Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases

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Abstract

Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by loss or mutations in SMN1. According to age of onset, achieved motor abilities, and life span, SMA patients are classified into type I (never sit), II (never walk unaided) or III (achieve independent walking abilities). SMN2, the highly homologous copy of SMN1, is considered the most important phenotypic modifier of the disease. Determination of SMN2 copy number is essential to establish careful genotype–phenotype correlations, predict disease evolution, and to stratify patients for clinical trials. We have determined SMN2 copy numbers in 625 unrelated Spanish SMA patients with loss or mutation of both copies of SMN1 and a clear assignation of the SMA type by clinical criteria. Furthermore, we compiled data from relevant worldwide reports that link SMN2 copy number with SMA severity published from 1999 to date (2834 patients with different ethnic and geographic backgrounds). Altogether, we have assembled a database with a total of 3459 patients to delineate more universal prognostic rules regarding the influence of SMN2 copy number on SMA phenotype. This issue is crucial in the present scenario of therapeutic advances with the perspective of SMA neonatal screening and early diagnosis to initiate treatments.

Keywords: Spinal muscular atrophy; SMN2 copies; Phenotype–genotype correlations; Prognosis considerations; Worldwide compilation

1. Introduction

Spinal muscular atrophy (SMA) is the second most common recessive genetic disease of infancy and early childhood, with an incidence of 1 in 5000–10,000 live births and a carrier frequency of 1/50 among Europeans [1]. Ultimately, the disease is caused by the degeneration and loss of alpha motor neurons in the spinal cord anterior horns, which leads to a progressive atrophy of proximal muscles, weakness, respiratory failure, and death in the most severe cases.

SMA patients are classified into different clinical groups based on the age of onset, clinical severity and, more importantly, achieved motor milestones. Type I or Werdnig–Hoffmann disease, the most severe form, appears within the first 6 months of life. Affected children are never able to sit and generally die of respiratory failure before the age of 2 years. In the intermediate
type II SMA form, symptoms appear between 6 and 18 months of life; these children are able to sit but they never walk unaided and usually reach adolescence and even adulthood. Type III patients (Kugelberg–Welander disease), learn to walk unassisted and retain this ability for a long time, but eventually become wheelchair-bound. These patients can be further subdivided according to the age of disease onset into type IIIa (first symptoms appearing before 3 years of age) and IIIb (onset after 3 years) [2]. Finally, in the milder type IV SMA first symptoms manifest themselves between the second and third decade of life [2–4]. At present, no cure for SMA is available despite the growing number of clinical trials conducted during the last years (www.clinicaltrials.gov). However, the actual scenario of SMA therapy is evolving due to the recent approval of nusinersen/Spinraza by the FDA [5] and EMA (www.ema.europa.eu). Nusinersen, an antisense oligonucleotide administered intrathecally to patients, represents the first effective tailored molecular therapy for SMA, and thus a categorical advance in disease treatment [6].

Loss or mutation of both copies of the survival of motor neuron (SMN1) gene causes SMA [7]. A centromeric and nearly identical paralog, SMN2, is uniquely found in humans and encodes in principle the same protein as SMN1 [7–9]. However, a silent transition within exon 7 of the SMN2 gene causes exon skipping from most SMN2 pre-mRNA transcripts, which results in a truncated, non-functional variant (SMN-Δ7) instead of the full-length protein, SMN [10]. SMN-Δ7 is highly unstable and rapidly degraded, and therefore SMN2 is unable to compensate for the absence or deficiency of SMN1 in SMA patients [11]. It has been estimated that each SMN2 copy can produce only around 10% of functional SMN protein, but the actual figures are likely to vary in different cells and tissues [12–14].

The number of SMN2 copies and the presence of its c.859G>C variant still remain as the major modifiers of SMA disease [15,16]. Indeed, numerous studies show that the higher the SMN2 copy number, and so the larger the amount of full-length SMN protein produced, the milder the associated SMA phenotype and vice versa [17–20]. However, this inverse correlation is not absolute and some patients with two SMN2 copies present milder SMA phenotypes, whereas some with three copies of the gene have been described as type I [17–20].

