Evaluation of c. * 3 + 80T> G or c. * 211_ * 212del duplication variants frequency in Italian population

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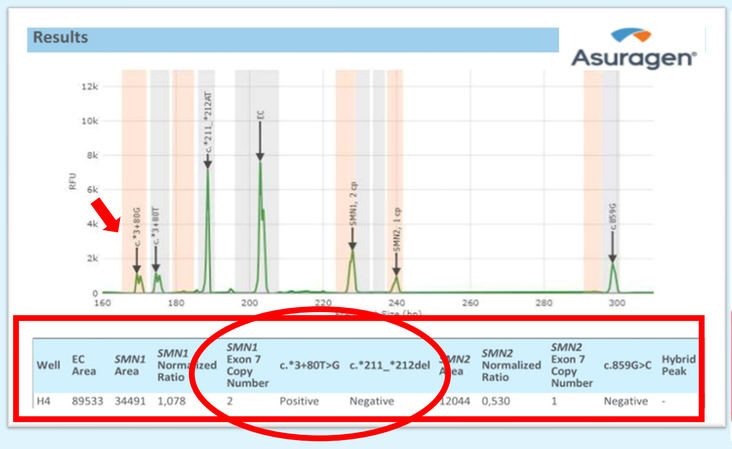
Abstract

The variants c.*3+80T> G and c.*211_*212del are associated with gene duplication of SMN1 in cis, therefore in subjects with two copies of SMN1, the co-occurrence of the duplication variants indicates an increased carrier risk. The finding of these variants in SMA carrier testing relates with an increased residual risk of being a carrier in all the ethnic groups and it varies between different populations, based on the prevalence of SMN1 duplication.

Methods and Specimens

SMN1-SMN2 genotype analysis was performed using the AmplideX SMN1 Plus CE Kit (ASURAGEN) (Fig. 1, Fig. 2). Variants were revealed by Asuragen test and confirmed by Sanger sequencing (Fig. 3, Fig. 4). Specimens included 122 SNA samples obtained from parents of subjects affected by SMA (with 0 SMN1 copies) and blood samples from 363 subjects of the general Italian population undergoing carrier testing. To better evaluate the association of the variants in subjects with 3 copies, the frequency of the variants in this subgroup was calculated including further 17 DNA samples with 3 SMN1 copies.



Well	EC Area	SMN1 Area	SMN1 Normalized Ratio	SMN1 Exon 7 Copy Number	c.*3+80T>G	c.*211_*212del	SMN2 Area	SMN2 Normalized Ratio	SMN2 Exon 7 Copy Number	c.859G>C	Hybrid Peak
H4	89533	34491	1,078	2	Positive	Negative	12044	0,530	1	Negative	-

Fig. 1: Asuragen Reporter Image: Silent Carrier Subject with 2 copies of SMN1 and c.*3+80T>G variant

Well	EC Area	SMN1 Area	SMN1 Normalized Ratio	SMN1 Exon 7 Copy Number	c.*3+80T>G	c.*211_*212del	SMN2 Area	SMN2 Normalized Ratio	SMN2 Exon 7 Copy Number	c.859G>C	Hybrid Peak
C8	60478	22810	0,983	2	Positive	Positive	825	0,473	1	Negative	-

Fig. 2: Asuragen Reporter Image: Subject of italian population with 2 copies of SMN1 and both c.*3+80T>G and c.*211_*212del variants

