European Journal of Human Genetics (2018) 26:1554–1557
https://doi.org/10.1038/s41431-018-0193-4

BRIEF COMMUNICATION

Utility of two *SMN1* variants to improve spinal muscular atrophy carrier diagnosis and genetic counselling

Laura Alías[1,2] · Sara Bernal[1] · Maite Calucho[3] · Elisabeth Martínez[1] · Francesca March[1] · Pia Gallano[1,2] · Pablo Fuentes-Prior[4] · Anna Abuli[3,5] · Clara Serra-Juhe[3,5] · Eduardo F. Tizzano[2,3,5]

Received: 5 September 2017 / Revised: 27 April 2018 / Accepted: 9 May 2018 / Published online: 14 June 2018

Abstract

Spinal muscular atrophy (SMA) is caused by deletions/mutations in *SMN1*. Most heterozygous SMA carriers have only one *SMN1* copy in one of the alleles (1/0 carriers). However, a few carriers lack *SMN1* in one of their chromosomes, but present two gene copies in the other. These "2/0 carriers" are undistinguishable from non-carrier individuals (1/1) with currently available methods. Previous association of *SMN1* variants c.*3 + 80 T > G and c.*211_*212del with two *SMN1* copies in *cis* in Ashkenazi population prompted us to analyze them in 270 Spanish individuals (SMA carriers, patients and general population). Both variants were much more frequently detected in chromosomes with 2 *SMN1* copies in *cis* in comparison with chromosomes carrying one copy (17.9 vs. 0.7%; $p < 0.001$). In particular, one-fifth of 2/0 SMA carriers harboured one or both variants compared to none of 99 non-carriers with two *SMN1* copies ($p < 0.001$). The c.*211_*212del variant was also much more frequent in exon 8 of *SMN2–SMN1* hybrids than in that of intact *SMN1* genes (20 vs. 0.83%, $p < 0.001$), suggesting its association with chromosomal rearrangements. Although absence of these variants does not exclude that a particular individual is a 2/0 SMA carrier, their presence is valuable to substantially increase residual risk in putative carriers, thus improving genetic counselling.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder with an estimated incidence of 1:6,000 to 1:10,000 live births and a carrier frequency of 1/35–1/60 [1, 2]. Clinically, SMA is a continuous spectrum of phenotypes ranging from severely compromised neonates and infants to adults with minimal manifestations. Patients are classified into four main groups based on age of onset and motor milestones [3].

Survival motor neuron 1 (SMN1) has been identified as the SMA disease-determining gene [4]. *SMN2* is a highly homologous copy of *SMN1*, which has been described as an SMA modifier [5, 6]. In 90% of SMA cases, the molecular pathology is absence of *SMN1* by deletion or gene conversion and in 5% *SMN2–SMN1* hybrid genes [7–10]. The remaining SMA cases are compound heterozygous [7, 8].

Current methods of *SMN1* dosage do not discriminate between 1/1 non-carriers and 2/0 carriers (individuals with two *SMN1* copies in *cis* [2]). Familial haplotype analysis with polymorphic markers of the SMA locus is helpful to detect these 2/0 carriers within blood relatives. However, diagnosis in partners of SMA carriers from the general population with two *SMN1* copies is challenging [2].

Two *SMN1* variants have been recently associated to chromosomes carrying two *SMN1* copies in *cis* in the Ashkenazi Jewish population [11]. These variants, c.*3 + 80 T > G corresponding to g.27134 T > G in intron 7 and c.*211_*212del corresponding to g.27706_27707delAT in exon 8 of the *SMN1* gene, had been previously described by Luo and co-workers [11] according to the first nucleotide of the gene, 5000 bases off from the NG_008691.1 reference sequence. Here, we tested these variants in a large set of Spanish individuals to confirm their utility to improve identification of 2/0 SMA carriers.

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41431-018-0193-4) contains supplementary material, which is available to authorised users.