Here, to establish accurate phenotype–genotype correlations we have determined SMN2 copy number in 625 unrelated Spanish SMA patients for whom phenotype information was available, using in all cases the same methodology (Multiplex Ligation-dependent Probe Amplification, MLPA). Furthermore, we have reviewed all relevant worldwide reports that link SMN2 copy number with SMA severity. In this manner, a total of 2834 patients with different ethnic backgrounds were incorporated in our analyses resulting in a total of 3459 SMA cases. Altogether, analysis of this large cohort has allowed us to compile and delineate more general prediction rules regarding the influence of SMN2 copy number on SMA phenotype.

2. Patients and methods

To define strict genotype–phenotype correlations, we studied SMN2 copy number in 625 unrelated (index) Spanish SMA patients with absence of both copies of SMN1 and an unambiguous assignment of the SMA type by motor milestones criteria. (For simplicity, in this work type IIIa, IIIb and IV cases have been grouped together as “walker” phenotype, as it is the highest milestone achieved.) We thus distinguish only between type I (never sit), II (never walk) or III (walker) patients. SMN2 copy number was determined by Multiplex Ligation-dependent Probe Amplification (MLPA) using a mixture of specific probes for the SMA locus essentially as previously described [21]. The c.859G>C variant in SMN2 exon 7 was detected by Sanger sequencing [16].

In addition, we reviewed all papers on the subject published from 1999 to date, and included in our further analyses those reports presenting a series of more than 10 genetically confirmed SMA patients and in which SMN2 copy number was assessed by different methods (Supplementary Table S1). In all cases, phenotypes were assigned according to the authors’ criteria of each paper. Earlier publications referring relative dosage of SMN2, reports describing a few isolated cases, type II and III patients grouped together as well as small series of less than 10 patients were not used to derive correlations.

2.1. Statistical analysis

Chi square test was used to compare the results of every SMA type between the Spanish cohort (I, I, and III) versus the worldwide cohort (I, II and III). We thus distinguish only between type I (never sit), II (never walk) or III (walker) criteria. (For simplicity, in this work type IIIa, IIIb and IV cases have been grouped together as “walker” phenotype, as it is the highest milestone achieved.) We thus distinguish only between type I (never sit), II (never walk) or III (walker) patients. SMN2 copy number was determined by Multiplex Ligation-dependent Probe Amplification (MLPA) using a mixture of specific probes for the SMA locus essentially as previously described [21]. The c.859G>C variant in SMN2 exon 7 was detected by Sanger sequencing [16].

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3. Results

3.1. Analysis of the Spanish cohort

We studied 625 Spanish SMA patients, which were distributed into the three major SMA types (272, 186 and 167 cases classified as type I, II and III, respectively). The major results of our study are summarized in Fig. 1. From the 272 type I patients, the vast majority showed two SMN2 copies (235/272, 86%), twenty had a single gene copy (7%), and the remaining seventeen had three copies (6%). Most of the type II patients presented three SMN2 copies (162/186, 87%), while 24 had two copies of the gene (13%). Finally, from the 167 type III SMA patients, 107 (64%) had three copies and 51 (31%) presented 4 SMN2 copies. Seven type III patients had two SMN2 copies (5%). The remaining two type III cases had five or six SMN2 copies (<1%).

MLPA methodology allows to quantify 5 of 9 exons of SMN1 and SMN2, which is useful to identify different genetic rearrangements in SMN genes. In our cohort of patients with homozygous absence of SMN1, we found 3’ deletions of SMN genes in 12 patients (10 with type I, one with type II and one with type III). However, it is not possible to ascribe these deletions to SMN1 or SMN2. Indeed, this type of deletion appears in approximately 23% of carriers and noncarriers which have presence of SMN1 (data not shown). Furthermore, we identified two clinically SMA patients with 5’ deletions of the SMN genes but these cases have not been included in this
The present cohort of SMA patients because they do not fulfill the criteria of inclusion of homozygous absence of exon 7 and or 8 of the SMN1 gene (manuscript in preparation).

Further analysis of discordant cases showed that 4 of the 24 type II patients with two SMN2 copies presented the c.859G>C variant in a heterozygous state. The positive modifier effect of this rare SMN2 polymorphism on the SMA phenotype has been previously described, and has been attributed to its ability to enhance inclusion of exon 7 in SMN2 transcripts [15,16,22].

Moreover, four of the seven type III cases with only two SMN2 copies had the variant in one of the SMN2 copies (Table 1).

