Molecular Analysis of SMN2, NAIP and GTF2H2 Gene Deletions and Relation with Clinical Subtypes of Spinal Muscular Atrophy

Nilgun Karasu
Erciyes University
Hamit Acer
Denizli State Hospital
Hilal Akalin
Erciyes University
Mikail Demir
Erciyes University
Izem Olcay Sahin
Erciyes University
Nuriye Gokce
Erciyes University
Ayten Gulec
Erciyes University
Asli Ciplakligil
Erciyes University
Ayse Caglar Sarilar
Erciyes University
Isa Cuce
Erciyes University
Hakan Gumus
Erciyes University
Huseyin Per
Erciyes University
Mehmet Canpolat
Erciyes University
Munis Dundar (dundar@erciyes.edu.tr)
Erciyes University

Research Article

Keywords: Spinal muscular atrophy, SMN2, NAIP, GTF2H2 (P44), CNVs

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1442537/v1

Abstract

SMA (spinal muscular atrophy) is an autosomal recessive neuromuscular disease that causes muscle atrophy and weakness. SMA is diagnosed by homozygous deletion in exon 7 of the *SMN1* gene. However, mutations in other genes in the SMA region may contribute to the disease. These include *SMN2*, which is a pseudogene of *SMN1*, as well as *NAIP* and *GTF2H2*. Within the scope of our study, 58 SMA patients and 40 healthy controls were analyzed in 2018–2021. *SMN1* and *SMN2* copy numbers were retrospectively included in the study. *NAIP* gene analyzes were performed by multiplex PCR method and *GTF2H2* analyzes were performed by RFLP method respectively. We detected a significant correlation between clinical subtypes (type 2 and type 3) and ambulation status ($p = 0.003$) and HFMSE scores ($p = 0.0063$) of 27 pediatric SMA patients compared with separately. Highly differences were determined between *SMN2* copy numbers and the SMA subtypes ($p = 0.00001$). Also, the *NAIP* gene ($p = 0{,}0095$) and the *GTF2H2* gene ($p = 0{,}0049$) revealed a significant difference between the healthy subjects and SMA subjects, whereas in the SMA subtypes indicated no significant difference. Our investigation is the first to examine the relationship between SMA clinical severity and SMN locus genes in the Turkish population. This small-scale study may be regarded as a pilot study, and it may pave the way for future research to better understand the molecular pathophysiology of SMA disease.

Introduction

Spinal muscular atrophy (SMA) is a neuromuscular recessive disease in which the anterior horn cells of the spinal cord are damaged and is characterized by hypotonia and decreased deep tendon reflexes (Nouri et al. 2014). SMA is caused by a lack of the SMN (survival motor neuron) protein, which is involved in the biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs) (Blatnik et al. 2021). Moreover, SMN deficiency is related to abnormalities in differentiation, neurite extension, and neuromuscular junction (NMJ) development in neuronal cells (Goulet et al. 2013). The prevalence of SMA is known to be 1–2 per 100,000 persons and the incidence of SMA is approximately 1 per 10,000 live births. Also, the carrier frequency of the disease is quite high and it is estimated that affects one in every 40–60 persons in the general population (Verhaart et al. 2017). Although SMA is a rare disease, its frequency increases in societies where consanguineous marriage is common (Dundar et al. 2010). The disease is caused by a defect in the survival motor neuron 1 (*SMN1*) gene, which manifests as homozygous deletion of exon 7 and exon 8. The *SMN1* gene is located in the 5q12.2-q13.3 region (Ahn, Eun-ji, Yum, Mi-Sun 2016). There is also a centromeric copy located in the same region as the telomeric copy *SMN1* gene. This is the *SMN2* gene and a highly homologous copy of *SMN1* and also varies from *SMN1* by only one nucleotide in the coding sequence. Loss of *SMN1* determines disease pathogenesis, while the *SMN2* copy number correlates with disease severity (D'Amico A, Mercuri E, Tiziano FD 2011). Muscle hypotonia, which is the most common symptom in SMA, is also seen in many other diseases. Since there is no specific clinical sign, the diagnosis of SMA is based on molecular genetic testing. Detection of homozygous deletion in exon 7 and exon 8 of the *SMN1* gene provides a definitive diagnosis (He et al. 2013).

