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MLPA analysis for molecular diagnosis of spinal muscular atrophy and correlation of 5q13.2 genes with disease phenotype in Egyptian patients

Heba A. Hassan[1*], Nagia A. Fahmy[3], Nagham M. El-Bagoury[1], Noura R. Eissa[1], Wessam E. Sharaf-Eldin[1], Mahmoud Y. Issa[2], Maha S. Zaki[2] and Mona L. Essawi[1]

Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease representing the most prevalent monogenic cause of infant mortality. It results from the loss of *SMN1* gene, but retention of its paralog *SMN2* whose copy number can modulate the disease severity and guide the therapeutic regimen.

Methods: For SMA molecular analysis, 236 unrelated Egyptian patients were enrolled at our institution. The Multiplex ligation-dependent probe amplification analysis (MLPA) was applied to investigate the main genetic defect in the enrolled patients (*SMN1* loss) and to determine a possible genotype–phenotype correlation between the copy number of other genes in the *SMN* locus (5q13.2) and disease severity in Egyptian patients with SMA. A small cohort of healthy subjects ($n=57$) was also included to investigate the possible differences in the distributions of *SMN2* and *NAIP* genes between patients and healthy individuals.

Results: Disease diagnosis was confirmed in only 148 patients (62.7%) highlighting the clinical overlapping of the disease and emphasizing the importance of molecular diagnosis. In patients with homozygous *SMN1* loss, the disease was mediated by gene deletion and conversion in 135 (91.2%) and 13 (8.8%) patients, respectively. In the study cohort, *SMN2* and *NAIP* copy numbers were inversely correlated with disease severity. However, no significant association was detected between *GTF2H2A* and *SERF1B* copy numbers and patient phenotype. Significant differences were demonstrated in the copy numbers of *SMN2* and *NAIP* between SMA patients and healthy subjects.

Conclusion: Molecular analysis of SMA is essential for disease diagnosis. Consistent with previous studies on other populations, there is a close relationship between *SMN2* and *NAIP* copy numbers and clinical phenotype. Additionally, potential differences in these two genes distributions are existing between patients and healthy subjects. National program for carrier screening should be established as a preventive disease strategy. On the other hand, neonatal testing would provide accurate estimation for disease incidence.

Keywords: Spinal muscular atrophy, SMN1, SMN2, NAIP, MLPA, SMA, Copy number variations

Introduction

Spinal muscular atrophy (SMA) is a major genetic cause of infant mortality worldwide [1]. It is characterized by degeneration of α-motor neurons in the anterior horn of the spinal cord, i.e., lower motor neurons, leading to progressive muscle weakness and atrophy [2]. The incidence

*Correspondence: heba.amin@yahoo.com; Heba.aminh@gmail.com; ha.abdel-hameed@nrc.sci.eg

[1] Department of Medical Molecular Genetics, Human Genetics and Genome Research Institute, National Research Centre, 33 El-Bohouth Street, Cairo 12311, Egypt
Full list of author information is available at the end of the article

of SMA is 1 in 6000–10,000 live births. In the Middle East, SMA incidence is up to 40-fold higher than the Western world due to the increased rate of consanguineous marriages [3]. In the Egyptian population, consanguinity was reported in about 50% of SMA patients [4]. On the other hand, SMA has a carrier frequency of 1in 25–50 in most populations, with lower rate in some ethnicities. For example, the frequency of SMA carriers in the Chinese population was estimated as low as about 3% only [5]. However, the carrier rate in the Middle East seems to be relatively higher (1 in 20) [3].

Disease symptoms vary greatly among different patients. In general, the disease is classified into four types according to the age of onset and the ultimately gained motor function. Type 1 (infantile SMA/Werdnig-Hoffmann disease, OMIM #253300): Symptoms begin within the first six months of life. Affected infants are unable to sit without support, and have trouble breathing, feeding, and swallowing. They often pass away by the age of 2 years. Type 2 (intermediate SMA/Dubowitz disease, OMIM #253500): Manifestations start between 6 and 18 months of age. Children with this type cannot walk independently. They might survive into adulthood by virtue of improved standards of healthcare. Type 3 (juvenile SMA/Kugelberg-Welander disease, OMIM #253400): Symptoms usually appear around 18 months of age or in early childhood. These patients have walking difficulties and might eventually require the use of wheelchairs. They generally have an almost normal life span. Type 4 (late SMA, OMIM #271150): It is a very rare type that usually starts in young adulthood resulting in mild motor impairment [6]. Some classifications tend to categorize patients with significant muscle weakness and respiratory distress at birth into a distinct type designated as SMA type 0/prenatal SMA. Indeed, such infants presented with reduced fetal movements in utero and they rapidly progress to respiratory failure often by the first month of life [7].

