

The Contribution of the PCR-Enzymatic Digestion in the Positive Diagnosis of Proximal Spinal Muscular Atrophy in the Moroccan Population

H. Merhni, A. Sbiti, I. Ratbi, A. Sefiani

Abstract—The proximal spinal muscular atrophy (SMA) is a group of neuromuscular disorders characterized by progressive muscle weakness due to the degeneration and loss of anterior motor neurons of the spinal cord. Depending on the age of onset of symptoms and their evolution, four types of SMA, varying in severity, result in a mutations of the *SMN* gene (survival of Motor neuron). We have analyzed the DNA of 295 patients referred to our genetic counseling; since January 1996 until October 2014; for suspected SMA. The homozygous deletion of exon 7 of the *SMN* gene was found in 133 patients; of which, 40.6% were born to consanguineous parents. In countries like Morocco, where the frequency of heterozygotes for SMA is high, genetic testing should be offered as first-line and, after careful clinical assessment, especially in newborns and infants with congenital hypotonia unexplained and prognosis compromise. The molecular diagnosis of SMA allows a quick and certainly diagnosis, provide adequate genetic counseling for families at risk and suggest, for couples who want prenatal diagnosis. The analysis of the *SMN* gene is a perfect example of genetic testing with an excellent cost/benefit ratio that can be of great interest in public health, especially in low-income countries. We emphasize in this work for the benefit of the generalization of molecular diagnosis of SMA by the technique of PCR-enzymatic digestion in other centers in Morocco.

Keywords—Exon7, PCR-digestion, SMA, *SMN* gene.

I. INTRODUCTION

SPINAL muscular atrophy (SMA) is one of the most frequent autosomal recessive diseases which is due to a degeneration of motor neurons in the anterior horn of the spinal cord, and as a consequence a skeletal muscle atrophy and generalized. The incidence of SMA is approximately 1/6,000 to 1/10,000 live births [1].

SMA is caused by mutations of the *SMN* gene located in 5q13.2, which encodes a protein involved in the survival of motor neurons. It is subdivided into four groups of decreasing severity ranging from type 1 to type four or adult SMA. The *SMN* gene exists in two copies: *SMN1* and *SMN2*, which differ by only five nucleotides. In about 95% of cases, SMA is due to a homozygous deletion of exon7 of the telomeric. A gene

conversion of the telomeric copy to the centromeric was also described [1].

On 5q13.2 locus is located *NAIP* gene, wich copies number is directly correlated with the age of onset of clinical signs and prognosis. It follows that the severity of the SMA phenotype is inversely proportional to the copy number of the modifying genes, mainly *SMN2* and *NAIP* [1].

SMA is estimated to be the second most common autosomal recessive disease with an overall incidence of around 1 in 10,000 live births and a carrier frequency that may be as high as 1 in 35 [2].

II. PATIENTS AND METHODS

This is a retrospective and prospective study over a period of 18 years from January 1996 to October 2014. We included in our study 133 patients from 295, in whom the diagnosis of SMA was suspected. The patients came from different regions of the kingdom. The pre-established genetic files of the patients were exploited to specify the clinico-biological and neurophysiological characteristics of each patient according to a data collection grid.

An informed consent was obtained from all families to carry out the molecular study. A sampling of 3 ml peripheral blood of each patient on EDTA tube (ethylene diamine tetra acetic acid) was carried out. An oral smear was done especially in newborns. Each sample was recorded and coded according to the processes in the laboratory and then stored at +4°C or -20°C until further use. DNA was extracted from peripheral blood lymphocytes with one of two methods: QIAamp DNA mini kit (Qiagen, GmbH, Germany) or the inorganic solvent (NaCl). The molecular diagnosis of *SMN* gene deletions was carried out by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) [3].

The telomeric and centromeric copies in exon 7 of *SMN* gene differ from each other by single base changes that can be identified by selective restriction enzyme digestion of DNA (Fig. 1).

