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Original Article

A Study of The Spinal Muscular Atrophy Cohorts in The Eastern Anatolia Region of Türkiye

Oğuzhan Yaralı,[1] Ozge Beyza Gündoğdu Öğütlü,[1] Serdar Sarıtaş,[2] Mustafa Can Güler,[3] Filiz Keskin[2]

[1]Department of Medical Genetics, Erzurum Regional Training and Research Hospital, Erzurum, Türkiye
[2]Department of Pediatric Neurology, Erzurum Regional Training and Research Hospital, Erzurum, Türkiye
[3]Department of Physiology, Faculty of Medicine, Ataturk University, Erzurum, Türkiye

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Correspondence: Oğuzhan Yaralı, Department of Medical Genetics, Erzurum Regional Training and Research Hospital, Erzurum, Türkiye
E-mail: medikant@gmail.com

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ABSTRACT

Objective: The purpose of this research is to carry out a genetic cohort study of SMA patients in the Eastern Anatolia region of Turkey, investigating the genetic causes of the illness, specifically the impact of the number of SMN1 and SMN2 gene alleles on the course of the disease.

Methods: The Erzurum Medical Faculty of Health Sciences at Erzurum City Hospital gave ethical approval for the study to be conducted. A total of 348 patients with an initial diagnosis of SMA underwent genetic testing. Using the PCR-RFLP approach, deletions of exons 7 and 8 in the SMN1 and SMN2 genes were examined.

Results: In examining the allele counts in the exon 7 and exon 8 regions of the SMN1 and SMN2 genes, 41 patients were found to have no copies of the SMN1 gene (0 alleles), while 112 people were identified as possible carriers. Analysis of the SMN2 gene's allele distribution revealed a substantial relationship between the number of alleles and the clinical severity of the disease.

Conclusion: The number of alleles in the SMN1 and SMN2 genes influences the course of the disease, as demonstrated by the genetic cohort analysis of SMA patients in the Eastern Anatolia region of Turkey presented in this paper. The findings indicate that sex does not influence the frequency of the disease or carrier status, offering significant new insights into the genetic diagnosis and treatment of SMA. The study also emphasizes the importance of establishing local genetic screening programs and counseling services to facilitate early diagnosis and treatment.

INTRODUCTION

Reduced motor neurons in the spinal cord and brainstem nuclei are the result of deletions or mutations in the SMN1 gene, which causes the uncommon autosomal recessive neuromuscular disease known as spinal muscular atrophy (SMA).[1] The currently used classification is shown in Table 1.[2]

The main symptoms of SMA include gastrointestinal issues, hypoventilation, and muscle weakness. However, as life expectancy has grown, more symptoms have been discovered, indicating that low SMN protein levels affect more organ systems. These symptoms include sensory dysfunction, cardiac arrhythmias, vascular issues like distal digital necrosis, decreased bone mineral content, and abnormal glucose metabolism.[3]

The genetic etiology of about 96% of SMA cases is SMN1 deficiency or conversion to SMN2, which usually results in homozygous deletion of exon 7 or both exons 7 and 8 of SMN1 (Figure 1).[4] In type II and type III SMA, there is frequently a gene conversion from SMN1 to SMN2, with an increase in the number of copies of SMN2. In type I SMA, most individuals have a genuine deletion of SMN1. Exon 7 of SMN2 and exon 8 of SMN1 can coexist in hybrid SMN1/SMN2 genes when this conversion isn't complete.[5,6] In 96% of type I, 94% of type II, and 86% of type III SMA patients, homozygous deletions are found through genetic screening. Subtle mutations are more common in those

Figure 1. The phenotype-genotype correlation and genetic basis of spinal muscular atrophy (SMA).

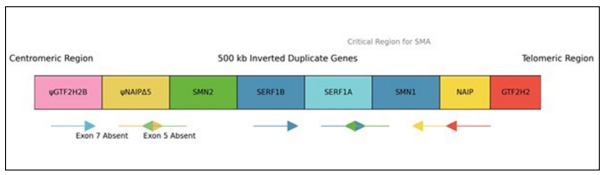

Figure 2. Organization of the 5q13 region on chromosome 5.

with milder manifestations of the disease than in those with more severe symptoms.[7,8]

About 4% of SMA patients have subtle abnormalities on chromosome 5 that result in the deletion of SMN1. Rarely, consanguineous marriages result in children with two mildly different SMN1 alleles. About 2% of SMA instances are de novo mutations, which are frequently the consequence of uneven recombination because of the high instability of the SMA locus at 5q13.[9,10] There are now 108 pathogenic SMN1 variants known to exist. A 4 bp deletion (c.399_402delAGAG) that causes a frameshift in Spaniards, p.Tyr272Cys in Germans, p.Thr274Ile in Poles, and a frameshift mutation p.Gly261fs*269 from an 11 bp duplication (c.770_780dup11) seen in Spanish, French, and US populations are examples of common subtle mutations.[11,12]

