Short Report

Improving detection and genetic counseling in carriers of spinal muscular atrophy with two copies of the \textit{SMN1} gene

Alías L., Barceló M.J., Bernal S., Martínez-Hernández R., Also-Rallo E., Vázquez C., Santana A., Millán J.M., Baiget M., Tizzano E.F. Improving detection and genetic counseling in carriers of spinal muscular atrophy with two copies of the \textit{SMN1} gene.


Spinal muscular atrophy (SMA) is an autosomal recessive disease caused by mutations in the survival motor neuron 1 gene (\textit{SMN1}). Global carrier frequency is around 1 in 50 and carrier detection is crucial to define couples at risk to have SMA offspring. Most SMA carriers have one \textit{SMN1} copy and are currently detected using quantitative methods. A few, however, have two \textit{SMN1} copies in \textit{cis} (2/0 carriers), complicating carrier diagnosis in SMA. We analyzed our experience in detecting 2/0 carriers from a cohort of 1562 individuals, including SMA parents, SMA relatives, and unrelated individuals of the general population. Interestingly, in three couples who had an SMA child, both the parents had two \textit{SMN1} copies. Families of this type have not been previously reported. Our results emphasize the importance of performing a detailed carrier study in SMA parents with two \textit{SMN1} copies. Expanding the analysis to other key family members might confirm potential 2/0 carriers. Finally, when a partner of a known carrier presents two \textit{SMN1} copies, the study of both parents will provide a more accurate diagnosis, thus optimizing genetic counseling.

Conflict of interest
The authors declare no conflict of interest.

The autosomal recessive spinal muscular atrophy (SMA), characterized by a progressive degeneration of the spinal cord motor neurons, is caused by mutations in the survival motor neuron 1 gene (\textit{SMN1}) (1). Given that SMA is one of the most common lethal genetic disorders, with a high carrier frequency of 1/35–1/60 (2), detecting carriers of \textit{SMN1} deletions are crucial to define couples at risk for SMA offspring. Familial carrier analysis includes testing both the parents of a patient with mutations in the \textit{SMN1} gene or when a patient suspected to suffer SMA died without molecular studies. Other requests are testing in siblings, uncles, aunts and cousins and individuals from the general population when they are partners of confirmed carriers. The protocol is based on the \textit{SMN1} dosage. However, about 2–5% of SMA carriers have two \textit{SMN1} copies in one chromosome and 0 copies in the other (carriers 2/0 instead of the common carriers with 1/0 genotype) (3). \textit{SMN1} dosage studies do not allow discrimination of non-carriers (typically 1/1) from 2/0 carriers and can give a false negative result in SMA carrier diagnosis (4). Thus, the finding of two \textit{SMN1} copies significantly reduces the risk of being a carrier, although a residual risk of being a carrier remains (5). Carrier diagnosis is further complicated by \textit{de novo} mutations and germ line and somatic mosaicism, as has been reported in some SMA families (6, 7).
Here, we report our experience with SMA carrier diagnosis in 1562 individuals. Our aim was to characterize parents and blood relatives of SMA patients with two SMN1 copies. We further analyzed individuals from the general population, in particular, partners of known SMA carriers, with the aim to improve detection and genetic counseling in these cryptic 2/0 carriers.

Materials and methods

Samples

We tested 1562 individuals to determine their SMA carrier status: 488 were parents of genetically confirmed SMA children; 665 were blood relatives of patients; and 409 were from the general population.

Carrier testing

SMN1 dosage was quantified by a quantitative real-time polymerase chain reaction (PCR) assay (Light-Cycler instrument, Roche Diagnostics, Basel, Switzerland), as previously described (8). Carriers with two SMN1 copies were re-tested with the multiplex ligation-dependent probe amplification (MLPA) methodology (MLPA p021 MMA kit) (MRC-Holland, Amsterdam, The Netherlands) (http://www.mrc-holland.org) to confirm results. The ‘at-risk’ haplotype was defined using markers D5S1556(Ag1-CA=C272), D5F149S1(C212), D5S629 and D5S610 (1).

