

## Molecular and Epidemiological Characterization of SMN Genes in Cuban Patients with Spinal Muscular Atrophy

Lombillo-Alfonso Fabián<sup>1</sup>

Pita-Rodríguez Mariana<sup>2</sup>

<sup>1</sup>Departament of Physiological Sciences, Latin American School of Medicine, La Habana, Cuba

<sup>2</sup>Laboratory of Neurogenetics, Institute of Neurology and Neurosurgery, La Habana, Cuba

\*Author for correspondence. E-mail: [flombillo@elacm.sld.cu](mailto:flombillo@elacm.sld.cu)

### ABSTRACT

Spinal Muscular Atrophy (SMA) is a neuromuscular disease of autosomal recessive inheritance with variable expressivity, characterized by degeneration and loss of the anterior horn neurons of the spinal cord and brainstem, resulting in progressive symmetric muscle weakness. The main cause of this disease is due to homozygous mutations in the SMN1 gene. The aim of the current study was to characterize molecularly the SMN genotypes in Cuban patients affected with Spinal Muscular Atrophy by PCR of Restriction Fragment Length Polymorphism (RFLP), establishing their association with molecular and biostatistical techniques, with gender, age and skin color. This study has two main lines of work: the genotypic characterization by the detection of homozygous deletions of SMN1 and SMN2 by PCR-RFLP and the establishment of the relationship with the clinical phenotype, demographic or ethnic characteristics using statistical tests. The proportion of patients by provinces, type of SMA, sex and skin color was calculated. 74% of the patients presented homozygous deletion of SMN1 and 2, 56% had deletion of SMN2. In this study, cases with SMA were confirmed by molecular study, finding that the clinical and electrophysiological characteristics coincided with the data reported in the medical literature. 74% of the patients who fulfilled the clinical and electrophysiological criteria presented homozygous

deletion of exon 7 of the SMN1 gene, which represents the mutation most frequently observed in patients with SMA. The distribution showed that the highest percentage of patients are concentrated in the western provinces of Cuba, especially in Havana, where the greatest number of specialized medical services are found where SMA patients are treated and the diagnosis is made. The results of this research seek to improve the genetic counseling of individuals carrying mutant SMN genes in order to improve quality of life by optimizing the diagnosis, prognosis and clinical management of these patients.

**Keywords:** Spinal Muscular Atrophy, deletion, proportion, SMN1, SMN2,.

## **METHODS**

**Selection of individuals for the study:** For this study, population sample was subscribed to patients clinically diagnosed with SMA whatever type. Each individual will be classified according to age, skin color, gender, and SMA type is also taken into account and the age of diagnosis. All selected individuals will be confirmed by molecular diagnosis of SMN genes.

**Genomic DNA extraction:** DNA extraction was carried out from peripheral blood samples taken from patients. The method used is a based microsalting-out method standardized for the particular conditions of our laboratory. As validation criteria extraction consistent check against a gel electrophoresis 0.8% agarose and staining laboratory protocol a solution of ethidium bromide.

**Amplification of exon 7 of NMSs genes by PCR-RFLP:** Once the genomic DNA was obtained and tested extraction we proceed with amplification of exon 7 of SMN genes for each samples. This had required a standardized PCR-RFLP protocol which will leave a total volume of 25 uL for a single sample. The PCR program is also standardized in the Thermocycler (Minicycler TMMJ Research). Once you finished proceed was checking by gel electrophoresis 2% agarose at 100V. Finally staining was performed with a solution of ethidium bromide and the amplified fragments were displayed on a UV transilluminator (Multidoc-It Digital Imaging UVP).