<table>
<thead>
<tr>
<th>SMA type</th>
<th>Type I (n = 272)</th>
<th>Type II (n = 186)</th>
<th>Type III (n = 167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMN2 copies</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Patients with c.859G&gt;C variant</td>
<td>0</td>
<td>0</td>
<td>4/24 (17%)</td>
</tr>
<tr>
<td>Total positive</td>
<td>0</td>
<td>4 (2.2%)</td>
<td>6 (3.6%)</td>
</tr>
</tbody>
</table>

In type III cases with three SMN2 copies, the variant was present in just one of these copies. *denotes heterozygous; †denotes homozygous.

As expected, type I SMA patients in this cohort presented a single (68/984; 7%) or, more commonly, two copies of the SMN2 gene (684/984; 69%). There were, however, also a significant number of discordant cases, as 228 individuals in this group had three (23%) or, exceptionally, even 4–5 SMN2 copies (<1% each). Regarding type II patients, three quarters of them showed three gene copies (740/974), but a minority presented only two copies (168/974; 17%). Particularly discordant cases were reported as both “better-than-expected” (four patients with a single gene copy; <1%) or, more commonly, as “worst-than-expected” cases (59 individuals (6%) that had four, and 3 (<1%) with five SMN2 copies).

Roughly half of the 876 patients with type III SMA presented either three (408; 47%) or four gene copies (404; 46%). Forty-seven cases (5%) can be considered to behave better-than-expected, as these patients had only two SMN2 copies. Few type III patients (2%) had five SMN2 copies, while 2 patients (<1%) even presented six gene copies. For 311 patients of this group, subtypes IIIa (n = 161) and IIIb (n = 150) have been separately reported. Most type IIIa patients presented three gene copies (97/161; 60%), while 57 (35%) had four copies. In type IIIb patients the situation is reversed: 24% of these individuals (36 out of 150) presented three SMN2 copies, whereas 69% (103/150) had four copies. Interestingly, the two subgroups differ also in the nature of discordant cases: seven type IIIa SMA patients (4%) had only two SMN2 copies, while both better-than-expected (5 individuals with two gene copies; 3%) and worst-than-expected cases (six individuals that had 5 copies; 4%) were reported among type IIIb patients.

3.2. Reassessment of data compiled from published studies

The results of the literature compilation are summarized in Supplementary Figs S1 and S2 and in Supplementary Table S1. From the 2836 patients studied worldwide by other groups in articles published from 1999 to date, two outlier cases were excluded from the final analysis: one patient with a phenotype reported as mixed type II/III, and one type IV SMA patient presenting 8 SMN2 gene copies [23]. Thus, the definitive analysis has been done with 2834 patients worldwide.

Again, the vast majority of these SMA patients were roughly equally distributed across the three major disease types: 984 individuals were type I, 974 were reported as type II, and 876 developed type III SMA. Only 26 patients (=1%) were described with the milder type IV disease form. For the sake of simplicity, in Supplementary Fig. S1 we merged together as ‘type III’ all type IIa (161 patients), IIb (150 individuals) and IV (26 cases) that had been differentially reported in the original publications, although their SMN2 copy numbers are separately given in Supplementary Fig. S2. Altogether, 876 patients are considered as type III “walkers”; this convention will be maintained throughout the current manuscript, unless otherwise specified.

Fig. 1. Distribution of SMN2 copy numbers according to SMA type. Number of Spanish SMA patients studied from our cohort of 625 index cases.
most patients reported as type IV presented four gene copies (21/26; 81%), 2 had five copies (8%) [24], and one individual showed 6 SMN2 copies (4%) [18].

### 3.3. Compilation of data from all cohorts

Combining our cohort of 625 SMA patients with the 2834 SMA cases reported worldwide from 1999 to date results in a cohort that includes 3459 patients. The results of data compilation are presented in Table 2. Analysis of this large patient cohort allows for quantitative estimates of the probability of developing a particular SMA type as a function of SMN2 copy number, as discussed below.

### 4. Discussion

We present a thorough analysis of the largest series of genetically confirmed SMA patients classified by widely accepted clinical criteria (age of disease onset, highest achieved motor milestones, and evolution of the disease). For all these individuals we have determined SMN2 copy number using a robust and highly reliable method, MLPA [21]. Furthermore, we compiled the most relevant data published to date on the correlation between SMN2 copy number and SMA phenotype, and merged these results with those obtained with our cohort of Spanish SMA patients.

