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# Prevalence of exon 7/exon 8 deletion in patients with hypotonia and spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a neuromuscular disease due to degeneration of the anterior horn cells of the spinal cord. The estimated incidence of SMA is 1:6,000-1:10,000. The complete deletion of exon 7 of the *SMN1* gene is the hallmark of 95-98% of SMA patients in most population. The first line of investigation for a child or young adult patient suspected to have SMA should be Multiplex ligation-dependent probe amplification (MLPA) testing for homozygous deletion of exons 7 and exon 8 in the *SMN1* gene. In this paper, we report the results of *SMN 1* exon 7 deletion tests in children who attended the Genetic clinic of a tertiary care hospital in Kerala with one or more of the symptoms especially floppy infants, hypotonia, muscle weakness, tongue fasiculations *etc*. *SMN1* exon 7 and exon 8 deletion was confirmed in 58% cases (19) of the total 33 hypotonia patients. SMA Type I, Type II and Type III were 68.4% (13), 21% (4) and 10.5% (2) respectively among the SMA positive cases. Carrier testing of the non-consanguineous parents showed that all parents were heterozygous carriers. Until 2016, the treatment for this disease was supportive only. Recently Nusinersen, Zolgensma and Risdiplam have become available for SMA patients. The carrier testing in parents with previous SMA child history is essential for the implementation of prenatal diagnosis of this disorder in future pregnancies. The paper emphasizes the importance of this rare neuromuscular disease.

**Keywords:** Hypotonia, MLPA, SMA, *SMN1*, *SMN2*.

Hypotonia is caused by variety of disorders like spinal muscular atrophy, central nervous system disorders, muscular dystrophy or Prader-Willi syndrome[1]. Spinal muscular atrophy (SMA) (classified as SMA I, II and III, each successively milder and have a later age of onset) is due to involvement of anterior horn cell due to genetic mutation in the *SMN1* gene and inherited as autosomal recessive[2]. The overall prevalence of this genetic condition is about 1 in 6000 to 1 in 10,000 newborns and carrier frequency of most population is 1 in 40 to 1 in 60[3]. *SMN1* and *SMN2* are the two genes which play a pivotal role in SMA. These two genes are highly similar located on long arm of chromosome 5 at telomeric (*SMN1*) and centromeric (*SMN2*) position[4]. These genes encodes 9 exons and a critical difference between *SMN1 and SMN2* is a single base C to T substitution transition that creates an exon splicing suppressor in exon 7 of *SMN2*, hence *SMN2* is translated much less in to a functional SMN protein[5]. So *SMN2* cannot compensate for *SMN1* which is the determinant factor in SMA, but the increased *SMN2* copies may alter the severity of the SMA condition. The homozygous deletion of exon 7 of the *SMN1* gene is the hallmark of 95-98% of SMA patients in most population[6]. Inactive *SMN1* gene due to point mutation or a deletion of exons 1-6 was also rarely reported[7]. Genetic testing for confirmation of the diagnosis and further quantification of the number of alleles of *SMN2* to identify the severity of the disorder is important for genetic counselling, prognostication and to consider the various new molecular therapies in patients with SMA[8,9].

The first line of investigation for a child or young adult patient suspected to have SMA should beMultiplex ligation-dependent probe amplification (MLPA) testing for homozygous deletion of exon 7 and exon 8 in the *SMN1* gene. The same test can be used to quantify the number of alleles in *SMN2* gene which will help to predict the severity of SMA as Type I, II or III[10]. Since diagnosis of SMA is important in any person with hypotonia, we studied the prevalence of SMA diagnosis by genetic testing

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(MLPA for exon 7 and exon 8 deletion) in a cohort of patients with hypotonia attended in genetic clinic in a tertiary care hospital in Kerala.

## Materials and Methods

### Study participants and methods

This prospective study was conducted at a tertiary care hospital in Kerala which serves as a referral centre for most of South Kerala and adjoining districts of Tamil Nadu. A total of 33 infants in the age range of 1 month to 13 years of age who attended the genetic clinic with phenotypic feature of hypotonia with suspicion of smuscular atrophy during the period 2016-2021 were included in the study. The female-male ratio was 6:5. All patients underwent clinical genetics evaluation, and the relevant clinical information was collected in a proforma. The parents of the participants were all non-consanguineous couple. Carrier testing was performed in parents of SMA positive cases. 2 mL of blood sample in an EDTA vacutainer was collected from the children after taking consent from the parent/care taker for genetic testing. The Institutional Ethical Committee clearance [HEC.No.02/35/2020/MCT] was obtained for the study.