✉ Eduardo F. Tizzano
etizzano@vhebron.net

[1] Servei de Genètica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

[2] CIBERER (CB06/07/0011 group), Barcelona, Spain

[3] Medicine Genetics, VHIR, Barcelona, Spain

[4] Molecular Bases of Disease, IIB Sant Pau, Barcelona, Spain

[5] Department of Clinical and Molecular Genetics, Hospital Valle Hebron, Barcelona, Spain

Table 1 Screening for the presence/absence of variants c.*3 + 80 T > G and c.*211_*212del in 270 Spanish individuals

	SMA patients		SMA carriers			Normal individuals		
	Del/Del	Hybrid	1/0 carriers	2/0 carriers	3/0 carriers	2 *SMN1* copies	3 *SMN1* copies	4 *SMN1* copies
c.*3 + 80 T > G	0	0	1 (2.4%)[a]	7 (21.8%)	0	0	11 (18.9%)[b]	0
c.*211_*212del	0	4 (20%)	1 (2.4%)[a]	6 (18.7%)[c]	0	0	11 (18.9%)[d]	0
Total Ind. studied ($n = 270$)	16	20	41	32	1	99	58	3[e]

[a]Correspond to the same individual (mother of a SMA patient with homozygous deletion of the *SMN1* gene and without hybrid genes)

[b]Ten of them presented the two variants, and other individual carried only the c.*3 + 80 T > G variant in intron 7

[c]All six individuals have the two variants

[d]Ten of them presented the two variants, and other individual carried only variant c.*211_*212del in exon 8

[e]Note that the variants under study were not present in none of the three individuals carrying four *SMN1* copies