SMA is caused by homozygous mutations in the *SMN1* gene (survival motor neuron 1, OMIM #600354), at the 5q13.2 locus, where SMN protein produced at low insufficient levels. In more than 95% of cases, the disease results from the loss of *SMN1* gene. However, intragenic gene mutations, including missense, nonsense, frameshift and splice-site variations, account for the remaining 5% of cases [6, 8]. The *SMN1* is located within a genomic segment of inverted duplication. This segment is unique to human lineages and contains 4 main genes: *SMN1* (survival motor neuron 1), *NAIP* (neuronal apoptosis inhibitor protein, OMIM#606831), *GTF2H2A* (general transcription factor IIH, *p44,* OMIM#601748) and *SERF1A* (small EDRK-rich factor 1A, *H4F5A,* OMIM# 603011). The duplicated genes are either identical to their partner gene (*SERF1B*), differ by a small number of nucleotides but still produce some functional protein molecules (*SMN2*, OMIM#601627) or are pseudogenes (*ΨGTF2H2B* and *ΨNAIPΔ5*) [9]. Importantly, numerous studies have elaborated that *SMN2*, *NAIP*, *GTF2H2A* and *SERF1A* can affect SMA severity by certain degrees, where *SMN2* is the primary disease-modifying gene [10].

The major clinically relevant difference between *SMN1* and *SMN2* is the C-to-T transition (c.850C > T) in exon 7. This position is located in the middle of the exonic splicing enhancer (ESE) sequence affecting the inclusion of exon 7 in SMN transcripts and leads to exon exclusion (SMNΔ7) from the majority (~ 90%) of SMN2-derived mRNAs. This negatively affects protein stability and self-oligomerization [11]. Noteworthy, when exon 8 of *SMN1* is retained in SMA patients, *SMN2* exon 7 recombines with *SMN1* exon 8, forming a hybrid *SMN* gene. This phenomenon is known as gene conversion representing one of the mechanisms that accounts for increased *SMN2* copy number and associated decreased disease severity [12].

Three gene-targeting SMN replacement therapies; nusinersen (Spinraza; Biogen), onasemnogene abeparvovec (Zolgensma; Novartis), and risdiplam (Evrysdi; Roche) have been currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Onasemnogene abeparvovec replaces the full-length SMN mRNA and protein, while nusinersin and risdiplam act by increasing exon 7 inclusion in SMN2 transcripts emphasizing the need for *SMN2* copy number investigation to instruct therapeutic plans [13].

Multiplex ligation–dependent probe amplification (MLPA) analysis greatly improves SMA diagnostics. Single reaction can simply and rapidly detect the main genetic defect of SMA, evaluate the *SMN2* copy number and determine the extent of deletion in the 5q13.2 region [14]. In the current study, MLPA was applied for molecular analysis of Egyptian patients with provisional diagnosis of SMA, and to investigate a possible genotype–phenotype correlation between the copy numbers of other 5q13.2 genes and disease severity in patients with confirmed diagnosis. A small cohort of healthy subjects was also analyzed to assess the possible differences in *SMN2* and *NAIP* distributions between subjects with and without SMA.

Subjects and methods

Subjects

From January 2017 to December 2020, 236 unrelated Egyptian patients were recruited from Clinical Genetics Department at National Research Centre (NCR) and Neuromuscular Unit at Faculty of Medicine—Ain Shams University for molecular diagnosis of SMA. The study

also included 57 healthy individuals. For patient selection and clinical classification, the International Spinal Muscular Atrophy Consortium criteria were applied. These criteria include hypotonia, which is typically symmetrical, more proximal than distal, and with preserved sensation, as well as EMG, which indicates a motor neuron disease [15].

Three milliliters of peripheral blood were withdrawn from all studied subjects on 0.5 M EDTA tubes.