To distinguish the two copies of the *SMN* gene, an amplification of the exon 7 is carried out by Polymerase Chain Reaction method and followed by an enzymatic digestion with the *DRA1* restriction enzyme. Then, the presence of ethidium bromide in NuSieve-Agarose gel provides to visualize the two distinct copies after electrophoresis [5].

The electrophoretic profile of a normal subject corresponds to the presence of two bands: An undigested heavy fragment,

H. Merhni and I. Ratbi are with the Human Genomics Center, Faculty of Medicine and Pharmacy, Department of Medical Genetics, National Institute of Health, Rabat, Morocco (e-mail: h_merhni89@hotmail.com, i.ratbi@um5s.net.ma).

A. Sbiti is with the Department of Medical Genetics, National Institute of Health, Rabat, Morocco (e-mail: aziza_sbiti@yahoo.fr).

A. Sefiani is with the Human Genomics Center, Faculty of Medicine and Pharmacy, Rabat, Morocco (sefianigen@hotmail.com).

which corresponds to *SMN1*, and a shorter digested fragment corresponding to *SMN2*, which migrates further. In all patients of our cohort, PCR-digestion revealed the homozygous deletion of exon 7 of the *SMN1* gene by the absence of the signal at the site of migration of the telomeric copy (Fig. 2).

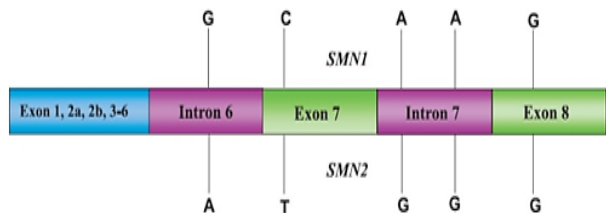


Fig. 1 Nucleotide differences in telomeric (*SMN1*) and centromeric (*SMN2*) copies of *SMN* gene [4]

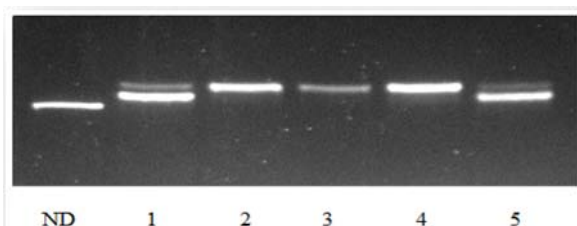


Fig. 2 Electrophoretic profile of *SMN1* and *SMN2* copies after PCR-digestion by *DraI*. ND=Not digested; 1=Normal control; Patients 2, 3 and 4=Deleted; Patient5=Normal

III. RESULTS

There were 133 unrelated probands with a sex ratio of 1.04 (all clinical forms combined) (Fig. 3). Consanguinity was found in 54 patients, namely 40.60% (Fig. 4). The graph of Fig. 5 shows the distribution of patients by age according to the classification of the international consortium. Their age varies from the neonatal period to the age of 40 years. The age group of 18 months to 21 years was the most represented. A clinical heterogeneity was observed in our cohort. The first sign in the majority of our patients was congenital hypotonia and motor regression, followed by motor delay. Severe respiratory distress was present in 04 infants with SMA type1 (Fig. 6). The notion of decreased fetal active movements since intrauterine life, difficult to evaluate during interrogation, was only reported by the mothers of 03 infants. The four degrees of SMA severity described according to the classification of the international consortium were found in our cohort with a variation of sex ratio between different types (Fig. 7).