**Digestion of the amplified products with restriction enzyme:** After amplification carried we proceed to digestion products to confirm whether or not deletion exon7 in the case of the SMN gene. For this, a total volume of 5 µL for each sample, the mixture composed of 2.5 uL of Buffer RestrictionEndonuclease SM, 1U volume DraI restriction enzyme

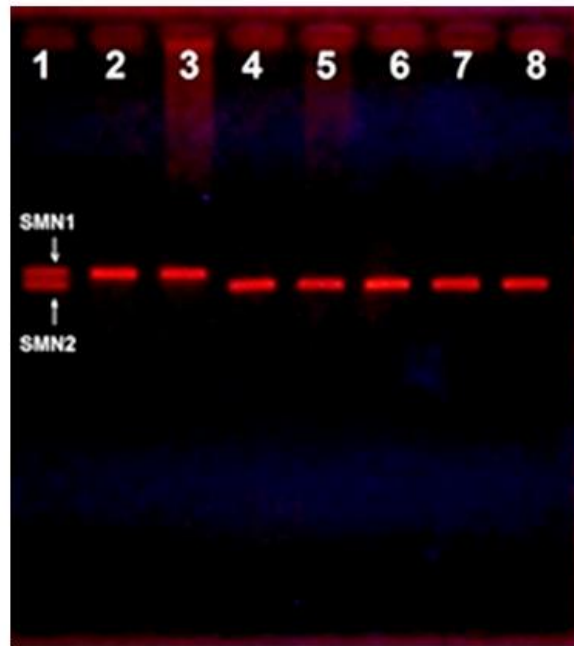
(Sigma Cat. # P3158) and 1.5 $\mu$ L RNAase free water be added. This mixture was incubated at 37 ° C bath temperature and then the results of digestion by electrophoresis V. be displayed agarose 3% between 80 and 100.

Statistical analysis: All data obtained from individuals was processed by statistical analysis, considering as variables: sex, age and skin color of the affected patients. After that, correlations were established between them and see if there was any relationship between the observed frequencies and those reported for different age groups, sex and skin color. To perform statistical tests that best meet the requirements of this study were chosen. The studied variables obtained from cases were expressed according to their summary measures. Chi square test of independence was used contingency tables to establish the association between categorical variables (genotypic variation, SMA type, etc.). The correlation coefficient Pearson and Spearman was used as appropriate in each variable (quantitative and qualitative respectively). Reason Odds (OR) was used to estimate the ratio of genotypic variants with phenotypic variants. We worked with a confidence level of 95%, prefixing the rejection zone (alpha) associated with the value of p less than 0.05 likely. It will work with the STATISTICA software v8.0.

## **RESULTS**

### *Analysis of deletions*

After molecular analysis performed as confirmatory study of clinical diagnosis it was observed that 58 patients had homozygous deletion of the SMN1 gene while 20 had not. It was further noted that a total of 32 patients had simultaneous deletion of exons 7 and 8, 32 cases with deletion only 7 and lost data were 14 cases which were not able to determine the existence of deletion of exon 8. We found that two patients showed a pattern of SMN2 deletion. Figure 1 shows an electrophoretic run corresponding to seven samples in which five (4-8) are seen with a pattern deletion SMN1 and two (2 and 3) corresponding to a pattern shown SMN2 deletion.



**Fig. 1.** Electrophoretic Run products enzymatic digestion with DraI. 1. Negative control without deletion 2 and 3: patients with deletion SMN2, 4-8: patients with SMN1 deletion pattern.

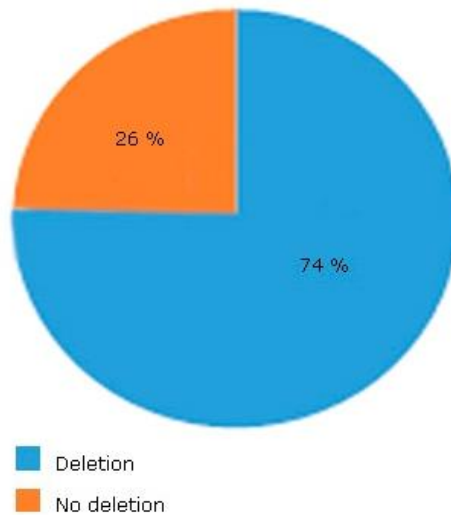
### *Frequency of deletion*

Of all patients analyzed 74.64% homozygous deletion SMN1 introduced gene (Fig. 2) and for the case of SMN2 only 2.56% of the patients showed a pattern of deletion of this gen. About the frequency deletion by sex of patients, no association between these variables was demonstrated. The association between sex and presence of the deletion was not significant with a value of  $p = 0.17$ .