Molecular analysis

DNA was extracted from peripheral blood leukocytes using PAXgene DNA blood extraction kit (Qiagen, Germany); according to the manufacturer's protocol. The MLPA assay was performed using one of two SALSA MLPA Probemixes; P021-A2 or P021-B1 (MRC-Holland, Amsterdam, Netherlands) as instructed by the manufacturer. Both probemixes include four probes specific for sequences in exon 7 or 8 of either *SMN1* or *SMN2* genes. In addition, the 2 probemixes contain some probes detecting sequences that are present in both *SMN1* and *SMN2*. The probemix P021-A2 contains 4 general probes for both *SMN* genes; one for exons 1, 4, 6 & 8. However, the probemix P021-B1 holds 17 general probes; at least one for exons 1, 2a, 2b, 3, 4, 5, 6 & 8 and introns 6 & 7 with one additional probe for exons 1, 2b, 3 & 8 and three additional ones for intron 7. On the other hand, they contain one probe specific for exon 5 of *NAIP* which is absent in *NAIPΨ*. Furthermore, P021-A2 probemix also includes one probe for exon 13 of both *NAIP* and *NAIPΨ* genes, one probe for exon1 of *SERF1B* gene and three probes for exons 4, 7 and 10 of *GTF2H2* genes. The MLPA products were analyzed using ABI 3500 genetic analyzer (Applied Biosystems), with GeneScan™ 500 LIZ™ Dye Size Standard (ThermoFisher Scientific, UK). Data analysis and interpretation were done by Coffyalyser.Net software (www.mlpa.com).

Statistical analysis

Statistical analyses were performed using the statistical package for the social sciences (SPSS Statistics for Windows, Version 25.0; IBM Corp., Armonk, NY, USA). Qualitative variables were reported as the number of cases (percentage) and compared using the Pearson's chi-square ($\chi 2$) test. Quantitative variables were expressed as mean ± standard deviation (SD) and compared using one-way analysis of variance (ANOVA). The correlation between different types of SMA and copy numbers of various modifier genes was assessed using Spearman's rank correlation coefficient. A two-sided probability (*P*) value was used for all statistical analyses and a *P* value of < 0.05 was considered statistically significant.

Results

The study included 236 patients with provisional diagnosis of SMA (132 males and 104 females) whose age ranged from 2 months to 55 years. According to their age of onset, they were classified as 32 (13.6%) type 1, 78 (33.1%) type 2, 114 (48.3%) type 3 and 12 (5.1%) type 4 (Table 1). MLPA analysis revealed homozygous deletions of *SMN1* in 148 patients (62.7%). Among them, 13/148 patients (8.8%) demonstrated the absence of exon 7 only, while exon 8 is retained indicating the occurrence of gene conversion in those patients. While, normal *SMN1* copy numbers, ranging from 2 to 4 copies, were indicated in 74 patients (31.6%). The remaining 9 patients (3.8%) had heterozygous deletion (1 copy) of *SMN1*. Among these patients, one patient showed heterozygous deletion of exons 1 to 6 of either *SMN1* or *SMN2* (Fig. 2A). Thus, there would be 4 expected genotypes for this patient, where only one would confirm SMA diagnosis (Fig. 2B).

Patients with homozygous gene deletions ($n = 148$) were classified into 25 (16.9%) type 1, 52 (35.1%) type 2, 69 (46.6%) type 3 and 2 (1.4%) type 4. They had *SMN2* copies ranging from 2 to 6, with no heterozygous or homozygous SMN2 deletions (Table 2 & Fig. 1). Using

Table 1 Number and gender of the studied patients (n = 236) and the patients with confirmed molecular diagnosis (n = 148) in the four clinical SMA types

	All studied patients			SMA patients with homozygous deletion of *SMN1* gene		
	Number	Gender		Number	Gender	
		Female	Male		Female	Male
SMA type 1	32 (13.6%)	15	17	25 (16.9%)	13	12
SMA type 2	78 (33.1%)	37	41	52 (35.1%)	26	26
SMA type 3	114 (48.3%)	47	67	69 (46.6%)	25	44
SMA type 4	12 (5.1%)	5	7	2 (1.4%)	1	1
Total	236 (100%)	104 (44.1%)	132 (55.9%)	148 (100%)	65 (43.9%)	83 (56.1%)

Table 2 Copy number variations of *SMN2* and *NAIP* genes in controls (n = 57) and SMA patients (n = 148) with homozygous deletion of exon 7 of *SMN1* gene