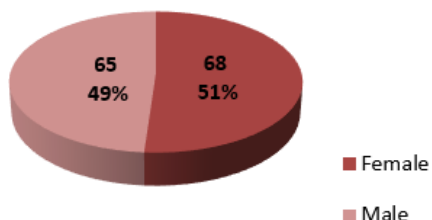


Fig. 3 Distribution of our cohort according to gender

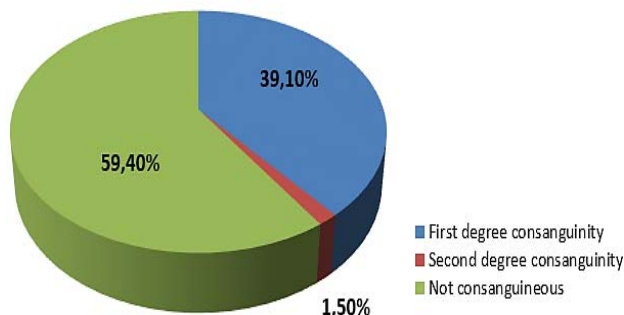


Fig. 4 Types and distribution of consanguinity

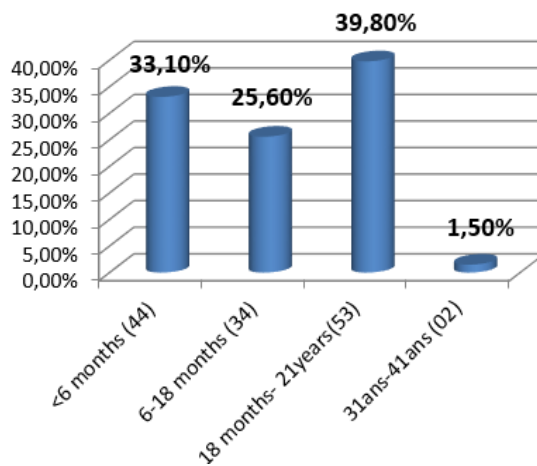


Fig. 5 Distribution of our patients by age of onset

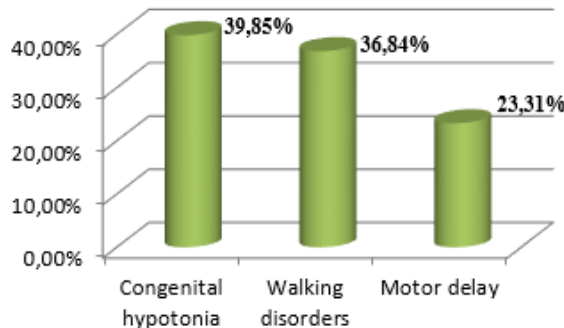


Fig. 6 Clinical features in our cohort

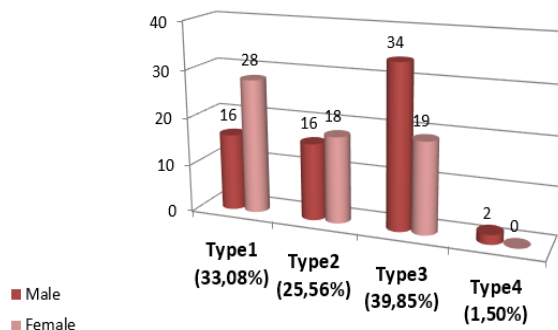


Fig. 7 Distribution of patients according to the type of SMA and gender

IV. DISCUSSION

This study focused on 295 patients referred to our department for suspicion of proximal SMA. The homozygous deletion of exon 7 of the *SMN2* gene was identified in 133 patients, or 45.1% (Fig. 8). SMA is among the relatively common genetic pathologies in children and has an estimated prevalence of 1 in 10,000 in Caucasians. In Morocco, because of the high rate of consanguineous marriages (15.25%), the frequency of this pathology is probably higher than European populations [6], [7].

Very few epidemiological studies on SMA have been carried out. Using a molecular epidemiology approach, Lyahyai et al. Have estimated that the frequency of heterozygotes corrected by the inbreeding rate would be 1/25. Assuming that our population is in Hardy-Weinberg equilibrium, we would estimate that the calculated prevalence of SMA in Morocco is 1/2,500. These results show that the frequency of heterozygotes for SMA in Morocco is higher than that estimated in European populations (from 1/50 to 1/80). The frequency of heterozygotes in other countries varies according to population [8], [9] (Table I).