Results

From 488 parents, 466 (95.5%) showed one SMN1 copy, 21 had two SMN1 copies and 1 had three copies (21 + 1/488, 4.5%) (Table S1, Supporting Information). In progenitors with more than one SMN1 copy, we conducted an in-depth study based on the inheritance of the at-risk alleles according to marker analyses and on the results of quantitative analyses of their respective parents or siblings. As illustrated in Figs 1 and 2, 2/0 carriers were considered when one of their parents had three copies and the other had one SMN1 copy. Fifteen of the 21 parents were fulfilled this criteria and confirmed as 2/0 carriers. The remaining five (two females, three males) were considered de novo-mosaic deletion cases giving that their parents had two SMN1 copies. In the remaining case, we did not have information to discriminate between 2/0 and a de novo-mutation. In three families, both the parents of the affected patient had two SMN1 copies. In one family, the father transmitted a de novo-mosaic deletion to his affected son and the mother was confirmed as a 2/0 carrier (Fig. 1a). In the remaining two families, both parents were confirmed as 2/0 carriers (Fig. 1b, c). In four other families (Fig. 2a–d) in which one of the parents had more than one SMN1 copy, genetic prenatal diagnosis in a new pregnancy of the couple was useful to determine whether the SMN1 deletion in the index case was inherited, confirming the parent as a 2/0 carrier (Fig. 2a, b) or de novo-mosaic deletion (Fig. 2c, d). Thus, all cases of 2/0 carriers were confirmed either by the analysis of their grandparents (Fig. 1) or their offspring (Fig. 2).

From 665 blood relatives, 259 were diagnosed as carriers with one SMN1 copy, 351 showed two SMN1 copies, 51 had three copies, and 4 had four copies. Following the same criteria as in the parent’s group, 33 (9.5%) individuals with two SMN1 copies were confirmed as 2/0 carriers in this group.

In the group of the general population (n = 409), 10 had one SMN1 copy (2.5%), 358 had two SMN1 copies (87.5%), 37 had three copies (9%), and 4 had four SMN1 copies (1%). From this group, we studied 85 individuals who were partners of a typical 1/0 SMA carrier. Five of these individuals showed one SMN1 copy and were considered as typical 1/0 carriers. Their respective couples were therefore defined as high risk to have an SMA child, and prenatal diagnosis was offered. The remaining 80 partners had two (61/85, 71.76%) or three (19/85, 22.35%) SMN1 copies (Table S1).

Discussion

We characterized all carriers of two SMN1 copies from a population of 1153 individuals made up of parents and blood relatives of SMA patients.

Around 4% of SMA parents showed two copies of the SMN1 gene

Even though the parents of SMA children are generally assumed to be carriers with one SMN1 copy, some parents (4.3% in our study) have two SMN1 copies. Interestingly, we have also detected a parent of an SMA child with three SMN1 copies (Fig. 2d). A high proportion of parents with two SMN1 copies (71% in our cohort) was defined as true carriers harboring two SMN1 copies in one chromosome and a null allele in the other. The remaining cases may be de novo-mosaic mutations as is the case of the parent with three SMN1 copies. The proportion of parents (4.3%) and blood relatives (9.5%) who had two SMN1 copies in one chromosome (2/0) differs little from the proportion of individuals in the general population who have three SMN1 copies (9%) (2/1). These results indicate a similar presence of alleles with two SMN1 copies in our three study groups, as reported previously (5, 7). In our experience, true carriers can be distinguished from de novo-mosaic cases by means of quantitative and marker analysis in other relatives. In Fig. 1, we illustrate three interesting families where both progenitors of an SMA case had two SMN1 copies. To the best of our knowledge, no such families have been previously reported. Family A (a de novo paternal deletion and a transmitted maternal deletion) was resolved by studying the patient’s grandparents and siblings. In the remaining two families, both parents were defined as 2/0 carriers based on the
Fig. 1. Spinal muscular atrophy (SMA) families with both the parents of an affected patient showing two SMN1 copies. (a) Three-generation studies in a family with an SMN1 de novo paternal deletion and a maternal transmitted deletion. The father (II: 1) is homozygous for the 20–21 C272 allele. This allele is deleted in the affected case (III: 1), compatible with a de novo deletion. It is assumed that the parent (II: 1, with two SMN1 copies) has a double copy of the C272 alleles (20–21) because of the intensity of the signal in the electropherogram in comparison with I: 1, I: 2, III: 2 and III: 3 which also present a 20–21 allele (data not shown). A recombination between C272 and the marker D5S610 was detected in the affected case. His sister (III: 2, a 2/1 non-carrier) inherited one of the non-recombinant paternal haplotypes and his brother (III: 3, a typical 1/0 carrier) the other haplotype. On the maternal side, the presence of three SMN1 copies in I: 3 and one SMN1 copy in I: 4 confirms that II: 2 and II: 3 were 2/0 carriers. (b) Family in which the quantitative and marker analysis of the healthy brother (II: 2) was useful to determine that SMA parents (I: 1 and I: 2) had two SMN1 copies in cis. Given that the signal in the electropherogram of the C272 allele (22) in the individual I: 1 was the same as his son (II: 2); we concluded that it was only present in the non-risk haplotype. The mother transmitted a deletion to her affected daughter and two SMN1 copies to her unaffected son. (c) Two generations of a family with consanguineous parents, three SMN1 homozygous deleted individuals (I: 1; II: 3 and II: 4) and an unaffected brother (II: 1). Markers C212, C272 and C281 allowed us to discriminate between maternal and paternal ‘at-risk’ haplotypes shared by the six members of the family from maternal or paternal ‘non-risk’ haplotypes. Quantitative methods revealed the presence of two SMN1 copies in both the parents, (I: 1 and I: 2), and in the healthy brother (II: 1). Both copies of the SMN1 gene were assigned to the same chromosome given the transmission of the same deleted allele to the three brothers. Note that the non-deleted allele with two SMN1 copies in cis is different in the parents.