Modifier gene	Copy no.	Patients (n = 148)	Controls (n = 57)	*P* value
SMN2 exon 7	0		6 (10.5%)	< 0.001*
	1		11 (19.3%)	
	2	34 (23.0%)	24 (42.1%)	
	3	38 (25.7%)	10 (17.5%)	
	4	68 (45.9%)	5 (8.8%)	
	5	3 (2.0%)	1 (1.8%)	
	6	5 (3.4%)		
SMN2 exon 8	0		5 (8.8%)	< 0.001*
	1	1 (0.7%)	9 (15.8%)	
	2	42 (28.4%)	22 (38.6%)	
	3	46 (31.1%)	20 (35.1%)	
	4	53 (35.8%)	1 (1.8%)	
	5	3 (2.0%)		
	6	3 (2.0%)		
NAIP exon 5	0	49 (33.1%)	1 (1.8%)	< 0.001*
	1	51 (34.5%)	4 (7.0%)	
	2	47 (31.8%)	36 (63.2%)	
	3	1 (0.7%)	16 (28.1%)	

Spearman correlation test, an inverse significant correlation was observed between phenotype severity and *SMN2* copy number in terms of both exons 7 & 8 (Additional file 1: Table 2). On the other hand, the distribution of *NAIP* copy number in patients with homozygous *SMN1* deletions were as follows: 49 showed homozygous deletion (33.1%), 51 revealed heterozygous deletion (34.5%), and 47 had 2 copies (31.7%). Only one type III patient (0.7%) had 3 copies (Table 2 & Fig. 1). A significant inverse correlation was also demonstrated between *NAIP* copy number and the disease severity ($r = 0.377$, $p = < 0.001$) (Additional file 1: Table 2). Furthermore, the copy numbers of *GTF2H2* and *SERF1B* were estimated in 63 patients with homozygously deleted *SMN1* identified using SALSA MLPA Probemix P021-A2. However, no significant association was detected between both genes copy numbers and the clinical severity (Additional file 1: Table 1). Simultaneous deletion of all 5q13.2 main genes was not detected in any patient of the studied cohort.

Most of the healthy subjects ($n = 30$) typically had 2 copies of *SMN1* exon 7 (52.6%). Only one copy was detected in 18 subjects (31.6%). Other subjects ($n = 9$) hold 3 or 4 copies (15.8%). The distribution of *SMN2* exon 7 copy numbers among healthy subjects was as follows: 0 copies in 6 individuals (10.5%), 1 copy in 11 (19.4%), 2 copies in 24 (42.1%), 3 copies in 10 (17.5%) and 4 copies in 6 (10.5%). On the other hand, the majority of healthy subjects (52; 92.9%) possessed 2 or 3 copies of *NAIP*, where only 4 subjects had homozygous ($n = 1$) or heterozygous deletion ($n = 4$) of *NAIP* (7%). Interestingly, statistically significant difference was revealed in the copy numbers of *SMN2* and *NAIP* between patients and healthy subjects ($P < 0.001$) (Table 2).

Discussion

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder. Survival Motor Neuron 1 gene (*SMN1*) located on 5q13 (SMA locus) is the main disease associated gene. The majority of SMA cases (94%) results from complete or partial deletion of *SMN1* gene. The most common partial deletion encompasses exons 7 and 8 (*SMN1Δ78*). However, other partial deletions have been identified, including exon 7 only (*SMN1Δ7*), exons 5 and 6 (*SMN1Δ56*), exons 2a through 5 (*SMN1Δ2a5*) and exons 1 through 6 (*SMNΔ16*) and exon 8 (*SMN1Δ8*). Even though exon 8 is downstream of the protein-encoding region, it may affect mRNA stability as well as post-transcriptional gene regulation [9]. Moreover, the launching of SMA genetic therapy targeting SMN2 gene increases the demand on molecular testing of both genes (SMN1 & SMN2). Variable methods have been used as PCR-RFLP, quantitative PCR (qPCR), multiple ligation probe amplification (MLPA) and multiplex droplet digital PCR (ddPCR). PCR-RFLP technique has limited sensitivity and reproducibility. qPCR requires normalization with standard curves. Whereas, MLPA is a comprehensive single reaction test for detecting CNVs in several SMA-related genes fragments. It can consistently distinguish between CNVs of SMN1 and SMN2 accurately. However, MLPA is a multi-step relatively time-consuming technique. Recently, ddPCR has overcome the pitfalls of MLPA, but it is a high-cost method that requires a dedicated instrument platform [16].