MLPA assay for the molecular diagnosis of SMA was performed in a thermal cycler using a kit (SALSA MLPA Kit; MRC Holland, Amsterdam, Netherlands). The kit contains specific probes for *SMN1* and *SMN2* genes[11]. Due to this specific probe set, MLPA assay for the SMA critical region is able to detect the copy number of both *SMN1* and *SMN2* genes. After amplification, fragment separation was done by capillary electrophoresis and analysed by Coffalyser.net[12,13]. Both homozygous and heterozygous *SMN1* deletions and assessment of *SMN2* copy number was documented.

## Results

A total of 33 cases were done genetic testing for SMA. Homozygous exon 7 deletion (with or without exon 8) in *SMN1* gene was identified in 58% of cases (19/33) which confirmed a diagnosis of spinal muscular atrophy [SMN1 (Exon7:0, Exon 8:0)] (Fig. 1). The SMA positive cases were 10 male and 9 female cases. Among 19 SMA patients, 13 patients were classified as Type I based on the onset of the weakness and its severity, and all of them had only 2 or less copies of exon 7/exon 8 in *SMN2* gene. In our cohort 4/19 (21%) was Type II SMA and 2/19 (10.5%) was Type III based on clinical phenotype and *SMN2* gene copy numbers (more than 2 copies). Carrier screening detected the heterozygous deletion of exon 7 and exon 8 of *SMN1* gene in the parents of SMA positive cases. All patients with SMA had other characteristic features like decreased movement of lower limbs, absent deep tendon reflexes, tongue fasciculation and normal cognitive function for their age. The negative cases were further evaluated as per protocol with other genetic testing and diagnosed with conditions like congenital muscular dystrophies, Prader-Willi syndrome, myotonic dystrophy, congenital disorders of glycosylation, and pompe disease. For the 13 SMA Type I patients, no definitive treatment was given to 10 patients, and they succumbed to death within 18 months of age. Three patients had some form of therapy like Zolgensma or Risdiplam and they are under supportive care. The life expectancy of Type I SMA without any treatment is under two years and new molecular treatment will definitely prevent mortality and life threatening events[14].Four Type II SMA patients were on regular Risdiplam medication. SMA Type III, mildest form with late onset, in two cases in the age group of 16-18 were facing difficulty in getting stairs and get up from squatting position. They are under physiotherapy follow-up.

## Discussion

SMA is a common neurodegenerative disorder and SMA type I was the most common type and Type IV is the milder form of SMA. Since SMA is inherited as autosomal recessive disorder, we expect a significant proportion of families with consanguinity. The reported consanguinity for SMA in the earlier studies ranged between 38.9% and 49%. High prevalence of SMA has been reported in Iran, Egypt, and Saudi Arabia where consanguineous marriage rates are high[15]. However among our diagnosed cases of SMA, no family gives a history of consanguinity. Carrier testing of the parents showed that all parents were heterozygous carriers. In Kerala, the frequency of consanguineous marriages is very low and one type of preferred marriage of the Dravidian marriage system uncle niece marriage is conspicuously absent. In the other states of South India, consanguinity and the coefficient of inbreeding are high[16]. Carrier frequency in north India is 1 in 30 to 1 in 38[17].This higher carrier frequencies in population may be the reason most of the cases are born to non-consanguineous parents.