The technique of multiple ligation-dependent probe amplification (MLPA) for the SMN1 and SMN2 genes is the gold standard for the genetic diagnosis of SMA.[13] The copy number of SMN2, compound heterozygotes with a single copy of SMN1 and a potential modest SMN1 variant, and healthy heterozygous carriers can all be identified using this technique. Patients with homozygous SMN1 deletions cannot be identified. However, neither the detection of minor mutations in SMN1 (which account for 6% of cases) nor the distinction between persons with one copy of SMN1 on each chromosome 5 and those with two copies are possible with this method (Figure 2).[14] This means that an individual carrying two copies of SMN1 may still be carrying SMA, resulting in a false negative rate of approximately 5%.[15]

Two methods will be used in order to find subtle differences in SMN1: In order to detect SMN1 gene carriers, two methods can be used: (a) long-range PCR of the whole 28 kb SMN1 genomic area using SMN1-specific primers, followed by exon re-amplification and sequencing; or (b) amplification and cloning of SMN cDNA products, followed by PCR.[16] The latter technique additionally finds exonic and intronic variations that could impact splicing.

Generally speaking, an individual with SMA will have fewer severe symptoms the more copies of SMN2 they have. There is a substantial correlation between the severity of SMA symptoms and the number of SMN2 copies. For instance, although 78% of type II patients have three copies, 73% of type I SMA patients have two copies. Three copies are present in roughly 50% of type IIIa patients, 61% of type IIIb patients, and 75% of type IV patients, respectively. The predictive value varies; 20% of type I patients, 78% of type II patients, and 51% of type III patients have three copies of SMN2.[9,17] People with five or more copies of SMN2 may continue to be asymptomatic, whereas having no copies of SMN2 can be deadly.[17] Nevertheless, there is no clear correlation between phenotypic and SMN2 copy number. Variability within families is possible.[18] SMA classification today is based on clinical symptom severity and functional capacity.[19]

MATERIALS AND METHODS

The Ethics Committee of the Medical Faculty at Erzurum University of Health Sciences granted ethical permission for this study (decision number BAEK 2024/03-73). Between 2018 and 2023, we tested 348 patients for genetics at Erzurum City Hospital from pediatric neurology, neonatal clinics, and family practices. Genomic DNA was extracted from 10 milliliters of peripheral blood with informed consent.

Using the primers 5'-AGACTATCAACTTAATTTCT GATCA-3', 5'-CCTTCCTTCTTTGATTTTGTTT-3', 5'-GTAATAACCAAAATGCAATGTGAA-3', and 5'-CTACAACACCTTCTCACAG-3', deletions in SMN1 gene exons 7 and 8 were found by PCR-RFLP. After 35 cycles of PCR, the samples were digested overnight at 37°C using the enzymes DraI and DdeI. Ethidium bromide-stained 4% agarose gels were used for the analysis of the digestive products.

The PCR-RFLP method was used for the SMN2 gene exon 7 p.G287R (c.859G > C) variant. Primers 5'-AGACTATCAACTTAATTTCTGATCA-3' and 5'-ATTTAAGGAATGTGAGCACCTTA-3' were used for 40 cycles. The results were examined on 4% agarose gels following DdeI digestion.

Multiplex PCR was used to analyze NAIP gene deletions in exons 5 and 6 utilizing primers 5'-CATTTGGCATGTTCTTCCAAG-3', 5'-AAAGCCTCTGACGAGAGGATC-3', and 5'-TGCCACTGCCAGGCAATCTAA-3' for exons 5 and 6, respectively, and exon 13 co-amplified using

Table 1. Current SMA classification

SMA Type	Subclassification	Onset	Acquired Motor Milestones	Evolution/Natural History	Prevalent SMN2 Copies
I	Ia (also called type 0)	Prenatal	None	Death within weeks, Contractures, Cardiomyopathy	1
I	Ib	<3 months	Weak or no head control	Feeding and respiratory problems, Linear decline, Death after the second or third year	2
I	Ic	>3 months	Head control	Feeding and respiratory problems, Plateau within the first 2 years	3
II	IIa	>6 months	Sitters	Scoliosis, May lose sitting ability	3
II	IIb	Usually after 12 months	Sitters	Scoliosis, Can stand with support	3
III	IIIa	Between 18 and 36 months	Walks unassisted	Scoliosis, Earlier loss of walking ability	3
III	IIIb	>3 years	Walks unassisted	Later loss of walking ability	3-4
IV	None	Second/third decade	Walks unassisted	Can walk most of life	3-5
V	None	Minimal symptoms or asymptomatic with absence of SMN1	All major milestones	Full lifespan walking ability	3-5

primers 5'-CCAGCTCCTAGAGAAAGAAGGA-3' and 5'-ATGCTTGGATCTCTAGAATGG-3'. Three percent agarose gels were used for electrophoresis.