Herein, 236 patients were studied, the most prevalent SMA type was type III, followed by type II, then type I, where type IV was much lower. However, SMA type I is the most common type, these patients have high mortality rate. In other words, although they have the highest incidence rate; they have the lowest prevalence frequency [17]. Its relatively low predominance among the study cohort would reflect the high mortality rate of these patients, and emphasizing the significance of neonatal screening of SMA, to conquer the missed diagnosis of SMA at that early age. In this study, MLPA assay confirmed SMA diagnosis in 148 patients (62.7%). In two previous studies on Egyptian patients with provisional SMA diagnosis, homozygous absence of exon 7 of SMN1 gene was found in 54.5% and 80% of patients, respectively [18, 19].

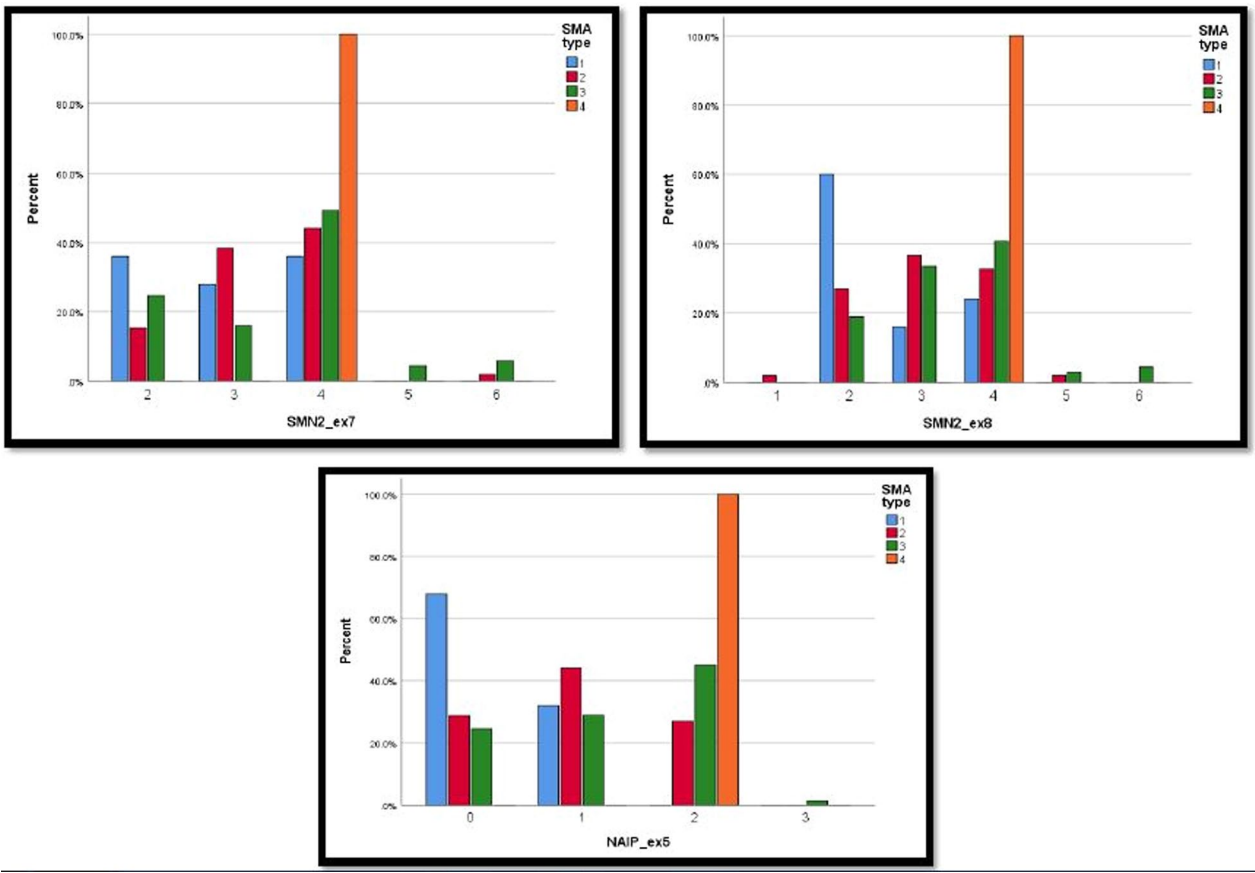

Fig. 1 Bar charts showing Copy number variations of *SMN2* and *NAIP* genes in SMA patients (n = 148) with homozygous deletion of exon 7 of *SMN1* gene