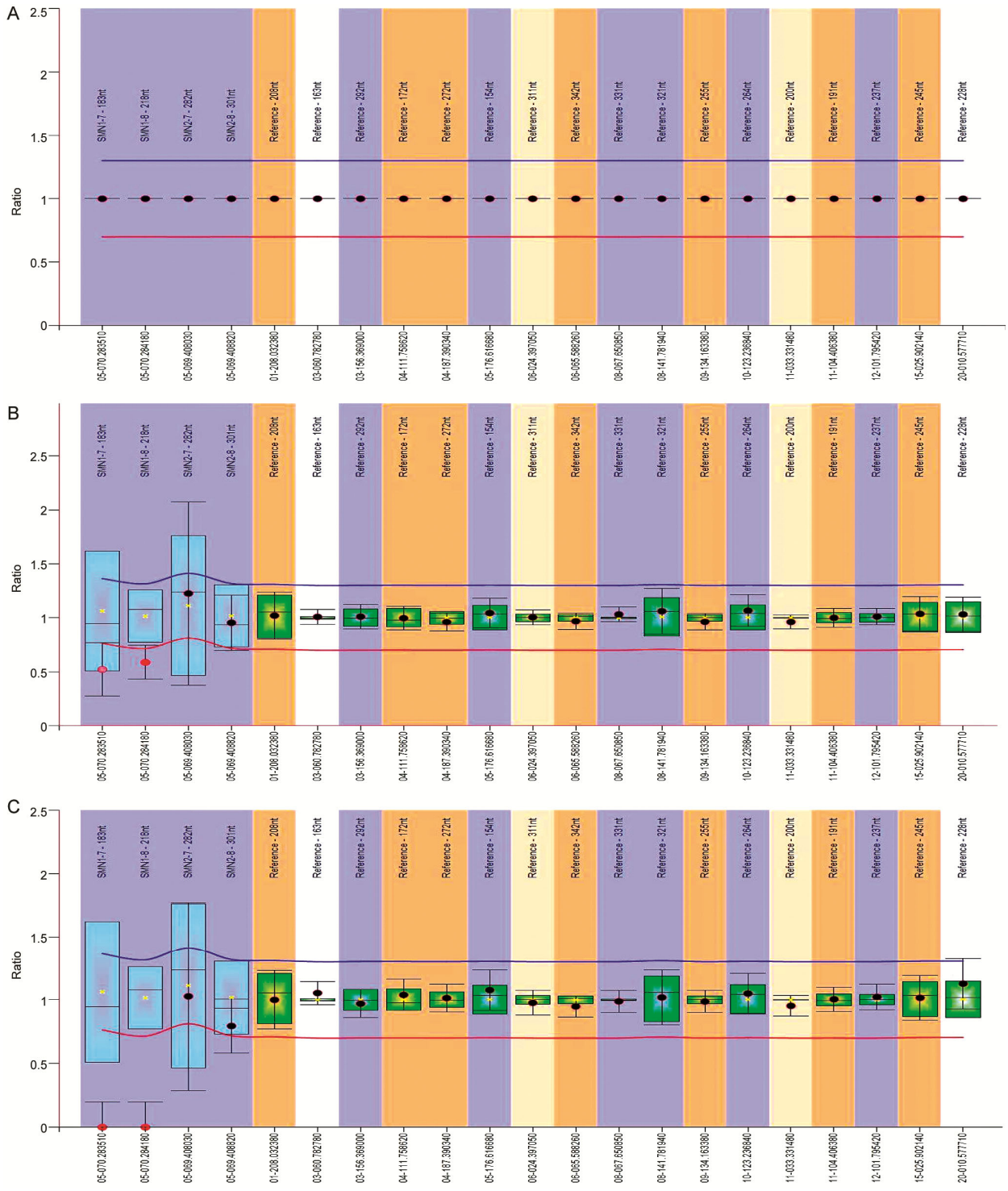


Fig. 1 — Coffalyzer ratio plot showing (A) SMA negative (normal ratio: 0.80<DQ<1.20) (B) SMA carrier (heterozygous deletion ratio: 0.40<DQ< 0.65) (C) SMA positive (homozygous deletion ratio: DQ= 0)

This shows the importance of population screening programme to detect the carriers as a preventive strategy[18].

SMA II shows a milder course with onset between 6 and 18 months. Patients are able to sit, but never ambulates and develop respiratory involvement that usually requires the use of non-invasive ventilation before adulthood and orthopedic complications such as severe scoliosis and joint contractures[19]. Among

the four SMA type II cases, one who diagnosed with Type II SMA at 3 years had frequent respiratory distress, hand tremors and muscle weakness was under respiratory support and the other three were under medication. SMA Type III, mildest form with late onset, in two cases in the age group of 16-18 were facing difficulty in getting stairs and get up from squatting position. They are under physiotherapy follow-up.

Currently there is no available complete cure for SMA; one possible intervention could be to reduce the consanguinity by educating the population. Even the non-consanguineous parents are even at the risk of having SMA Child; carrier testing for SMA should be effectively implemented in society for the management of SMA[20]. SMA patients require a multidisciplinary supportive approach including respiratory, orthopedic, psychological, physio- and speech-therapist and nutritional care for their rehabilitation[21].