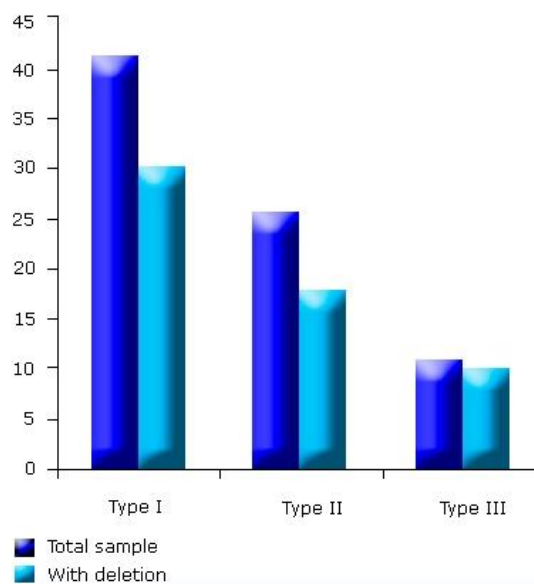
### *Distribution of patients according to the type of SMA*

In the graph of figure 3 shows the distribution of patients of the sample is shown according to the type of SMA in the same shows that the greatest number of cases diagnosed clinically confirmed, correspond to Atrophy Type I, followed by type II and less frequently the type III. The dark bar represents the total patients in the sample, while the clear, and corresponds to those with deletion of exon 7 of SMN1.

The most common type of SMA is the type I with 52.6% of cases, followed by type II to 33.3% and finally the type III with 10.14%.



**Fig. 2.** Percentage of cases with and without homozygous deletion of SMN1 patients shows that patients confirmed represent 74% of the sample, while 26% no deletion.



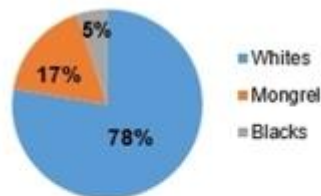
**Fig. 3.** Patient distribution of the sample according to the type of SMA. The highest frequency corresponds to the type I, followed by II and finally III, both total patients, as confirmed.

The distribution of patients showed that type I are the most frequent, followed by type II and type III. Similar distributions in relation to the prevalence of different groups classification, have been reported extensively in the literature, which Cuba exhibits similar to the rest of the world in terms of the frequency of each type of SMA behavior. Because of its severity, patients with SMA type I have a short life expectancy of about one year of age in patients with type I (Chung et al., 2004; Oskoui et al., 2007; Finkel et

al. 2014), whereas type II 75-93% of patients survive more than 20 years of age (Zerres et al., 1997; Farrar et al, 2017) and life expectancy for type III is believed to be close of the normal population (Pearn, 1978, Lung and Wang, 2008).

#### *Distribution by skin color*

The composition of the sample selected by clinical diagnosis is given by 60 white mongrel, 15 and 3 black. Confirmed patients by molecular study 77.6% were whites, followed by mongrel with 17.2% and finally blacks with only 5.2% (Figure 4). The association between skin color and the presence or absence of deletion had no significant differences so that the skin color is not a determining factor being the bearer deletion.



**Fig. 4.** Percentage of cases with deletion according skin color.

The analysis of the relative frequency of patients, both clinically diagnosed as confirmed molecularly, for each group of skin color showed that SMA is distributed in the Cuban population so that the most represented are white, followed by mongrel and last black.

In Figure 5 the number of cases are represented by regions of greater proportion and plotted with darker, the intermediate ratio to the less dark and the low ratio is the lighter shade. Most confirmed by molecular studies possess the provinces of Pinar del Rio, Havana and Matanzas cases, while Isla de la Juventud, Granma and Holguin are that fewer patients are observed deleted.

The provincial distribution of all cases with clinical diagnosis by type of SMA showed that type I, in which most patients were observed are Pinar del Rio and Havana, for type II Havana and Mayabeque and type III, Havana again, being this province which has more patients than three types of SMA (Fig. 6). The provinces are less frequently Las Tunas and Granma, with two cases of SMA type I, respectively.