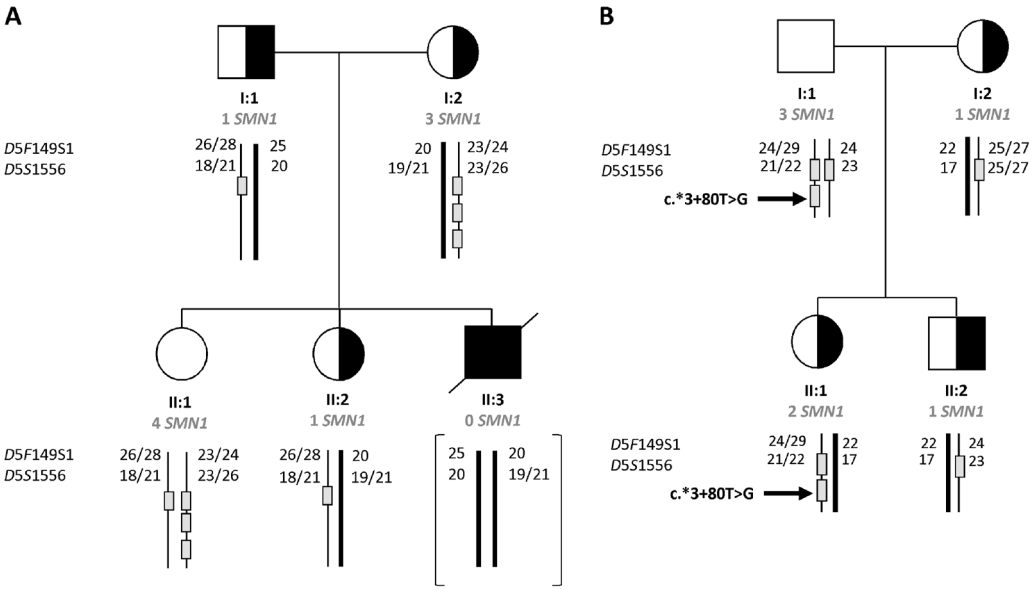

Fig. 1 Identification and genetic analyses of special SMA carriers. **a** Familial haplotype and *SMN1* quantitative analyses in a family with a 3/0 carrier. The family requested carrier studies because of the previous death of a son with clinical manifestations compatible with type I SMA. Due to the informative potential of the *D5F*149S1 and *D5S*1556 polymorphic markers and allele segregation, the more likely explanation is that the three copies of the *SMN1* gene detected in the mother (I:2) were located in *cis* in the same chromosome. Her non-carrier daughter (II:1) harboured 4 *SMN1* copies, three of them inherited from her maternal chromosome and the other one from her father. The carrier daughter (II:2) showed a single *SMN1* copy inherited from her father (shared also with her sister) and the *SMN1* deletion from her mother. The haplotype of SMA patient (II:3) is inferred. Black bars represent the chromosome that lacks *SMN1*. Each box represents a single *SMN1* copy. The number of each marker allele corresponds to the CA repeats. **b** Association between the presence of the c.*3 + 80 T > G variant in intron 7 of the *SMN1* gene and a chromosome carrier of two copies of this gene. By quantitative studies, we detected the presence of three *SMN1* copies in the father (I:1) and one copy in the mother (I:2). Haplotype analyses revealed that both siblings inherited the same maternal chromosome without the *SMN1* gene, while the paternal chromosome was different. The older daughter (II:1) was diagnosed as a 2/0 carrier with 2 copies of the *SMN1* gene and the presence of the c.*3 + 80 T > G variant inherited from her father, while the younger son (II:2) showed one copy of the gene without the variant

Materials and methods

A total of 270 Spanish individuals were analysed for the presence of the c.*3 + 80 T > G and c.*211_*212del variants in the *SMN1* gene. Carriers were divided into classical 1/0 SMA carriers ($n = 41$) and 2/0 confirmed SMA carriers ($n = 32$). An exceptional 3/0 carrier was included in the study. Non-carriers had two (1/1; $n = 99$) or more than two copies of the gene (3 *SMN1*, $n = 58$; 4 *SMN1*, $n = 3$). Finally, we studied a subset of 16 SMA patients who lack *SMN1* and another 20 with hybrid *SMN2–SMN1* genes [9, 10]. All individuals signed their informed consent.

Determination of *SMN1* copy number, sequencing methods and haplotype studies have been described elsewhere [2, 12, 13]. For detection of the variants, we used primers R111 in intron 6 and 541C1120 in exon 8 of the *SMN* genes [8, 10]. All variants were numbered according to the first translated base of the *SMN* gene (NM_000344.3 or NG_008691.1 for intronic changes) according to standard nomenclature guidelines [14]. Exons are numbered as in ref. [15]. All variants detected in this study were submitted to the LOVD database

(http://databases.lovd.nl/shared/genes/SMN1) with patients' IDs 00150115, 00150116, 00150120–00150129, and 00150239–00150250. X^2 statistics were calculated with SPSS package, and p values < 0.05 were considered significant.

Results

Our major results are summarised in Table 1. In general, the studied variants were almost completely absent from chromosomes with a single *SMN1* copy (1/297; 0.33%), but they were frequently detected in those carrying two copies of the gene (18/96; 18.75%) ($p < 0.001$).

Carriers. One classical 1/0 carrier presented the variants (1/41; 2.4%) whereas 7 of the 32 2/0 carriers were positive (7/32; 21.8%). Interestingly, the 3/0 carrier was negative for both variants (Fig. 1a). All individuals with the variants were unrelated and shared a 20-repeat allele for marker *D5S1556*. Further, five of them, who were from the Canary Islands, showed a 24-repeat allele for marker *D5F149S1* (Supplementary Figure 1).

Non-carriers. All 1/1 individuals ($n = 99$) were negative for the studied variants, whereas 11 out of 58 subjects with three *SMN1* copies (2/1) were positive (19.3%; $p < 0.001$). Four individuals in this last subgroup had also homozygous absence of *SMN2* genes, three of whom showed the variants. A comparison between 2/0 carriers vs. 1/1 controls was also significant ($p < 0.001$) for the presence of the variants.

SMA patients. None of the variants were found in 16 SMA patients who lack *SMN1*. However, four out of 20 SMA patients with *SMN2–SMN1* hybrid genes presented the c.*211_*212del variant (20%) (Supplementary Figure 2).

Discussion

To improve genetic counselling for carriers of SMA, we aimed to validate in the Spanish population two variants of the *SMN1* gene, c.*3 + 80 T > G and c.*211_*212del, which had been previously associated to chromosomes with two *SMN1* copies in *cis* in the Ashkenazi population [11].