About 5% of SMA patients harbor compound heterozygous mutations of SMN1 gene [20, 21]. In our cohort, 3.8% (9/236) of patients had heterozygous deletions. Importantly, one of these patients showed heterozygous deletion of exons 1 to 6. Where the MLPA probemix analyzes the copy numbers of exons 1–6 of both SMN1 and SMN2 genes simultaneously, such result could not assure SMA diagnosis in this patient (Fig. 2b). In most cases, compound heterozygosity results from the deletion of one SMN1 allele and an intragenic mutation within the other allele. More than 100 subtle pathogenic variations have been identified in the SMN1 gene [9]. However, due to the great homology between SMN1 and SMN2, combination of long-range PCR (LR-PCR) and nested PCR should be considered in the analysis of SMN1 intragenic mutations [22].

Patients with two or more copies of exon7 of the SMN1 gene may have causative pathogenic mutations in non-5q genes. This group accounts for about 6% of all SMA patients where, at least 16 different genes have been found to be associated with these non-5q forms with considerable phenotypic variability and diverse inheritance patterns [23]. Recently, SMN1 deletions or mutations in suspected SMA patients are detected in about 50% of them only. This could be due to clinical overlapping with other neuromuscular disorders, e.g., hereditary motor neuropathies (HMN), myasthenic syndromes [24]. Whole exome sequencing (WES) for this group of patients would be the best choice for detecting the causative gene.

Homozygous deletion of *SMN1* exon 7 with the retaining of *SMN1* exon 8 is known as hybrid SMN gene or gene conversion [12, 25]. It was reported that the frequencies of hybrid SMN gene in SMA patients vary from 5 to 30% among different ethnic groups [12]. Combining data from the current study and other previous research [19], it has been determined that the frequency of the hybrid SMN gene in Egyptian patients seems to be between low (< 10%) and medium (10–20%) frequencies, with a total of 9.8% (16/164) and only one patient of Type I SMA. Reduced phenotype in case of gene conversion might be justified by the rise of the CNVs of *SMN2*

