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# The c.859G>C variant in the *SMN2* gene is associated with both type II and III SMA and originates from a common ancestor

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**Running title:** Positive modifier effect of the *SMN2* c.859G>C variant in SMA

**Homozygous mutations of the telomeric *SMN1* gene lead to degeneration of motor neurons causing spinal muscular atrophy (SMA).<sup>1</sup> A highly similar centromeric gene (*SMN2*) can only partially compensate for *SMN1* deficiency. The c.859G>C variant in *SMN2*, has been recently reported as a positive disease modifier.<sup>2,3</sup> We identified the variant in 10 unrelated chronic SMA patients with a wide spectrum of phenotypes ranging from type II patients who can only sit to adult walkers. Haplotype analysis strongly suggests that the variant originated from a common ancestor. Our results confirm that the c.859G>C variant is a milder *SMN2* allele and predict a direct correlation between SMN activity and phenotypic severity.**

Based on age at onset and achieved milestones, SMA is classified into types I (the most severe form, characterised by generalised muscle weakness and hypotonia within the first six months of life); II, which manifests after the age of 6 months, affected children are able to sit but they never walk unaided; and III, in which patients eventually lose their ability to walk unassisted. Type III SMA is further divided into IIIa, with clinical symptoms appearing before the age of 3, and IIIb, with onset between 3 and 20 years.<sup>4</sup>

Although both *SMN1* and *SMN2* encode in principle identical proteins, a C-to-T transition within exon 7 of *SMN2* leads to a high proportion of transcripts that lack this exon, resulting in an unstable, non-functional protein<sup>5</sup>. However, the expected inverse correlation between *SMN2* copy number and severity of SMA disease (most type I, II and III SMA patients carry two, three, and three or four *SMN2* copies, respectively) is not absolute,<sup>6,7</sup> and some patients with two *SMN2* copies show mild phenotypes, suggesting that other factors modulate disease progression.

We studied 261 unrelated Spanish SMA patients (Table 1), and six pairs of SMA discordant siblings with homozygous absence of the *SMN1* gene, 10 parents of patients with the c.859G>C variant, and 105 unrelated healthy individuals, five of whom lack the *SMN2* gene. Informed consent was obtained from all subjects studied. Criteria to evaluate

phenotype variability of the patients were age of onset, progression of motor milestones and age of loss of walking ability. We used LightCycler and multiplex ligation-dependent probe amplification (MLPA) for an accurate estimation of *SMN* gene copy number as described elsewhere.<sup>7,8</sup>

By direct sequencing using primers and methodology reported previously,<sup>9,10</sup> we identified the *SMN2* c.859G>C variant in 10 unrelated type II or III SMA patients (nine males, one female; patients' clinical and molecular data are summarised in Table 2). Five of these individuals had first clinical symptoms of the disease between 4 and 15 years of age, indicating type IIIb SMA; they all maintained walking capacity for several years. Three of these cases (patients 1-3) showed two *SMN2* copies carrying the c.859G>C nucleotide change in homozygous state. The remaining two patients had three *SMN2* copies and the variant in heterozygous state, with two copies of wild-type *SMN2* and one that carries the c.859G>C variant. Patient 6 (type IIIa) carries two *SMN2* copies and the variant in heterozygous state. He started to walk at 18 months, but muscle weakness manifested around two years and was generalised at six years old. The other four individuals with 2 *SMN2* copies and the variant in heterozygous state (patients 7-10) developed type II disease. Altogether, 8 out of 16 patients with the chronic forms but only two *SMN2* copies were positive for the variant (Table 1). The analysis of ten parents confirmed that the variant has been transmitted either maternally and/or paternally (Table 2).

The variant was not detected in any of the non-discordant cases: 126 type I SMA patients with two *SMN2* copies, 55 type II patients with three *SMN2* copies, and eight walkers with four *SMN2* copies. Furthermore, the variant was neither found in the six pairs of SMA discordant sibling or individuals without *SMN2* genes but was present in 2 of 105 healthy individuals analysed.

Haplotype characterisation<sup>10</sup> showed that the 10 patients with the variant presented a common allele of 193bp or (CA)<sub>21</sub> in the C272 multicopy marker, which is closest to the 5'-end

of the *SMN* genes. Analysis of the C212 marker revealed two alleles of 227bp or (CA)<sub>30</sub> repeats (n=7) and 225bp or (CA)<sub>29</sub> repeats (n=3); this minimal size difference could be explained by replication slippage in a previous generation (Table 2 and Supplemental Figure 1). In striking contrast, these alleles were rarely associated with *SMN2* in SMA patients without the variant. Thus, the linkage disequilibrium of the c.859G>C variant with these alleles strongly suggests that it originated from a common ancestor.

**Table 1.** Distribution of the c.859G>C variant among 261 SMA patients according to their phenotype and number of *SMN2* copies. Note that the variant is positive in 8 of 16 (50%) discordant type II and III cases with two *SMN2* copies.