analysis of the patient’s siblings. In family B, the brother of the affected patient showed four SMN1 copies and the alleles inherited from their parents were different. In family C, three affected cases born from consanguineous parents showed homozygous SMN1 absence, sharing the same alleles. In this family, as expected, the origin of the chromosomes with the SMN1 deletion was the same. Surprisingly, we noticed that the origin of the chromosomes predicted to harbor the two SMN1 genes in cis was different. A de novo-mosaic case was unlikely in each parent given the recurrent transmission of the deleted alleles (7 times). This couple, living in Canary Islands, was originally from North Africa. According to a pan-ethnic population study of the SMN1 gene (2), Afro-Americans have a high frequency of cases with three or more copies of the gene, which would explain the unusual finding in this family.

Carrier diagnosis of 2/0 cases in blood relatives requires both quantitative and marker studies

In a SMA carrier study of blood relatives, the presence of two SMN1 copies together with the non-inheritance of the at-risk haplotype is helpful to exclude carrier status providing unambiguous results. On the other hand, in the presence of two SMN1 copies together with the inheritance of the at-risk haplotype, the diagnosis of a 2/0 carrier should be considered and evaluated in the context of the results in other relatives of the family. In recent years, many laboratories have implemented SMA carrier diagnosis based only on quantitative analysis. This was mainly the result of easy-to-implement techniques such as MLPA. However, the exclusive use of such techniques and the analysis of only isolated individuals in a given family may lead to errors in defining carrier status. The examples in Figs 1 and 2 demonstrate the utility of marker
SMA carriers with two SMN1 copies

Fig. 2. Prenatal analysis in spinal muscular atrophy (SMA) families to confirm the existence of 2/0 carriers or de novo cases. (a) Family with an SMA patient (II: 1) having homozygous deletion of the SMN1 gene. The mother (I: 2) had one SMN1 copy whereas the father (I: 1) had two SMN1 copies. His brother (II: 2) had three SMN1 copies, compatible with a maternal transmission of the normal allele with one copy and a paternal transmission of a normal allele with two SMN1 copies. Testing of the fetus (II: 3) showed the presence of the deletion from the father confirming that he was a 2/0 carrier. (b) This case is similar to the family in A. The father (I: 1) had one SMN1 copy, and the mother had two SMN1 copies. Quantitative study of a new pregnancy (II: 2) showed three SMN1 copies. The mother (I: 2) is thus likely to be a 2/0 carrier, but could be confirmed if her deletion is transmitted to a new offspring. (c) Family with an SMA patient (II: 1) having homozygous deletion of the SMN1 gene. Quantitative analysis confirmed that her father (I: 1) was a common 1/0 carrier and her mother (I: 2) had two SMN1 copies. Studies in two new pregnancies (II: 2 and II: 3) showed the transmission of the two complete maternal haplotypes, determining that a ‘de novo’ deletion occurred in the SMA patient with the loss of C212 and C272 alleles. (d) In this family, the homozygous deletion of the SMN1 gene in patient (III: 1) was the result of a transmitted deletion from the mother (II: 2, with one SMN1 copy) and a de novo mutation from the father (II: 1, with three SMN1 copies). Note in the SMA patient the loss of the paternal alleles 23 and 17 of markers C212 and C272 which are present in the non-affected fetus (III: 3). The sister (III: 2) is a 2/0 carrier given that she inherited the paternal chromosome with two SMN1 copies and the maternal chromosome with the SMN1 deletion.

Two SMN1 copies in partners of SMA carriers

Once a SMA carrier is identified by molecular analysis, in most cases his/her partner requests carrier diagnosis. The a priori risk of being a carrier will depend on the ethnic origin of the partner (2). According to this work, in our Spanish population, the carrier frequency is 1/41 (Table S1). Assuming this figure (approximately 1/40), the probability of a known carrier and an individual of the general population having an affected child is around 1/160 (1/40 × 1/4) (Table 1). This figure is very ambiguous and usually induces the couple to request SMA carrier testing for the partner. After testing for SMN1 copies in the partner, if one copy is present then the risk may be as high as 1/4. On the other hand, when two copies are present, false negative results may be obtained. Three main reasons may explain a residual risk of being a carrier: (i) having two SMN1 copies in cis (2/0 carrier); (ii) being a carrier of a point mutation; and (iii) harboring mutation/deletions in germinal cells. Considering all these reasons, the probability of being an SMA carrier with two SMN1 copies is 1/781 (around 1/800; Table 1), not unlike figures in previous reports (2, 7).