Finally, the SALSA P060-B1 SMA kit was used to perform the MLPA method in order to determine the copy number of the SMN1 and SMN2 genes. Following five minutes of denaturation at 98°C, an overnight hybridization at 60°C, fifteen minutes of ligation at 54°C, and PCR using FAM-labeled primers, the DNA samples were processed. PCR products were examined using Coffalyzer software on an ABI3130 Genetic Analyzer.

RESULTS

This study examined the number of alleles in the exon 7 and 8 regions of the SMN1 and SMN2 genes in a total of 348 patients. Forty-one patients were identified as having no copies (0 alleles) of exons 7 and 8 of SMN1, which are critical for diagnosing SMA. This is in line with the expected general prevalence of SMA, representing 11.8% of patients. Additionally, 112 individuals with single-copy loss (1 allele) of the SMN1 gene were identified as potential carriers, representing 32.2% of the population.

The distribution of SMN2 gene allele counts plays a significant role in influencing the course of the illness. There were two SMN2 alleles for exon 7 in 31 patients, three in four patients, and four in three patients among the 41 individuals who lacked copies of the SMN1 gene. Twenty-nine patients had two alleles for SMN2 exon 8, eight patients had three alleles, and one patient had four alleles. This distribution implies that the clinical severity of the condition is significantly influenced by the number of SMN2 copies. Milder symptoms might be indicated by higher SMN2 copy levels.

A total of 348 newborn infants with hypotonia and a family history of SMA were screened as part of this study. Of those who were screened, 166 were male and 182 were female. The distribution of the diagnosis of SMA and the carrier status according to sex was analyzed.

The prevalence of SMA was 9.0% (15/166) for men and 8.8% (16/182) for women. Regarding carrier status, 34.3% (57/166) of men and 30.2% (55/182) of women were identified as carriers. Although this gender difference in carrier rates may not be statistically significant, gender-specific carrier status is important for genetic counseling and family planning.

In summary, our research indicates that gender has no bearing on the frequency and carrier status of SMA and emphasizes the significance of screening for the condition in patients with hypotonia and a family history of it (Table 2-3).

Table 2. Frequency distribution of SMN1 and SMN2 exons

Allele Count	0	1	2	3	4
SMN1 Exon 7	41	112	185	8	1
SMN1 Exon 8	41	112	185	8	1
SMN2 Exon 7	11	91	220	22	3
SMN2 Exon 8	11	91	220	24	2

Table 3. Allele count of SMN2 exon 7 and exon 8 in patients with 0 copies of SMN1 Exon 7 and Exon 8

Exon/Allele Count	2 Alleles	3 Alleles	4 Alleles
SMN2 Exon 7	31	4	3
SMN2 Exon 8	29	8	1

DISCUSSION

The study focused on 348 spinal muscular atrophy (SMA) patients at Erzurum City Hospital, Eastern Anatolia, Turkey, analyzing the effect of SMN1 and SMN2 alleles on disease progression. The results indicate a significant relationship between allele variations and SMA severity, contributing to a broader understanding of the disease when compared to existing literature.

The identification of 41 patients (11.8% of the cohort) without any copies of SMN1 gene exons 7 and 8 underlines the diagnostic importance of the gene, in line with global prevalence rates. Notably, the study also identified 112 individuals (32.2% of participants) with a single-copy loss in the SMN1 gene. This suggests a significant carrier prevalence.

Current research shows that SMA type 1 patients typically suffer rapid and irreversible motor neuron loss starting perinatally. This leads to extensive motor unit loss within months.[20] Delays in diagnosis are common. For SMA types 1, 2, and 3, there are several months between symptom onset and diagnosis.[21]

In particular, the number of SMN2 alleles influences the clinical course of patients with missing SMN1 copies, with higher SMN2 levels correlating with milder symptoms. This highlights the potential therapeutic importance of SMN2.

The study found a minimal effect of sex on SMA prevalence and carrier status, consistent with the autosomal recessive nature of SMA. This suggests that sex should not be considered in genetic counseling or SMA risk assessment.