<b>SMA type</b>	<b>I (n=126)</b>	<b>II (n=66)</b>		<b>III (n=69)</b>		
<b><i>SMN2</i> copies</b>	<b>2</b>	<b>2 (n=11)</b>	<b>3 (n=55)</b>	<b>2 (n=5)</b>	<b>3 (n=56)</b>	<b>4 (n=8)</b>
<b>Patients with the c.859G&gt;C variant</b>	0	4 (36%)	0	4 (80%)	2 (3.6%)	0
<b>Total positive</b>	0	4		6		

**Table 2.** Clinical and molecular data of 10 Spanish SMA patients with homozygous absence of the *SMN1* gene and with the c.859G>C variant in the *SMN2* gene. *NAIP* + indicates at least one copy of the *NAIP* gene. Alleles associated with the c.859G>C variant for C272 (Ag1-CA) and C212 markers are in bold. NA= parents were not available for study. \*Used only to cover long distances. \*\*This patient had an affected sister who died at the age of six due to pneumonia (further clinical data not available). \*\*\*Never walked. \*\*\*\*This patient is not yet using wheelchair because of her age although was never able to walk unaided.

Patient	1	2	3	4	5	6	7	8	9	10
Gender	Male	Male	Male	Male	Male	Male	Male	Male	Male	Female
Age (years)	65	36	22	59	34	18	30	12	5	3
SMA type	IIIb	IIIb	IIIb	IIIb	IIIb	IIIa	II	II	II	II
Age at onset of weakness (mths / yrs)	15 yrs	14 yrs	4 yrs	14 yrs	13 yrs	< 3 yrs	7 mths	8-9 mths	12 mths	14 mths
Walked unaided	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Wheelchair bound (age)	Yes (59 yrs)	No	No	Recently*	Recently*	Yes (6 yrs)**	Yes***	Yes***	Yes***	-****
<i>SMN2</i> copies	2	2	2	3	3	2	2	2	2	2
c.859G>C in <i>SMN2</i>	Homoz.	Homoz.	Homoz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.	Heteroz.
Telomeric <i>NAIP</i>	+	-	-	+	-	-	-	-	-	-
Parental inheritance	NA	Both	NA	NA	NA	Maternal	Paternal	NA	Paternal	Maternal
C272 alleles	<b>193</b>	<b>193</b>	<b>193</b>	189 191 <b>193</b>	181 189 <b>193</b>	181 <b>193</b>	183 <b>193</b>	181 <b>193</b>	183 <b>193</b>	181 <b>193</b>
C212 alleles	<b>225</b>	<b>227</b>	<b>227</b>	<b>225</b> <b>227</b> 233	219 221 <b>225</b>	217 <b>227</b>	217 <b>225</b>	215 <b>227</b>	217 <b>227</b>	215 <b>227</b>

Two previous reports of the c.859G>C variant described patients with type III SMA.<sup>2,3</sup> However, in our series it was also found in four unrelated patients with type II disease (two *SMN2* copies with the variant in heterozygous state; Table 2). Underscoring the impact of *SMN* dosage on disease phenotype, we detected the c.859G>C variant in heterozygous form in two type IIIb cases with three *SMN2* copies, while a patient also carrying the variant in heterozygous state but only two *SMN2* copies suffered from the more severe type IIIa form. A homozygous type IIIa patient who inherited two copies of the c.859G>C variant from his mother in the same chromosome in *cis* position has been previously reported.<sup>3</sup> The parents of one of our patients homozygous for the *SMN2* variant were consanguineous, and both transmitted the variant (patient 2). Thus, the phenotype spectrum associated with the *SMN2* c.859G>C variant ranges from classical type II patients to adult type IIIb walkers depending on the homozygous or heterozygous state of the variant, the copy number of *SMN2* genes, and whether variant *SMN2* copies are present in *cis* or *trans*.

The c.859G>C variant was not found in 126 patients with type I SMA and two *SMN2* copies, similar to previous observations in 82 cases.<sup>2,3</sup> Altogether, over 200 patients with the most severe disease form from various origins have been studied, which strongly suggests that the variant should not be expected in these patients. Instead, the c.859G>C mutation appears to be restricted to discrepant cases of milder phenotype, and might be found in approximately half of the patients with only two *SMN2* copies but chronic SMA. When stratifying these patients according to SMA type, the frequency reaches 80% in our type III cases (Table 1). Interestingly, all but two type IIIb patients who carry the c.859G>C variant lack *NAIP*, which contrasts with the low frequency of *NAIP* deletion reported in chronic SMA cases (10-15%).

In conclusion, we confirm that the *SMN2* c.859C>G variant is a milder SMA allele, which is present in a minority of patients with chronic SMA, but not in any with type I disease.

Our results indicate that there is a direct correlation between SMN activity and disease severity.

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**Competing interests:** None.

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## Supplemental Figure Legend

**Figure 1.** Electropherogram of the 10 patients with the c.859G>C variant in the *SMN2* genes.

(A) Results of the C272 marker showing the common 193 peak corresponding to (CA)<sub>21</sub> repeats. Note that the homozygous patients for the variant showed only one peak;

(B) Results of the C212 marker showing two common peaks of 225 (CA)<sub>29</sub> repeats and 227 (CA)<sub>30</sub> repeats. Thus, the common haplotypes associated with the *SMN2* variant are 193-225 or 193-227. In contrast, the 193-bp allele was detected in only 7 of 120 *SMN2* genes in 50 SMA patients without the c.859G>C variant (6%), while the 225- and 227-bp alleles of C212 were identified in 17 and 5 *SMN2* genes (14 and 4%, respectively). None of these samples showed haplotypes 193-225 or 193-227. M=ROX molecular weight marker

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