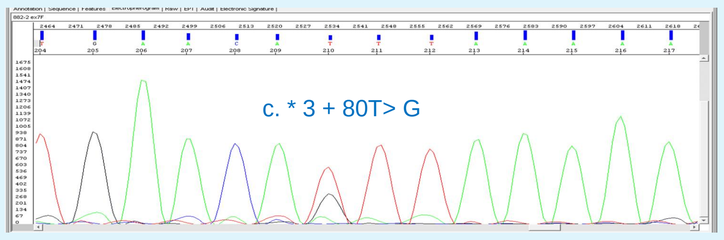


Fig. 3: c.*3+80T> G sequence

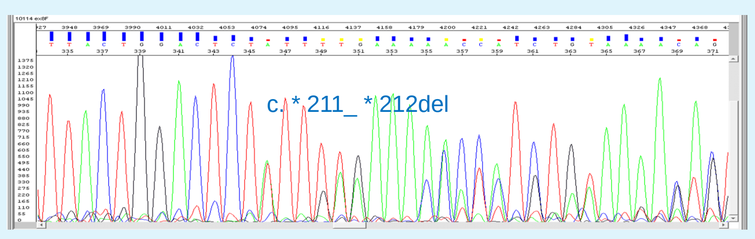


Fig. 4: c.*211_*212del sequence

Aim of the Study

The study aims to evaluate the frequency of carriers of c. *3+80T>G and c.* 211_*212del in the Italian population, after all, to date there are no specific data of frequency and correlation of these variants with SMN1 duplication in this ethnic group.

Results

Among parents of affected children, the analysis revealed a genotype 1 SMN1 copy in 112 (91,8%) individuals, in all of them no case with variants was observed. Moreover, we identified 2 SMN1 copies in 10 subjects (8.2%); 1 subject (10%) out of these carried the c. * 3 + 80T> G variant. Among subjects of general Italian population were observed: 7 cases (2%) with 1 copy of SMN1 (carriers), 334 cases (92%) with 2 copies of SMN1 and 22 (6%) with 3 copies of SMN1; among them, 6 cases (1.6%) were found to be carriers of the duplication variants and 2 out of these had 2 SMN1 copies (0.6%), the remaining 4 subjects had 3 SMN1 copies. With these cases should be considered other 17 subjects known to have 3 copies of SMN1 for a total of 39 cases, 6 of which showed up duplication variants (15,4%).

Parents of affected children* (*with 0 SMN1 copies)

SMN1 copy number	General italian population	%	Tot subjects with duplication variants	% subjects with duplication variants
1 copy	112	91,8%	0	**0%**
2 copies	10	8,2%	1	**10%**
3	-	-	-	-
Tot	122	100%	1	**0,8%**

General italian population

SMN1 copy number	General italian population	%	Restrospective cases with 3 SMN1 copies	Tot subjects with duplication variants	% subjects with duplication variants
1 copy	7	2% (carriers)	-	0	**0%**
2 copies	334	92%	-	2/334	**0,6%** (Increased risk to be silent carriers)
3	22	6%	17(§)	6/39	**15,4%**
Tot	363	100%	na	na	na

Discussion and Conclusions

Gene duplication variants in SMN1 can adjust the risk of being a silent carrier and help informed reproductive decisions for couples. This study confirms the association ($p<0.01$) of c.*3+80T>G and c.*211_*212del variants with SMN1 duplication in Italian population: frequencies of 15,4% in 3 copies SMN1 individuals and 0,6% in 2 copies SMN1 individuals as evaluated for European Caucasian population, versus 0 cases with variants detection in carrier 1+0.

Analysis of these variants is important to better understand the residual risk of being a silent carrier (2+0) in subjects with 2 copies of SMN1 who undergo carrier screening. In fact, 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 .

Bibliography

1 1.Alías L. et al. Utility of two SMN1 variants to improve spinal muscular atrophy carrier diagnosis and genetic counselling. Eur J Hum Genet. 2018; 26:1554–1557.
2.Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. Genet Med. 2014; 16(2):149-56.
3.MacDonald WK, et al. S. SMA carrier testing: a meta-analysis of differences in test performance by ethnic group. Prenat Diagn. 2014; 34(12):1219-1226.
4 Milligan J. et al. Multisite Evaluation and Validation of a Sensitive Diagnostic and Screening System for Spinal Muscular Atrophy that Reports SMN1 and SMN2 Copy Number, along with Disease Modifier and GeneDuplication Variants J. Mol. Diagnostics, 2021; 23(6): 753-764

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