Regarding the first reason for false negatives – having two SMN1 copies in cis – genetic counseling could be improved by testing the parents of the partner. In our study, all blood relatives characterized as 2/0 carriers (33 individuals, 9.5% of cases with two SMN1
Table 1. Risk calculation when an individual of the general population is tested for the SMN1 gene and the result is two copies (left column)

<table>
<thead>
<tr>
<th>Two SMN1 copies</th>
<th>Testing the partner and both parents screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior</td>
<td>1/40</td>
</tr>
<tr>
<td>Conditionals</td>
<td></td>
</tr>
<tr>
<td>A. Two SMN1 copies</td>
<td>3/100</td>
</tr>
<tr>
<td>B. Point mutation carrier</td>
<td>1/100</td>
</tr>
<tr>
<td>C. To be a mosaicism</td>
<td>1/100</td>
</tr>
<tr>
<td>Joint</td>
<td>0.05/39.05</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.00128</td>
</tr>
<tr>
<td>Final risk</td>
<td>1/781a</td>
</tr>
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</table>

aThe final risk could be considered approximately 1/800. Therefore, the risk of a known carrier and a partner with two SMN1 copies having an affected child may be as low as 1/3200 (1/800 × 1/4). Genetic counseling is more accurate if both the parents are tested and point mutations in exons 3 and 6 are screened (right column).

bProbability of an individual being a 2/0 carrier considering that both progenitors have two SMN1 copies (approximately 1/40 × 3/100 = 1/1300; 1300 × 1300 = 1/1690000).

copies) were identified by studying their respective parents, and all had one parent as a 1/0 carrier and the other with three SMN1 copies (most probably genotype was 2/1). On the other hand, if both progenitors have two SMN1 copies, virtually the only probability that the partner is a 2/0 carrier is that both progenitors are also 2/0 carriers. Although very unlikely in practice (1/1300 × 1/1300 = 1/1690000; see Table 1), this situation occurred in one of our families presented above (Fig. 1c). A higher incidence of 2/0 carriers is expected in SMA families with African ancestors and the figure risk should be adapted (2). Thus, quantitative testing of both the parents of a partner of an SMA carrier may be an option to improve identification of 2/0 individuals. Geographic origin, cost effectiveness, and anxiety of the couple should be evaluated in each circumstance.

The second situation for false negatives is the presence of a point mutation. Exons 3 and 6 are the most frequently mutated exons in Spanish SMA patients (1). These mutations are routinely screened in our patients under study, reducing the residual risk from 1/800 to 1/1000. Considering that both parents of a partner are tested for quantitative SMN1 analysis and that the frequent point mutations are screened, the residual risk from 1/800 of being a carrier could decrease to 1/4000. Accordingly, the risk of the couple to have an affected child is around 1/16,000, clearly optimizing genetic counseling (Table 1).

The probability of false negatives in the third situation, being a mosaic, is very low (approximately 1%) (6). Molecular methodologies to reduce the residual risk in this situation are lacking.

A partner from the general population harboring three or four SMN1 copies is not uncommon (around 10% in our results) and these cases are considered as 2/1 or 2/2 non-carriers given that the risk of being a 3/0 or 4/0 carrier is highly unlikely, albeit not impossible. We have no knowledge of this type of carriers being reported.

In conclusion, a thorough molecular analysis should be performed in parents and blood relatives of SMA patients when initial results indicate two SMN1 copies. Studies may be necessary in other members of the family to characterize an individual with two SMN1 copies as a 2/0 carrier or as a de novo-mosaic mutation. Recent studies estimate that the detection rate of SMA carriers in the general population is about 90%, facilitating informative reproductive options (2, 9). It is important to minimize false negative results in population screening programs. As mentioned above, the probability that both partners are carriers presenting two SMN1 copies is negligible. However, when a screening program detects that one member of a couple has one SMN1 copy, we should rule out the possibility that the other member has two SMN1 copies through quantitative studies of their parents. This strategy can provide more accurate diagnosis and more appropriate genetic counseling, thereby lessening the couple’s anxiety.

Supporting Information

The following Supporting information is available for this article:

Table S1. Results of the SMN1 gene dosage analysis in 1562 individuals.

Additional Supporting information may be found in the online version of this article.

Acknowledgements

This work was supported by CIBERER (to L.A.), GENAME Project (to S. B. and R. M. H.), and FIS 05-2416 (to E. A.); Grants: FIS 11-2606 (E. F. T.). We wish to thank T. Jaijo for providing DNA samples, V. V olpini for helpful comments on risk calculations and the consenting patients and parents who made this study possible.

References