The 5q forms of SMA are primarily caused by homozygous deletions or, less frequently, other mutations in the SMN1 gene, notwithstanding the great clinical diversity of SMA (OMIM 600354).[22] The SMN2 gene (OMIM 601627), which functions as an alternate copy, primarily determines the severity of the disease; the phenotype tends to become milder as SMN2 copy numbers grow.[17] Furthermore, uncommon variations in SMN2 and other genetic variables such as plastin 3 (PLS3) or neurocalcin delta (NCALD) may have an impact on the severity of the condition.[23]

There is a significant variation of mutations found in SMA patients. Point mutations account for 4% of the total, while homozygous deletion of SMN1 accounts for 96%. Owing to the intricate genetic structure, gene conversions and de novo rearrangements occur often. Additional SMN2 variations or independent genes like PLS3 and NCALD can exacerbate the illness.[24,25] Research has shown that in people with two copies of SMN2, SMA-I develops in 79% of cases, whereas SMA-III occurs in 5% of cases; in people with three copies of SMN2, SMA-II occurs in 54% of cases, SMA-III in 31% of cases, and SMA-I in 16% of cases. Milder types of SMA are more common in patients with four copies of SMN2. Of those diagnosed, just 1% have SMA-II and 11% have SMA-I.[26]

By conducting this study in Eastern Anatolia, it is possible to examine regional genetic diversity and environmental influences on SMA prevalence and carrier rates. This highlights the importance of tailored regional screening and genetic counseling services.

Conclusion

In conclusion, by demonstrating the benefits of regional genetic screening and counseling in improving early diagnosis and disease management, this study at Erzurum City Hospital provides critical insights into the genetic basis and management of SMA. Such localized studies enhance national and international efforts in the screening and management of genetic disorders. They also aid in the development of strategies for genetic counseling and early diagnosis.

Ethics Committee Approval

The study was approved by the Erzurum Medical Faculty of Health Sciences Ethics Committee (Date: 14.03.2024,

Decision No: BAEK 2024/03-73).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: O.Y., Ö.B.G.Ö.; Design: O.Y., M.C.G.; Supervision: S.S.; Fundings: O.Y.; Materials: F.K.; Data: Ö.B.G.Ö., S.S.; Analysis: O.Y., F.K.; Literature search: O.Y., Ö.B.G.Ö.; Writing: O.Y.; Critical revision: S.S.

Conflict of Interest

None declared.

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Türkiye'nin Doğu Anadolu Bölgesi'ndeki Spinal Müsküler Atrofi Kohortları Üzerine Bir Çalışma

Amaç: Bu çalışmanın amacı, hastalığın genetik temelini ve özellikle SMN1 ve SMN2 genlerinin alel sayısının hastalığın ilerlemesi üzerindeki etkisini araştırmak için Türkiye'nin Doğu Anadolu bölgesindeki SMA hastalarında genetik bir kohort çalışması yapmaktır.

Gereç ve Yöntem: Çalışma, Erzurum Şehir Hastanesi'nde Erzurum Sağlık Bilimleri Üniversitesi Tıp Fakültesi'nin etik onayı ile yürütülmüştür. SMA ön tanısı olan toplam 348 hastaya genetik test uygulandı. SMN1 ve SMN2 genlerindeki ekzon 7 ve 8 delesyonları PCR-RFLP yöntemi ile analiz edildi.

Bulgular: SMN1 ve SMN2 genlerinin ekzon 7 ve ekzon 8 bölgelerindeki alel sayıları incelenerek SMN1 geninin hiç kopyası olmayan (0 alel) 41 hasta ve potansiyel taşıyıcı olarak değerlendirilen 112 birey belirlendi. SMN2 genindeki alel sayısının dağılımı analiz edilmiş ve hastalığın klinik şiddeti üzerinde önemli bir etkisi olduğu gösterilmiştir.

Sonuç: Bu çalışma, Türkiye'nin Doğu Anadolu bölgesindeki SMA hastalarının genetik kohort analizini sunmakta ve SMN1 ve SMN2 genlerindeki alel sayısının hastalığın ilerlemesi üzerindeki etkisini ortaya koymaktadır. Bulgular, cinsiyetin hastalık yaygınlığı ve taşıyıcılık durumu üzerinde sınırlı bir etkiye sahip olduğunu öne sürerek, SMA'nın genetik teşhisi ve yönetimi konusunda önemli bilgiler sağlamaktadır. Araştırma ayrıca bölgesel genetik tarama ve danışmanlık hizmetlerinin geliştirilmesinin kritik önemini vurgulamaktadır.

Anahtar Sözcükler: Genetik varyasyon; SMA; SMN1; SMN2.