Our results confirm that these variants are only present in *SMN1*. First, we did not detect them in patients with total absence of the *SMN1* gene. Second, in subjects with *SMN2–SMN1* hybrids we detected only the c.*211_*212del variant, which corresponds to the *SMN1* half of the hybrid. Finally, we identified both variants in three individuals with three *SMN1* copies each, but who lack the *SMN2* gene.

We have also corroborated the utility of both variants for genetic testing of SMA carriers, as they are much more frequent in chromosomes with two *SMN1* copies in *cis* ($p < 0.001$), and are also present in almost 20% of cases with three *SMN1* copies (Table 1).

We report for the first time a 3/0 SMA carrier, identified in the context of a SMA family study but without the variants (Fig. 1a). Individuals with 3 *SMN1* copies are usually thought to harbour two copies in one chromosome and one in the other (2/1). Our finding emphasizes the complexity of the SMA region and points to possible pitfalls in interpreting the results of non-carriers. Genetic counselling in these cases should be carefully evaluated in the context of haplotype results [13].

Chromosomes with two *SMN1* copies are more frequent in the African population [2, 16] and, concomitantly, they present both *SMN1* variants at higher frequencies [11]. In our cohort, six of seven cases with the variants were from the Canary Islands, a region with African genetic influence due to the territorial proximity. Most of these cases share a common haplotype (Supplementary Figure 1).

Among non-carriers, the variants were absent in 1/1 individuals but present in almost every fifth (19.3%) of the 2/1 individuals studied ($p < 0.001$), reinforcing their association with chromosomes harbouring 2 *SMN1* copies in *cis*. These variants were previously reported in 116 out of 200 individuals (58%) with three *SMN1* copies [11]. Ninety of these positive cases were from African origin, likely explaining the differences with our cohort.

Given that the variants are linked to *SMN1* but not *SMN2* (ref 10. and this work), they are not detected in SMA patients who lack *SMN1*. However, 20% of patients with hybrid *SMN2–SMN1* genes, who usually have intron 7 of the *SMN2* gene and exon 8 from *SMN1* [9, 10], present the exon 8-linked c.*211_*212del variant. As expected, none of them had the intron 7-associated variant. These observations suggest that some hybrid genes originate from chromosomes with two *SMN1* copies in *cis*. However, it is not known whether the presence of the variant makes *SMN1* genes prone to rearrange in hybrid structures. It is also possible that individuals who only present the intron 7 variant may represent hybrid *SMN1* intron 7 and *SMN2* exon 8 structures.

In conclusion, our results indicate that *SMN1* variants c.*3 + 80 T > G and c.*211_*212del are associated to chromosomes that underwent rearrangements such as those with two *SMN1* copies in *cis* and those with hybrid *SMN2–SMN1* genes (around 20% of the cases). However, absence of both variants in a subject with two *SMN1* copies does not preclude the 2/0 carrier status limiting the utility of this analysis. Since most of the two-*cis* chromosomes and hybrids do not show these variants, their study in SMA carrier testing may have a limited geographical application, assuming a higher frequency in the Ashkenazi Jews and African population due to an increased number of chromosomes with two *SMN1* copies. However, the study of the variants is useful to select individuals with increased risk of being 2/0 carriers. Indeed, the presence of one or both variants notably increases the residual risk from 1/781 to ~1 (Table 2). In these cases, testing of the parents of the individual [2] would be necessary to confirm his/her 2/0 carrier status.

Table 2 Usefulness of the c.*3 + 80 T > G and c.*211_*212del *SMN1* variants for assessing the risk of being a 2/0 SMA carrier

Risk of an individual to be a carrier		Comments
A priori risk for the general population	1/40	Spanish population (ref. 2)
Risk of an individual with two SMN1 copies to be a carrier		
Without testing of variants	1/781	Residual risk includes probability of 3% of being a 2/0 carrier or carrier of a point mutation or being a mosaic (ref. 2).
Presence of variants	~ 1	All individuals with two *SMN1* copies that present the variant(s) have the two copies in *cis* (Present work).
Absence of variants	1/888	Around 80% of 2/0 carriers do not have the variant(s) (Present work).
Testing of both parents for *SMN1*	1/4,000	When both parents have two *SMN1* copies (ref. 2)