SMA is classified into five subtypes (0, I, II, III, and IV) according to the clinical manifestations and age of onset of the disease. In SMA Type 0, symptoms appear at birth and, this is the most severe type with respiratory distress and severe hypotonia. Infants with SMA can survive up to 6 months (Hassan et al. 2020)(Canpolat et al. 2016). SMA Type I is the most common and most severe form and accounts for about half of SMA patients (D'Amico A, Mercuri E, Tiziano FD 2011). Clinical manifestations occur in SMA Type 1 patients before 6 months and usually present with muscle weakness and hypotonia. Children with SMA Type 1 usually do not live beyond 2 years of age. In SMA Type 2, symptoms usually appear between 6–18 months. Children with SMA Type 2 cannot walk or sit unaided. In the SMA Type 3, the symptoms begin to appear after 18 months, and they able to walk alone. SMA Type 4 occurs in the 20s or 30s and is the adult form of SMA. Compared to other types, the symptoms are milder, and SMA type 4 form is rarely seen (Zhang et al. 2020).

The distance between *SMN1* and *SMN2* is 848 kb, and they are in the same direction. Two *NAIP* pseudogenes, the *SERF1A* and *GTF2H2B* genes are located between *SMN1* and *SMN2*. The *SMN1* gene is located adjacent to the neuronal apoptosis inhibitory protein (*NAIP*) gene and next to the *NAIP* the general transcription factor IIH, polypeptide 2 (*GTF2H2)* gene is located (Blatnik et al. 2021). *SERF1(H4F5)*, *NAIP*, and *GTF2H2(p44)* gene variations within the *SMN* locus have also been thought to contribute to the SMA phenotype (Liu et al. 2016). One of the first discovered mammalian apoptosis inhibitors (IAP), Neuronal Apoptosis Inhibitor Protein (*NAIP*), was cloned as a potential gene for SMA (Roy et al. 1995) (Maier et al. 2007). The *NAIP* gene is located in the same region as *SMN* (Ahn, Eun-ji, Yum, Mi-Sun 2016). The centromeric truncated *NAIP* gene is a pseudogene (*NAIP* ψ), is not include two coding exons, 4 and 5 via deletion (Nouri et al. 2014). Akutsu *et. al.* reported that there is a relationship between the severity of SMA and the *NAIP* gene deletion in 2002 (Akutsu et al. 2002). Also, it is indicated that the *NAIP* gene has an act in the differentiation and survival of neuronal cells (Mercer et al. 2000). Another gene *GTF2H2* (General Transcription Factor IIH Subunit 2) (OMIM #601748) is located in the 5q12.2-q13.3 region same as the *SMN* gene. In SMA patients, the deletion of the *GTF2H2* gene attends the deletion of the *SMN1* gene, but it is unclear whether or how it does contribute to the SMA phenotype (Medrano et al. 2016). These genes (*NAIP* and *GTF2H2*), which are located in the 5q13 region, have been remarked as disease-modifying genes (He et al. 2013). Also, the relevance of these genes to specific tools that assess motor function or clinical features in patients with SMA is unclear. A better understanding of the pathogenesis of the disease may enable novel treatment options in the light of biotechnological developments (Gartland et al. 2013).

In the present study, we aimed that to exhibit the *NAIP* and *GTF2G2* gene mutation profile in adult and pediatric SMA patients. Also, we focused to examine the relationship between the *SMN2* gene, the *NAIP* gene, and the *GTF2H2* gene and thus determine the frequency of *NAIP* and *GTF2H2* deletions in SMA patients in our country and revealing its effect on the disease. Further, another goal of our study is to compare the molecular analyses results according to the clinical features and Hammersmith Functional Motor Scale (HFMSE) scores.