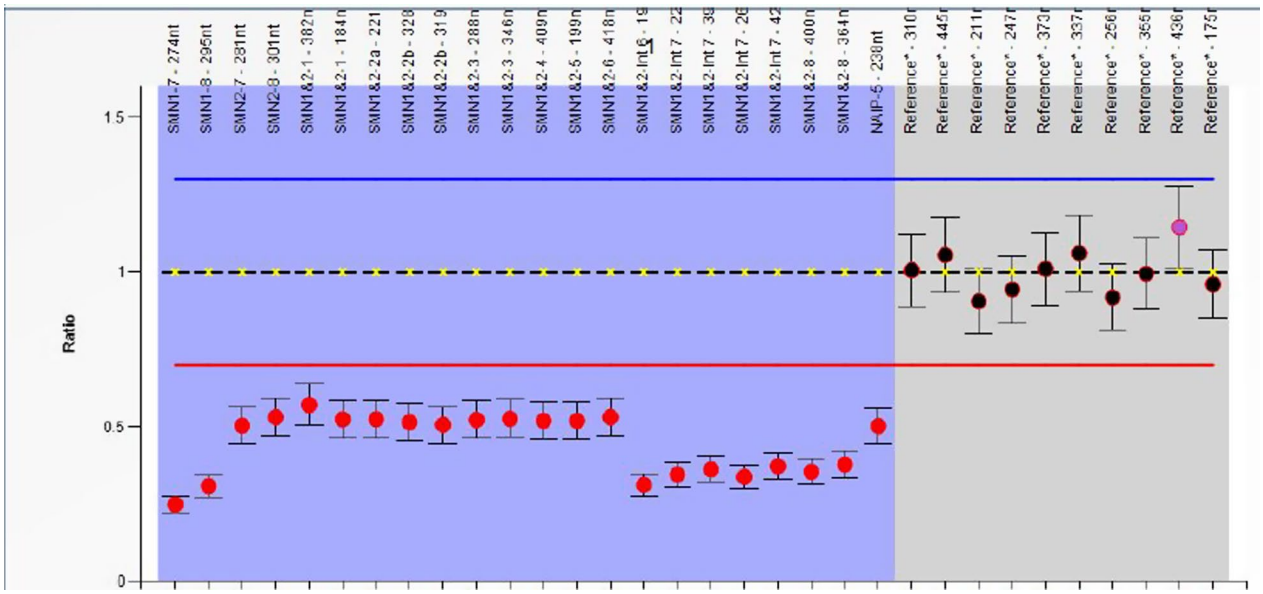

Fig. 2 Analysis of SMA patient with type III showing heterozygous deletion of exons 7 and 8 of *SMN1* gene, and expected heterozygous deletion of exons 1–6 of *SMN1* gene. **A** MLPA analysis showing median value of 0.755 for the 10 probes detecting exon 1–6 of both *SMN1* and *SMN2* genes (3 copies) and median values of exons 7 and 8 of SMN1 gene and SMN2 gene between 0.52–0.63 (1 copy of each). **B** Expected distributions of *SMN1* & *SMN2* genes exons showing (a) heterozygous deletion of the whole SMN1 gene from one allele, which doesn't explain the phenotype, (b) heterozygous deletion of exons 7 & 8 of SMN1 gene from one allele and heterozygous deletion of exons 1–6 from the other allele, which could be pathogenic for SMA., (c &d) heterozygous deletion of exons 1–6 of SMN2 gene

which is considered the most reliable modifier gene. It is a highly homologous paralog of *SMN1* gene acting as a hypomorphic allele [26]. *SMN2* produces non-functioning truncated SMN protein, as exon 7 is mainly missed from its transcript (SMNΔ7). However, approximately 10% of *SMN2* transcripts encodes functioning full-length SMN protein due to alternative splicing. Thus, the higher the number of copies of *SMN2*, the larger the amount of the full length SMN protein produced, and thus the less SMA phenotype severity [27]. Therefore, the majority of gene conversion is associated with less severe phenotypes, as observed in Egyptian SMA patients who were mostly Type II or III.

The majority of SMA patients have 2 copies or more of SMN2 [28] and numerous studies have demonstrated a strong inverse relationship between *SMN2* copy number and clinical severity [13]. In the current study, *SMN2* copy number ranged from 2 to 6 copies in SMA patients and significant inverse correlation in terms of both exons 7 and 8 was estimated between phenotype and CNVs. Interestingly, increased CNVs of *SMN2* have been identified in a number of asymptomatic subjects who had homozygous deletion of *SMN1* [29–31]. It is noteworthy that the copy number variant (CNV) is not the only modifying factor of *SMN2*. Certain structural intronic variants in *SMN2*, such as c.835-44G>A and c.888 + 100A>G can affect exon 7 inclusion in SMN transcripts producing greater amounts of SMN protein and resulting in milder phenotype [32]. Furthermore, *SMN2* is a primary target for the development of therapeutics for SMA. Thereby determination of *SMN2* copy number becomes a crucial criterion for therapeutic application in SMA patients. Hence, analysis of *SMN2* copy number is used not only as a prognostic tool, but also to guide gene therapy based therapeutic strategies. Importantly, the Egyptian National Drug Authority has registered Risdiplam in June 2021 as the first SMA therapy. It has been approved to treat SMA patients starting from age of 2 months, with a once daily dose determined by patient's age and bodyweight [33].

Other genes in the *SMN* gene locus might also affect clinical phenotype. In the current study, simultaneous deletions of *SMN1* and *NAIP* were detected in 49 patients (33.1%). In patients with *type I SMA, all patients had either homozygous (n = 17) or heterozygous* (n = 8) *NAIP* deletion. Several studies have also demonstrated that lower copy numbers of *NAIP* were associated with more severe phenotype [10, 34, 35]. Consistently, we revealed an inversely significant correlation between *NAIP* copy number and disease severity ($p = < 0.001$). On the other hand, large-scale deletions extending to *SERF1* and *GTF2H2* genes are also observed in patients with type I SMA [36]. In the current study, none of *SMN1* deleted patients showed simultaneous deletion of *SERF1* and *GTF2H2*. In the context of these 2 distributions, Amara

et al. [37] reported significant association between SMA disease severity and *SERF1* but not *GTF2H2* in Tunisian patients. *SERF1* is more closely related to *SMN1* on the genomic organization, enhancing its possible frequency for deletion with *SMN1* gene in SMA patients. Conversely, no relationship was observed between the 2 genes and SMA clinical severity in other study cohorts [38, 39]. Here, we also failed to estimate any significant association between *GTF2H2* or *SERF1* copy numbers and SMA phenotype. Generally, weak correlation seems to be existing between these two genes and disease phenotype, so that they have been currently excluded from the prognostic molecular testing.