#### 4.1. Spanish cohort

Our analysis of 625 SMA patients corroborates the existence of a strong, inverse correlation between SMN2 copy number and disease severity. Thus, one and four SMN2 copies are the genotypes most closely linked to a particular SMA phenotype: 100% of these individuals suffer from the most life-threatening type of the disease or the milder type III form, respectively. In particular, the presence of a single SMN2 copy implies that minimal SMN protein production is tightly linked to particularly severe phenotypes, sometimes referred to as type 0 or type Ia SMA [25].

Nevertheless, most patients of our cohort present either two (43%) or three copies of the SMN2 gene (46%), and for them genotype–phenotype correlations are less clear-cut. Although the majority of children with two copies develop classical type I SMA several weeks after birth, some may manifest first symptoms of the disease as early as in the first week of life with marked hypotonia and weakness. Three SMN2 copies were detected in patients of all three main SMA types, the vast majority being type II or III cases (57% and 37%, respectively). However, a few patients who harboured three copies of the SMN2 gene were unambiguously diagnosed with type I SMA (17/286; 6%) based on a disease onset between 3 and 6 months and the fact that they never sit (Fig. 1). These patients usually have better clinical evolution and higher survival rates compared to “classical” type I cases, and can be classified as type Ic [26]. The natural history of these patients may be explained by the non-equivalency of their SMN2 copies, which renders their phenotype more severe than expected in patients with three SMN2 copies. DNA (hyper)methylation resulting in partial inactivation of one or several of the gene copies is a likely explanation to account for this discordance [27], but this issue has not been specifically investigated to date in this subgroup of patients. In addition, we cannot rule out the existence of unknown variants or cryptic partial deletions in any of these copies as a possible explanation for the worst-than-expected phenotype of these patients because the quantification by MLPA does not allow them to be identified (Table 3). On the other hand, this methodology may identify 5’ or 3’ deletions of the SMN genes. Our results indicate that these events are less frequent in SMA patients (approx 2%) than in carriers or control population (approx 23%) suggesting that they are more likely to occur in SMN1 and is considered a polymorphic loss of SMN exons 7 and 8 [28]. In our work, we identified this event mostly in type I SMA patients but the total number is very low to consider a particular modifier effect in the final phenotype. It is worth to note that cryptic intragenic deletions may account for some phenotypic discrepancies.

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Table 2
Summary of combined data from our cohort of 625 Spanish patients and of 2834 SMA patients worldwide, as extracted from articles published from 1999 to date.

<table>
<thead>
<tr>
<th>SMN2 copy number</th>
<th>Type I ((n = 1256))</th>
<th>Type II ((n = 1160))</th>
<th>Type III ((n = 1043))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88 (7%)</td>
<td>4 (&lt;1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>919 (73%)</td>
<td>192 (16%)</td>
<td>54 (5%)</td>
</tr>
<tr>
<td>3</td>
<td>245 (20%)</td>
<td>902 (78%)</td>
<td>515 (49%)</td>
</tr>
<tr>
<td>4</td>
<td>3 (&lt;1%)</td>
<td>59 (5%)</td>
<td>455 (44%)</td>
</tr>
<tr>
<td>5</td>
<td>1 (&lt;1%)</td>
<td>3 (&lt;1%)</td>
<td>16 (2%)</td>
</tr>
<tr>
<td>6</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (&lt;1%)</td>
</tr>
</tbody>
</table>

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Table 3
Causes of discrepancies in the quantification of SMN2 copies and phenotype–genotype correlations (see Discussion for further explanations and references).

- **Biological**
  - (Hyper)methylation of one or several SMN2 gene copies (when the patient has a more severe than expected phenotype)
  - Negative modifier effect of cryptic mutations or partial deletions of SMN2 genes (when the patient has a more severe than expected phenotype)
  - Positive modifier effect of c.859G>C variant in SMN2 or other unknown variants (when the patient has a milder than expected phenotype)
  - Effect of other positive or negative modifier genes and/or other so far undiscovered (epi)genetic or environmental factors
  - Clinical evolution of the patients might result in a reassessment of the SMA type with time