Patients And Methods

Patients

Patients, who applied to the Department of Child Health and Diseases and also the Department of Adult Neurology were pre-diagnosed with SMA were referred to the Department of Medical Genetics for genetic testing. A total of 58 patients were determined between 2018 and 2021 diagnosed with SMA according to clinical findings and genetic test results, and those who fulfilled the inclusion criteria were included in the study. 39 children (0-18 years) and 19 adults (18-50 years) a total of 58 patients, 28 children and 12 adult healthy controls were analyzed in terms of *NAIP* and *GTF2H2* gene deletions. The study was conducted both retrospectively by examining the Multiplex Ligation Dependent Probe Amplification (MLPA) results of SMA patients, and prospectively by studying multiplex PCR for the *NAIP* and *GTF2H2* gene with samples taken from these patients and the healthy control group.

The patients included according to the diagnostic criteria for SMA defined by the International SMA Consortium. Patients or their parents were informed about the study and taken a written informed consent for the genetics analysis from all participants. This study was approved by Erciyes University Clinical Research Ethics Committee.

Establishment of healthy control groups:

To the healthy control group; children and adults who applied to the Pediatric Neurology outpatient clinic and Adult Neurology outpatient clinic and who did not have any SMA or other neuromuscular disease findings as a result of the clinical examination, who do not have any chronic disease according to the anamnesis and who do not use regular medication, was included.

Methods

A retrospective examination of SMA patients who had previously genetically analyzed by using the MLPA method:

2cc peripheral blood samples were taken from these patients with an EDTA tube and DNA was extracted. Mutation status in exon 7 and exon 8 regions of *SMN1* and *SMN2* genes was analyzed with MLPA method according to the manufacturer's protocol. In this context, the SALSA MLPA P021 kit was used and the MLPA PCR products were loaded on the ABI Prisma 3500 genetic analyzer (Applied Biosystems) with LIZ 500 as the size standard. The results were evaluated in the Coffalyser program and the copy numbers were determined. The files of the patients were investigated who were found to have homozygous deletions in the *SMN1* gene.

Prospective molecular analysis of the NAIP gene and the GTF2H2 gene associated with SMA disease:

NAIP gene exon 4 and 5 deletions, were analyzed in 58 patients (39 children + 19 adults) diagnosed with SMA and compared with 40 healthy control. The DNA products that available in the Department of

Medical Genetics of pediatric and adult patients who were previously positive for SMA type1 homozygous deletions were used. As the control group, 2 ml of EDTA blood taken from healthy volunteers at the same age as our patients, and DNA was extracted. The DNA quality and concentrations were measured in the nanodrop.

PCR amplification for *NAIP* gene was performed by primer oligonucleotide sequences of exon 5: 5F, 5'TGCCACTCCCAGGCAATCTAA-3', 5R, 5'-CATTTGGCATGTTCCTTCCAAG-3', and exon 4: 4F, 5'-AAAGCCTCTGACGAGAGGATC-3' and 4R, 5'-CTCTCAGCCTGCTCTTCAGAT-3'. Also, the primer exon 13: 13F, 5'-ATGCTTGGATCTCTAGAATGG-3' and 13R, 5'-CCAGCTCCTAGAGAAAGAAGGA-3' was used as an internal control. Exon 4, 5 and 13 of *NAIP* were amplified with multiplex PCR. (Liang et al. 2009). Multiplex PCR was performed on Verriti Thermal Cycler. Each reaction was carried out in a 50-µl volume containing 10 mmol/L reaction buffer, 1.5 mmol/L MgCl2, 200 µmol/L each dNTPs, 0.2 µmol/L each primer, approximately 200 ng genomic DNA, and 5U/µl Taq polymerase (Thermo). Multiplex PCR was carried out with an initial denaturation at 94 °C for 6 min, followed by 35 cycles (95 °C, 59 °C, and 72 °C for 1 min each) and a final extension step at 72 °C for 7 min. The PCR products were run for 45 minutes on a 3% agarose gel with 100 volts current. The results were evaluated under ultraviolet (UV) and the presence or absence of exon 4 and 5 of the *NAIP* gene was verified.