Recently Nusinersen (2016), the gene replacement by *Onasemnogene abeparvovec* (Zolgensma), (2019) and Risdiplam (2020) have become available for SMA patients[22].The new therapeutic options have brought about financial challenges for the SMA community. Nusinersen, the first approved drug for SMA is an oligonucleotide administered in the central nervous system (CNS) using intrathecal injection has the role to increasing the expression of the survival motor neuron protein. Of the four initial loading doses; the first three loading doses are given at 14-day intervals, while the fourth loading dose is given 30 days after the third. After that, a maintenance dose is given every four month[23]. Risdiplam which is administred through orally is a small molecule that modulates *SMN2* gene splicing. The unique specificity of binding two sites in *SMN2* pre-mRNA 5′ splice site (5′ss) of intron 7 and exonic splicing enhancer 2 (ESE2) in exon 7 increases levels of full-length *SMN* mRNA and protein[24]. *SMN1* gene replacement therapy by *Onasemnogene abeparvovec* through one-time injection was approved by the FDA in May 2019 to treat children less than 2 years of age with mutations in the *SMN1*gene. This uses a non-replicating adeno-associated virus capsid (scAAV9) to efficiently deliver wild-type *SMN1* gene to motor neuron cells. This can cross the brain blood barrier, produces a sustained expression of *SMN* protein[25,26]. The main advantage of this therapeutic option is that a one-time injection is needed, but the high cost is a barrier for the patients.

## Conclusion

*SMN1* gene homozygous deletion of exon 7 and exon 8 was the most common genetic deletion found in this study. A total of 33 cases were undergone genetic testing for SMA, 58% of cases were identified with homozygous exon 7 and exon 8 deletion and were confirmed as SMA. With the advent of molecular biology techniques, SMN gene deletion studies have become the first line of investigation for confirmation of a clinical diagnosis of SMA. With the availability of genetic testing it is now possible to diagnose these children early so that appropriate counseling can be given to the family on the risk of future pregnancies. The carrier testing in parents with previous SMA child history is essential for the implementation of prenatal diagnosis for early detection, effective control and management of this disorder in future pregnancies. Antenatal diagnosis is also possible by the early identification through genetic testing.

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## Conflict of interest

The authors declare that they have no known conflict of interests.

## Reference

1 Gur S, Gurbuz G &Tozkir H, Radiological and Genetic Evaluation in Hypotonic Infants. *Age,* 41 (2023) 63.

2 Chaudhary R, Agarwal V, Rehman M, Kaushik AS & Mishra V, Genetic architecture of motor neuron diseases. *J Neuro Sci,* 434 (2022)120099.

3 Gailite L, Sterna O, Konika M, Isakovs A, Isakova J, Micule I, Setlere S, Diriks M & Auzenbaha M, New-Born screening for spinal muscular atrophy: results of a Latvian pilot study *Int J Neonatal Screen,*8(2022)15.

4 Lopez-Cortes A, Echeverría-Garcés G & Ramos-Medina MJ, Molecular pathogenesis and new therapeutic dimensions for spinal muscular atrophy. *Biology,* 11(2022) 894.

5 Singh NN, O'Leary CA, Eich T, Moss WN & Singh RN, Structural context of a critical exon of spinal muscular atrophy gene. *Front Mol Biosci ,* 9(2022) 928581.

6 Alghamdi A, AlDossary S, Alabdulqader WA, Amer F, Ali M, Almomen M & Alghamdi F, Identifying Clinical and Genetic Characteristics of Spinal Muscular Atrophy Patients and Families in Saudi Arabia. *Cureus,* 15(2023).

7 Fay A, Spinal muscular atrophy: A (now) treatable neurodegenerative disease. *Pediatric Clinics,* 70 (2023)963.

8 Zhou Y & Jiang Y, Current Advances in Genetic Testing for Spinal Muscular Atrophy.*Current Genomics,* 24 (2023)273.

9 Keinath MC, Prior DE & Prior TW, Spinal muscular atrophy: mutations, testing, and clinical relevance. *Appl Clin Genet*, (2021) 11.

10 Yasheswinee R, Dharmarajan A & Parvathi VD, Expanded Carrier Screening for Neuromuscular Disorders. *Biomed J Sci Tech Res,* 42(2022) 33778.