Fig. 5. Number of cases and proportion by provinces.

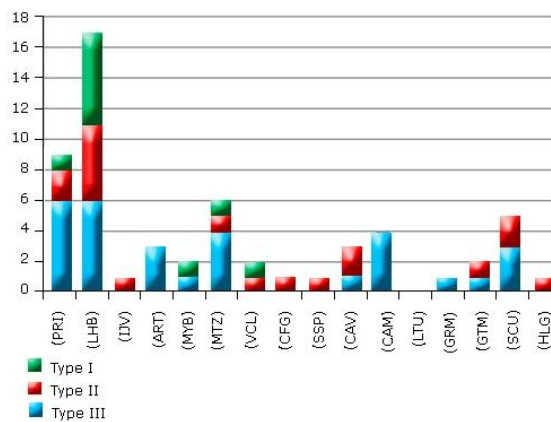


Fig. 6. Number of patients with deletion by province depending on the type of SMA. the provinces of the western region stand to present more patients of the three types, especially Havana.  
 Legend: PRI: Pinar del Rio, LHB: Havana, IJV: Isla de la Juventud, ART: Artemis, MYB: Mayabeque, MTZ: Matanzas, VCL: Villa Clara, CFG: Cienfuegos, SSP: Sancti Spiritus, CAV: Ciego de Ávila, CAM: Camagüey, LTU: Las Tunas, GRM: Granma, GTM: Guantánamo, SCU: Santiago de Cuba, HLG: Holguín.

## DISCUSSION

The results of molecular studies deletion in SMA patients showed that 74.64% of the sample had homozygous deletion of exon 7 of SMN1, a result confirming accurately clinical diagnosis of Spinal Muscular Atrophy; however, this percentage does not match that reported by Lefebvre et al (1995), who suggested that about 95% of patients have deletion. There are several reasons that may cause the difference in the percentages can be attributed to improper clinical diagnosis, the presence of other point mutations or SMA concerned autosomal dominant locus which is located outside the region 5q.

The difference showing the percentages of deletion in Cuban patients than that reported has great significance regarding the potential administration of any of the approved therapies because it is necessary to know the origin and cause of the disease. This is because the therapies recently approved are aimed mainly towards the restoration and

inclusion of exon 7 in mRNA of SMN gene, and thus increase levels of expression of complete and functional proteins, thus, a patient SMA this exon deletion and intragenic mutations that generate dysfunctional proteins will not respond equally to such therapy.

It is known that Spinal Muscular Atrophy is second most common genetic cause of infant mortality, with a reported incidence worldwide between 1 / 6,000 and 1 / 10,000 births and a rate of between 1/35 and 1/50 carriers. Until today there are countless reviews and publications referencing and reporting the values of different variables related to SMA such as the incidence and frequency of the number of copies of the SMN gene by ethnic group, gender and age.

Besides, several researchers have also conducted studies of correlation between several variables reporting significant results lead us to have a notion of how to understand the phenomenon of a disease that affects so many people worldwide and so tragic for individuals who suffer consequences. These studies represent the previous and the knowledge base we intend to employ to gain an understanding of how they might be behaving this phenomenon in our country, since the characteristics of our population the few studies that have been conducted, such as Zaldívar et al in 2005 reported that Cuba frequencies do not behave in the SMA way as in the rest of the world. That is why we have proposed a study of genotyping in order to corroborate that assertion and update information about this phenomenon.

Additionally provide valuable information will enable interdisciplinary groups with a view to implementing therapeutic clinical trials, as well as improving large amount of genetic counseling to carriers of SMN gene mutated in order to have successful pregnancies. And thus provide long-term significant improvement in survival and quality of life of these patients.

## **CONCLUSIONS**

- We characterized molecularly the SMN genes in terms of presence or absence of SMN1 and SMN2 genes of Cuban patients with SMA
- We established the association of different genotypes found with the main variables under study showing that most affected patients were white and children.



- We determined the differences between groups regarding the genotype-changing association study

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