- **Pitfalls**
  - Erroneous information on patient labelling/misclassification
  - DNA samples of poor quality and/or low concentration
  - Interpretation and control issues regarding the different methodologies employed for SMN2 copy determination
  - Genomic SNPs (variants) in the defined regions to hybridize (either by primer or probe approach) to perform diagnosis may result in ambiguous or unresolved data that makes interpretation more difficult or incorrect
Noteworthy, three SMN2 copies are associated with type II cases in a wide spectrum of phenotypes ranging from typical type II weak patients to children that are stronger and may stand up and take some steps aided. These observations reinforce the existence of at least two main clinical categories of type II patients, ‘weak’ and ‘strong’ [29–31]. On the other hand, an important proportion (37%) of type III patients also has 3 SMN2 copies. These clinical and genetic findings point to the fact that small but crucial differences in the concentration of SMN protein and/or its functionality at some point(s) of development may result in patients that present either a “sitter” or a “walker” phenotype. This fact, together with the overall genetic background and perhaps additional disease modifiers (see Table 3) could define the ultimate evolution and phenotype for a given individual. The ability to predict whether a patient with three SMN2 copies will end up as a sitter or a walker remains one of the most important open questions in SMA clinical research, and urges the discovery of biological markers that might help in decision making and standard of care measures.

A number of milder type II and even type III cases with two SMN2 copies are present in our series. The presence of the c.859G>C SMN2 variant in a subset of these cases is a satisfactory explanation for the better-than-expected behaviour of these patients, because the SMN2 genes that contain this rare variant would produce a higher number of full-length transcripts and thus of functional protein [15,16,22]. However, not all better-than-expected SMA cases with two gene copies can be explained by the presence of this SMN2 variant. This warrants further investigation of other variants of the gene, cis modifiers and/or long-range/distance regulators of gene expression as additional disease modifiers [14].

4.2. Worldwide cohort

The results of our study compare well with those of 2834 cases compiled from 33 previous investigations that focused on the correlation between SMN2 copy number and SMA phenotype (summarized in Supplementary Table S1 and Supplementary Fig. S1). Together with our cohort of Spanish patients, a total of 3459 individuals have been described. The results of the worldwide cohort, as expected, are more heterogeneous than in our cohort. The comparison of every SMA type between the two groups revealed statistical differences by chi square test (I vs II p = 0.000; III vs II p = 0.002; III vs III p = 0.001). The distribution of SMN2 copy number in all the Spanish SMA cases in comparison with worldwide cases was also significant. However, all major genotype–phenotype correlations and trends are maintained. Applying the Kruskal–Wallis test the mean number of SMN2 copies increases when the phenotype is milder (Supplementary Fig. S3). Interestingly, the proportion of type I patients was lower than expected (42.5% in our series and 36.3% in the meta-analysis vs the 50%–60% estimated by rate [32,33]). A likely explanation in our case for this discrepancy is that DNA samples from smaller numbers of type I patients are available for SMN2 copy analysis after the initial SMN1 test, because virtually all these patients die by the age of two years.
Furthermore, a few type I and II cases with five SMN2 copies have been also reported [34]. Finally, one Italian type IV patient had 8 SMN2 copies representing the clinical case with the highest number of SMN2 copies reported to date [23]. As mentioned above, the discrepancies in these exceptional patients can be explained by different intrinsic biological factors (Table 3). Along these lines, the clinical category to which a patient is initially assigned could change through the follow-up because of loss or acquisition of specific motor milestones, and might result in a reassessment of the SMA type in nearly 10% of patients over time [31]. In summary, patient-to-patient variability in the genetic/genomic background as well as the lack of functional equivalence of their SMN2 genes might explain these outliers.

However, some discrepancies observed in this compilation could also result from technical aspects such as DNA quality (decisive in any gene dosage quantification technique), but most notably of the interpretation and control issues regarding the different methodologies employed for SMN2 copy determination. Indeed, the choice of reference samples is important for the correct determination of SMN1 and SMN2 copy numbers (see refs [21,35], and Table 3). It is also possible that in a few individuals, genomic SNPs in the regions that are employed for diagnosis may result in ambiguous or unresolved data that makes interpretation more difficult [21]. In our experience, it is more accurate to distinguish between 1–3 SMN2 gene copies than between 4 or more copies [21]. Although digital PCR, a novel technology, is a reliable and accurate method to measure SMN1 and SMN2 copy numbers in the range from one to six [36,37], SMN2 copy number in some patients could be both under- and overestimated with some more widely used techniques. For example, marker analysis, real-time qPCR using LightCycler instrument and MLPA are more reliable when used together than separately [21]. Finally, most methods may present technical limitations such as difficulties to study hybrid SMN2-SMN1 genes, to detect the presence of deletions at the 3' end of SMN2 and the masking of intragenic deletions [21,28]. Thus, for accurate estimation of SMN2 copy number, particularly when dealing with outliers or exceptional cases, it is cautious to combine different approaches. Considering that the information available in clinical laboratories might not always be precise enough to characterize the phenotype of some reported patients, it is not unreasonable to speculate that some genetically confirmed SMA cases could have been mislabelled or misclassified (Table 3).

