lysis of cells as does standard western blotting and sandwich ELISA methods. In this study, we have validated the cell immunoassay for use in clinical trials and have determined the inherent reliability of the test. In order to avoid the plate-to-plate variability that was encountered in this study, we recommend that samples from all subjects at each time point in the trial should be collected, archived, and then run together on one plate at the end of the trial.

Our data indicate that SMN +7 mRNA and SMN protein levels in blood are related to both SMN1 and SMN2 gene copy number, whereas SMNΔ7 mRNA levels are strictly dependent on SMN2 gene copy number. In blood cells of SMA patients we demonstrate that 50% of transcript arising from SMN2 contains exon 7 in blood. Because exon 7 inclusion occurs with this frequency, patients with SMA with four copies of SMN2 alone are able to produce comparable blood levels of SMN+7 as individuals with two copies of SMN1 and one copy of SMN2. Unlike the SMN+7 mRNA levels, our data suggest that the SMN protein levels may plateau with increasing SMN2 gene copy number greater than two or three (see figure 3C). This may indicate that there are post-translational mechanisms that regulate SMN protein expression in human blood cells so that SMN protein levels are not always directly related to SMN+7 mRNA levels.

It has been accepted that SMA is caused by reduced levels of SMN protein; however, in this study we found that only patients with type I SMA had significantly reduced SMN+7 and SMN protein levels in blood compared to carriers and controls. Patients with type II and III SMA had relatively normal SMN+7 mRNA and SMN protein levels, but increased levels of SMNΔ7 mRNA. Other investigators have previously observed similar levels of SMN protein in lymphoblastoid and fibroblast cells lines isolated from some SMA type II and III patients compared to controls. In a limited number of patients, SMN protein levels were found to be reduced in fetal liver, muscle, and spinal cord tissues derived from Type II and III patients compared to controls, but not in a muscle biopsy obtained from a type III SMA patient postnatally. The relatively normal blood SMN levels we found in type II and III patients may indicate that the development of SMA does not strictly depend on a particular level of SMN protein. One function of SMN is to mediate snRNP assembly and this function has been shown to correlate with SMN levels. Perhaps SMN function is impaired even when the level is relatively normal. Alternatively, SMN levels may be different in peripheral blood and motor neurons. Perhaps the SMN2 gene is less transcriptionally active in motor neurons, or the splicing of SMN2-derived transcripts results in predominantly SMNΔ7 transcript in motor neurons. The role of SMNΔ7 mRNA in SMA disease pathogenesis remains unknown. Recent data indicate that SMNΔ7 may play a disease-modifying role, as increasing SMNΔ7 has been shown to prolong survival in SMA mice, however it has also been suggested that SMNΔ7 could be deleterious. Another possibility is that SMN levels may be reduced in both peripheral blood cells and motor neurons during a particular stage of early development in type II and III patients, but this reduction is no longer evident postnatally.

These SMN mRNA and protein assays can be used in clinical trials of drugs expected to increase SMN levels in patients with SMA. The data collected in this study establishing the reliability of these assays and the baseline temporal stability of SMN expression levels can also be used as the basis of a power analysis for the design of future clinical trials. Such clinical trials would determine whether measures of SMN in the peripheral blood have utility in predicting clinical response to SMN-modulating drugs in patients with SMA. These assays can also be adapted for other purposes. Because the RNA assays require small volumes of blood, they can be used as biomarkers in drug studies in animal models of SMA. In addition, the SMN protein measure can be automated and used in cell-based high throughput screens to identify drugs that increase SMN protein in cells. This might lead to the identification of new compounds with therapeutic value in patients with SMA.

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