11 Wang N, Jiao K, He J, Zhu B, Cheng N, Sun J, Chen L, Chen W, Gong L, Qiao K, Xi J, Diagnosis of Challenging Spinal Muscular Atrophy Cases with Long-Read Sequencing. *J Mol Diagn,* 26 (2024) 364.

12 Qu Y, Bai J, Jiao H, Qi H, Huang W, OuYang S, Peng X, Jin Y, Wang H & Song F. Variants located in intron 6 of SMN1 lead to misdiagnosis in genetic detection and screening for SMA. *Heliyon,* 10 (2024).

13 Milligan JN, Larson JL, Filipovic-Sadic S, Laosinchai-Wolf W, Huang YW, Ko TM, Abbott KM, Lemmink HH, Toivonen M, Schleutker J & Gentile C. 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 Gene Duplication Variants. *J Mol Diagn*, 23 (2021) 753.

14 Nishio H, Niba ET, Saito T, Okamoto K, Takeshima Y & Awano H. Spinal muscular atrophy: the past, present, and future of diagnosis and treatment. *Int J Mol Sci,* 24 (2023)11939.

15 Al Jumah M, Al Rajeh S, Eyaid W, Al-Jedai A, Al Mudaiheem H, Al Shehri A, Hussein M & Al Abdulkareem I. Spinal muscular atrophy carrier frequency in Saudi Arabia. *Molecular Genetics & Genomic Medicine*, 10 (2022) e2049.

16 Kalam MA, Sharma SK, Ghosh S & Roy S. Consanguinity, pregnancy outcomes and offspring mortality in India: Evidences from National Family Health Survey, 2015-2021(2024).

17 Aasdev A, Sreelekshmi RS, Iyer VR & Moharir SC. Spinal muscular atrophy: Molecular mechanism of pathogenesis, diagnosis, therapeutics, and clinical trials in the Indian context. *J of Biosci,* 49, (2024)1.

18 Nilay M, Moirangthem A, Saxena D, Mandal K & Phadke SR, Carrier frequency of SMN1 related spinal muscular atrophy in north Indian population: The need for population based screening program. *Am J Med Genet A*, 185 (2021) 274.

19 Lagae L, Proesmans M, Van den Hauwe M, Vermeulen F, De Waele L & Boon M. Respiratory morbidity in patients with spinal muscular atrophy—a changing world in the light of disease-modifying therapies. *Front Pediatr,* 12 (2024)1366943.

20 Rouzier C, Chaussenot A & Paquis-Flucklinger V, Molecular diagnosis and genetic counseling for spinal muscular atrophy (SMA). *Archives de Pédiatrie*, 27.7 (2020)7S9.

21 Messina S & Sframeli M. New treatments in spinal muscular atrophy: positive results and new challenges. *J Clin Med,* 9(2020)2222.

22 Erdos J & Wild C. Mid-and long-term (at least 12 months) follow-up of patients with spinal muscular atrophy (SMA) treated with nusinersen, onasemnogene abeparvovec, risdiplam or combination therapies: a systematic review of real-world study data. *Eur J Paediatr Neurol,* 39 (2022) 1.

23 Qiu J, Wu L, Qu R, Jiang T, Bai J, Sheng L, Feng P & Sun J. History of development of the life-saving drug "Nusinersen" in spinal muscular atrophy. *Front Cell Neurosci* ,16 (2022)942976.

24 Torroba B, Macabuag N, Haisma EM, O'Neill A, Herva ME, Redis RS, Templin MV, Black LE & Fischer DF. RNA-based drug discovery for spinal muscular atrophy: A story of small molecules and antisense oligonucleotides. *Expert Opin Drug Discov*, 18(2023)181.

25 Xie Q, Chen X, Ma H, Zhu Y, Ma Y, Jalinous L, Cox GF, Weaver F, Yang J, Kennedy Z, & Gruntman A. Improved gene therapy for spinal muscular atrophy in mice using codon-optimised hSMN1 transgene and hSMN1 gene-derived promotor. *EMBO Mol Med,2* (2024)1.

26 Schorling DC, Pechmann A & Kirschner J, Advances in treatment of spinal muscular atrophy–new phenotypes, new challenges, new implications for care. *J Neuromuscul Dis*, 7 (2020) 1.