GTF2H2 gene analyzed with RFLP (Restriction Fragment Length Polymorphism) method. PCR amplification for *GTF2H2* gene was performed by primer oligonucleotide sequences of exon 10: 10F, 5'-CGTACCATGTTATTTTAGATG-3' and 10R, 5'-TACGAATAAGTGAGCATTCAG-3'. PCR reaction was carried through with a 50-µl volume in same conditions with the *NAIP* gene and PCR program was set initial denaturation at 95 °C for 5 min, followed by 36 cycles (94 °C for 40 sec, 50 °C for 45 sec, and 72 °C for 45 sec) and a final extension step at 72 °C for 10 min (Carter et al. 1997). The PCR products were cut using restriction enzymes DdeI and Hinf1II at 37 °C overnight. On a 3 percent agarose gel with 90 volts current, the cut products with the enzyme were run for one hour. The cut and uncut conditions of the *GTF2H2* gene were determined under ultraviolet (UV) illumination.

Clinical evaluation

The clinical features of all SMA patients in our study group, including ambulation, muscle weakness, hypotonia and respiratory distress were noted. In addition, an expanded version of the HFMSE was used for the motor function of SMA type 2 and 3 patients. The HFMSE, a scale specifically designed for SMA, is scored from 0 to 66 points, and higher scores indicate higher motor function (O'Hagen et al. 2007)(Pera et al. 2017).

Statistical analyzes

Normality tests were performed in all comparisons. The relationship between various subtypes of SMA and ambulation status was determined by the non-parametric Fisher's exact test (SPSS version 26). To compare clinical subtypes and HMFSE scores, the non-parametric Mann Whitney U test was performed and shown as a bar graph (GraphPad Prism 8). The distribution of *SMN2* exon 7 copy number variants

(CNVs) by clinical subtypes was determined by Fisher's exact test (SPSS version 26), and categorical data were expressed as percentages. Also, in order to determine where the significance originates from, post-hoc analysis was performed in the comparison of subgroups.

Results

The study comprised 58 pediatric and adult SMA patients who were diagnosed in childhood. It had been detected homozygous deletion of exon 7 of the *SMN1* gene in all of patients via the MLPA method. The patients' average age at diagnosis was 3.8 ± 3.9 years. Males made up 63.8 percent of the patients, while females made up 36.2 percent, for a male/female ratio of 1.76.

We compared the ambulatory and non-ambulatory status of 27 pediatric SMA patients in relation to SMA clinical subtypes. In this analysis, we excluded all patients with SMA type 1 and under 1 year of age. Of the 17 non-ambulatory patients, 14 were type 2 (82.4%). In addition, 8 out of 10 patients who were ambulatory were type 3 (80%) (Fig. 1). Accordingly, there was a statistically significant difference between the SMA subtypes and the ambulation status of the patients (p = 0,003).

We compared the HFMSE scores with clinical subtypes in 27 pediatric SMA patients. According to the results of this analysis, the mean score of type 2 patients was 20.13, and the mean score of type 3 patients was 43. A significant correlation was found between HFMSE scores and clinical subtypes (p = 0,0063) (Fig. 2).

Figure 3 depicts the percentage of individuals with various subtypes of SMA and the matching CNVs of exon 7 of the *SMN2* gene. The number of copies was distributed as 2, 3 and ≥ 4. Significant differences were found between the copy numbers and the three clinical subtypes (p < 0,0001). All eight Type 1 individuals had 2 CNVs. A total of the 22 Type 2 patients'; three had 2 CNVs, 18 had 3 CNVs, and one had 4 CNVs. Three of the 28 Type 3 patients had 2 CNVs, ten had 3 CNVs, and fifteen had ≥ 4 CNVs.