Among healthy subjects, we identified 18 subjects with a single *SMN1* copy, with a carrier frequency of 31.6%. The great majority of SMA carriers can be identified by the presence of a single copy of *SMN1* exon 7. However, about 5% of SMA carriers have two *SMN1* copies on one chromosome and zero copies on the other [40]. In other words, these so-called silent carriers have a *cis* allelic distribution (2 + 0), rather than a *trans* allelic distribution (1 + 1). Standard assays including MLPA cannot distinguish '1 + 1' from '2 + 0' (silent carriers). On the other hand, differences in the copy numbers of exons 7 and 8 in both *SMN1* ($n = 23$) and *SMN2* ($n = 29$) have been detected in significant proportion of healthy subjects. This would be mainly attributed to the conversions between the 2 genes. Importantly, six possible hybrid *SMN* genes were determined [41]. Although, homozygous and heterozygous deletions of exons 7 and 8 of SMN genes were up to 20% in Caucasian populations [42]. Combined CNVs of *SMN1* and *SMN2* genes were consistent within other populations ranging from 3.6 copies in Asians to 3.8 copies in Africans. The median CNVs of *SMN2* in European and Asian populations were two copies, with ratios of 49.2% and 55.3%, respectively. Whereas, in Africans the median CNVs was 1 copy of *SMN2* (50.3%), while only 24.9% had 2 copies. The median CNVs of *SMN2* in this study was 2 copies of 42.1% [43].

In the current study, the distributions of *SMN2* and *NAIP* were significantly different between SMA patients and healthy individuals ($P < 0.001$) with increased copy numbers in patients. Prior studies among various ethnicities consistently revealed that CNVs of the *SMN2* gene were significantly lower in control subjects. [44, 45]. Additionally, both *SMN2* and *NAIP* genes copy numbers were significantly different ($P < 0.001$) between Chinese SMA patients and healthy subjects [28]. The proportion of SMA patients (33.1%) lacking *NAIP* gene was dramatically higher than that of healthy individuals (1.8%). In fact, *NAIP* gene is the gene that precedes *SMN1*, so that co-deletion may occur identifying a potential cause of direct correlation between CNVs of *SMN1* and *NAIP* genes.

Conclusions

Molecular analysis of SMA is essential to ensure diagnosis and guide therapeutic plans. MLPA is the gold standard of SMA diagnosis. It can detect *SMN1* copy number, investigate the mechanism responsible for SMN1 loss (i.e., deletion or conversion to *SMN2*), evaluate the *SMN2* copy number and determine the extent of deletion in the 5q13.2 region. Improvements in molecular diagnostic tools are associated with enhanced detection rate and accurate estimation for disease incidence. However, other disease causative variations rather than the most common *SMN1* deletion should be studied through either *SMN1* gene sequencing or whole exome sequencing. Neonatal screening would overcome the missed diagnosis of SMA, particularly for type I patients with short life expectancies. Carrier screening should be implemented as a national program in Egypt as a preventive disease strategy.

Supplementary Information

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Additional file 1. Supplementary tables 1 and 2.

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Author contributions

All authors have read and approved the manuscript. HH contributed in molecular studies and writing and revising the article. WS contributed in molecular studies, statistical analysis and writing the article. NRE contributed in molecular studies and writing the article. MZ contributed in clinical diagnosis and assessment of patients. NF contributed in clinical diagnosis and assessment of patients. NME contributed in molecular studies and writing the article. MI contributed in clinical diagnosis and assessment of patients. ME contributed in the study design, molecular studies and revising the article. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study protocol was reviewed and approved by Medical Research Ethics Committee (MREC) of the NRC [registration number: 16-100].

Consent to participate
According to Medical Research Ethics Committee (MREC) of the NRC, written informed consents were taken from all subjects or from their guardians approving to take part of this study and to publish their data in scientific journal.

Competing interests
The authors have no conflicts of interest to declare.

Author details
[1]Department of Medical Molecular Genetics, Human Genetics and Genome Research Institute, National Research Centre, 33 El-Bohouth Street, Cairo 12311, Egypt. [2]Department of Clinical Genetics, Human Genetics and Genome Research Institute, National Research Centre, 33 El-Bohouth Street, Cairo 12311, Egypt. [3]Neurology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

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