TABLE I
COMPARISON OF THE FREQUENCY OF HETEROZYGOUS FOR EXON 7 DELETION OF *SMN* GENE IN DIFFERENT POPULATIONS

Population	Number of individuals studied	Frequency of heterozygous %	References
USA Hispanic	1,030	0.8% (1/129)	Hendrickson et al. [10]
USA Afro-Americans	1,015	1.1%(1/92)	Hendrickson et al. [10]
USA Asian	1,027	1.8%(1/57)	Hendrickson et al. [10]
Korea	100	2%(1/50)	Seoyoung Yoon et al. [11]
USA Jewish-Ashkenazis	1,002	2.2%(1/64)	Hendrickson et al. [10]
China	1,712	2.39%(1/42)	Zhu Sheng Yuan et al. [12]
Brazilia	150	2.6%(1/38)	Bueno et al. [13]
USA Caucasians	1,028	2.7%(1/37)	Seoyoung Yoon et al. [11]
Germany	140	2.85%(1/35)	Thieme et al. [14]
Ukraine	370	3.23%(1/31)	O. Soloviov et al. [15]
Morocco	150	4%(1/25)	Lyahyai et al. [9]
KSA	ND	5%(1/20)	Hasanzad et al. [16]
Iran	200	5%(1/20)	Hasanzad et al. [16]

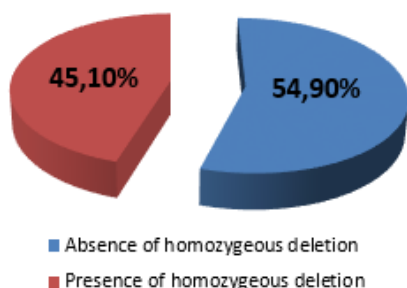


Fig. 8 Frequency of homozygous deletion of the exon7 of the *SMN* gene among the 295 patients

With this high prevalence of SMA in our population, a national health strategy must be followed to allow positive diagnosis, a heterozygous screening and an appropriate genetic counseling to the families. The rate of consanguinity in our cohort is close to 40.6%, therefore, the latter, as in all recessive diseases, is an important factor in the emergence of SMA. This rate is however close to that found in other series especially in the Middle East and Asia (Table II). The predominance of type III in our cohort has also been reported in the Tunisian population in a series of 33 patients all with SMA type III [17]. A predominance of type I was noted in other series: in Pakistan 37/67 patients and in Spain 26/37 patients [18], [19]. From these observations, two hypotheses could be raised:

- ❖ In the Maghreb population, and because of the clinical severity of SMA type I and less type II, few patients survive and are diagnosed. In type III and IV, life expectancy is not threatened and respiratory distress is not classical [9].
- ❖ The impact of the number of cDNA copies, modifying genes and extent of deletion that modulate the severity of SMA clinical expression including type I [20].

TABLE II
CONSANGUINITY RATE IN DIFFERENT POPULATIONS

Population	Consanguinity rate	References
KSA	64%	Al-Rajeh et al. [22]
Pakistan	68%	Ibrahim et al. [23]
Iran	65%	Salahshourifar et al. [24]
Oman	49%	Koul et al. [25]
Egypt	46%	Shawky et al. [26]
Morocco	15,25%	Cherkaoui et al. [8]

Out of 295 suspicions of SMA, who were referred to our department during the period between 1996 and 2014, this diagnosis was confirmed in 133 of them. In this study, it was demonstrated that the percentage of the homozygous deletion of the exon7 of the *SMN* gene in the Moroccan population is 45.1% of all types of SMA combined (Fig. 8). Similar results to ours were reported in Tunisia (45.4%) but this sample was represented only by patients with SMA type III; and also in Egypt (54.5% -80%) [17], [21].

These results indicate a geographical distribution in relation to ethnic origins. Severe forms of SMA may cause death of the newborn in the first few weeks due to a respiratory distress, even before a diagnosis is established.

V. CONCLUSION

In this study, we have shown that by a simple and low-cost genetic test, we can confirm the diagnosis of SMA in 45.1% of patients with clinical suspicion of SMA. Even in the absence of any paraclinical explorations that could guide the diagnostic procedure, such as electromyography and the serum level of muscle enzymes, molecular biology is a very useful tool for rapid diagnostic confirmation and appropriate therapeutic management, especially since the advent of targeted therapy.

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