4.3. Practical applications

The current management of SMA involves both supportive and preventive strategies [4,38]. However, during the last years new advanced therapies for SMA have been investigated preclinically, and are now in the developmental clinical phase with several ongoing clinical trials (www.clinicaltrials.gov). The first approved therapy, based on modulating the splicing behaviour of the SMN2 pre-mRNA [5] implies that potential new trajectories in the SMA phenotype might need to be considered from now on [39]. The efficacy of these advanced treatments may depend on early detection of the disorder, and therefore newborn screening using validated SMA tests rises as a means of ensuring early intervention [40]. Furthermore, results from animal models of SMA strongly suggest that patients would respond better to drugs administered before the onset of the disease and thus before irreversible neuronal loss occurs [41]. For this reason, it is important to establish protocols to accurately predict the SMA phenotype at presymptomatic stages to help with the follow-up and with adequate proactive measures. Once a presymptomatic case is genetically confirmed, the number of SMN2 copies could help to predict which SMA type the patient would probably develop. This assignment may allow the child to be enrolled in a clinical trial or therapeutic protocol before irreversible neuronal loss occurs, and would enable patients to receive more proactive treatments [38,39].

However, is it possible with currently available data to accurately predict an SMA phenotype based on SMN2 copy number alone? In our experience, with validated methodologies and after a thorough clinical examination and phenotype determination, a prediction based on the number of SMN2 copies could be attempted. In the Spanish cohort (Fig. 2a), all children with a single SMN2 copy and as much as 88% of those with 2 copies of the gene would develop type I disease. From these patients with two SMN2 copies, 9% and 3% would develop milder type II or III forms, respectively. About half of individuals in the latter group are expected to carry the c.859G>C SMN2 variant [16] predicting a walker or non-walker phenotype depending upon its presence in homozygous or heterozygous state, respectively (Table 1). With the exception of a few original case reports [15,22] no other series have been published including type II and III SMA cases with two SMN2 copies harbouring the positive modifier [16], this work.

Cases with three SMN2 copies are the most challenging given that the three major SMA types are present: 6% of the patients would develop the more severe type I form and would never sit, but much more common in this group are type II (sitters; 57%) and type III patients (walkers; 37%). Therefore, in the actual scenario of SMA treatment options, it is of utmost importance to discover biomarkers that predict whether a given patient with three SMN2 copies would be able to walk or not. Finally, all individuals with four or more gene copies are expected to be walkers developing type III or IV disease forms. These correlations are essentially reproduced in the compiled cohort of 2834 patients (Fig. 2b) and in the combined cohort that includes a total of 3459 cases (Fig. 2c). However, inclusion of cases reported by different groups results in a larger variability, most likely due to the various techniques employed for SMN2 copy number determination and some limitations of the available clinical information (Table 3). Thus, most cases with a single (96%) and two SMN2 copies (79%) will present the most severe form of the disease, and more than half of patients with three copies will suffer from type II SMA. Finally, patients with higher number of SMN2 copies (four, five and six) would predominantly have the milder type III form (88%, 80% and 100% of cases, respectively).

In conclusion, we have analyzed 625 SMA cases and compiled all relevant reports on the correlation between SMA type and SMN2 copy number in 2834 additional individuals.
This has allowed us to establish quantitative correlations for predicting the likely evolution of SMA patients. Novel therapies will have a definite impact on envisaged early intervention given that pre-symptomatic children with SMA could be identified either by testing of infants at risk (e.g., if the parents have a previous child affected with SMA) or from newborn screening [39,40]. Despite the progress in the methodologies and understanding of the phenotype–genotype correlations in SMA, there are still a number of issues that need to be clarified such as the link between disease onset and evolution in cases with three SMN2 copies. Discovery and validation of genetic and other biological markers of disease progression remains therefore an urgent matter in SMA research.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.nmd.2018.01.003.

References