The final risk to be a 2/0 SMA carrier for an individual from the general population with 2 copies of the *SMN1* gene is 1/781 (ref. 2), but it increases to ~ 1 when at least one of the variants studied (c.*3 + 80 T > G and c.*211_*212del) is detected. An individual with 2 *SMN1* copies but without the presence of the variants reduces to some extent the risk to be a 2/0 SMA carrier (1/888). Testing for *SMN1* in the parents of the consultant could categorically define the risk (1/4,000). (Bayesian calculations according to results of the present work and ref. 2. detailed in Supplementary Material)

Acknowledgements This work was supported by Grants from Fundación Privada Daniel Bravo Andreu (to EFT and PFP and supporting MC). LA is supported by CIBERER. We wish to thank the consenting individuals who made this study possible and colleagues for referral of families.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Sugarman EA, Nagan N, Zhu H, et al. Pan-ethic carrier screening and prenatal diagnosis for spinal muscular atrophy: Clinical laboratory analysis of 72,400 specimens. Eur J Hum Genet. 2012;20:27–32.
2. Alías S, Barceló M, Bernal S, et al. Improving detection and genetic counseling in carriers of Spinal Muscular Atrophy with two copies of the SMN1 gene. Clin Genet. 2014;85:470–5.
3. Talbot K, Tizzano EF. The clinical landscape for SMA in a new therapeutic era. Gene Ther. 2017;24:529–33.
4. Lefebvre S, Bürglen L, Reboullet S, et al. Identification and characterization of a Spinal Muscular Atrophy-determining gene. Cell. 1995;80:155–65.
5. Prior TW, Krainer AR, Hua Y. *et* al: A positive modifier of spinal muscular atrophy in the SMN2 gene. Am J Hum Genet. 2009;85:408–13.
6. Calucho M, Bernal S, Alias L, et al: Correlation between SMA type and *SMN2* copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2,834 reported cases. Neuromuscul Disord. 2018;28:208–15.
7. Wirth B. An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). Hum Mutat. 2000;15:228–37.
8. Alías L, Bernal S, Fuentes-Prior P, et al. Mutation update of Spinal Muscular Atrophy in Spain: molecular characterization of 745 unrelated patients and identification of four novel mutations in the SMN1 gene. Hum Genet. 2009;125:29–39.
9. Hahnen E, Schönling J, Rudnik-Schöneborn S, Zerres K, Wirth B. Hybrid survival motor neuron genes in patients with autosomal recessive spinal muscular atrophy: new insights into molecular mechanisms responsible for the disease. Am J Hum Genet. 1996;59:1057–65.
10. Cuscó I, Barceló MJ, del Rio E, et al. Characterization of SMN hybrid genes in Spanish SMA patients: de novo, homozygous and compound heterozygous cases. Hum Genet. 2001;108:222–9.
11. Luo M, Liu L, Peter I, et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. Genet Med. 2014;16:149–56.
12. Alías L, Bernal S, Barceló MJ, et al. Accuracy of marker analysis, quantitative real-time polymerase chain reaction, and multiple ligation-dependent probe amplification to determine SMN2 copy number in patients with Spinal Muscular Atrophy. Genet Test Mol Biomark. 2011;15:587–94.
13. Barceló MJ, Alías L, Caselles L, Robles Y, Baiget M, Tizzano EF. Two independent mutations of the SMN1 gene in the same Spinal Muscular Atrophy family branch: Lessons for carrier diagnosis. Genet Med. 2006;8:259–62.
14. den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. Hum Genet. 2001;109:121–4.
15. Bürglen L, Lefebvre S, Clermont O, et al. Structure and organization of the human survival motor neuron (SMN) gene. Genomics. 1996;32:479–82.
16. Hendrickson BC, Donohoe C, Akmaev VR, et al. Differences in SMN1 allele frequencies among ethnic groups within North America. J Med Genet. 2009;46:641–4.