58 SMA patients and 40 healthy controls were analyzed for *NAIP* and *GTF2H2* genes. *NAIP* exon 4 and exon 5 deletions were identified in 8 patients with multiplex PCR and *GTF2H2* deletions in 10 patients with the RFLP method. No *NAIP* or *GTF2H2* deletion could be detected in any of the 40 healthy controls. *NAIP* and *GTF2H2* deletion status were compared between healthy controls and SMA patients via Fisher's two-tailed test. There was a significant difference between the control and the patient group for the *NAIP* gene (p = 0,0095) and *GTF2H2* gene (p = 0,0049), but no significant difference was found between the SMA subtypes. The demographic characteristics of patients with *NAIP* and *GTF2H2* deletions were examined and the clinical features of patients with and without deletions were compared. Also, *SMN2* copy numbers were compared with deletions in *NAIP* and *GTF2H2*. We performed the Fishers exact test to determine the relationship between the del and non-del groups we created (Table 1) and the clinical subtypes and we detected a significant difference. As a result of post-hoc analyses to reveal the reason for this significance, we determined that it was caused by *SMN2* CNVs.

Table 1
Genotype combination and relation with the subtype of SMA

Genotype	Clinical subtype	Number of patients	Genotype	Clinical subtype	Number of patients
ND1 (*NAIP* del + 2 copy *SMN2*)	1	3	GD1 (*GTF2H2* del + 2 copy *SMN2*)	1	2
	2	0		2	1
	3	0		3	1
ND2 (*NAIP* del + 3 copy *SMN2*)	1	0	GD2 (*GTF2H2* del + 3 copy *SMN2*)	1	0
	2	3		2	2
	3	0		3	2
ND3 (*NAIP* del + ≥ 4 copy *SMN2*)	1	0	GD3 (*GTF2H2* del + ≥ 4 copy *SMN2*)	1	0
	2	0		2	1
	3	2		3	1
NN1 (*NAIP* nondel + 2 copy *SMN2*)	1	5	GN1 (*GTF2H2* nondel + 2 copy *SMN2*)	1	6
	2	3		2	2
	3	3		3	2
NN2 (*NAIP* nondel + 3 copy *SMN2*)	1	0	GN2 (*GTF2H2* nondel + 3 copy *SMN2*)	1	0
	2	15		2	15
	3	10		3	8
NN3 (*NAIP* nondel + ≥ 4 copy *SMN2*)	1	0	GN3 (*GTF2H2* nondel + ≥ 4 copy *SMN2*)	1	0
	2	1		2	1
	3	13		3	14

With and without deletion status was divided into 3 groups according to the *SMN2* copy number for both genes. ND: *NAIP* deletion, NN: *NAIP* non-deletion, GD: *GTF2H2* deletion, GN: *GTF2H2* non-deletion.

In our study, *NAIP* deletion was found in 3 (37.5%) of 8 type 1 patients, 3 (13.6%) of 22 type 2 patients, and 2 (7.1%) of 28 type 3 patients. Also, GTF2H2 deletion was found in 2 (25%) of 8 type 1 patients, 4 (18.1%) of 22 type 2 patients, and 4 (14.3%) of 28 type 3 patients (Fig. 4). There was a total of 5 patients with deletions in both *NAIP* and *GTF2H2* genes. One of them was in type 1, 2 of them were in type 2 and 2 of them were in type 3.

Discussion

SMA is a hereditary disease that leads progressive hypotonia and damage of lower motor neurons in the spinal cord's ventral horn (Keinath et al. 2021). SMA is mostly caused by a homozygous deletion of the *SMN1* gene (more than 95% of cases). The copy number of the *SMN2* gene becomes important in the absence of *SMN1* and the severity of the disease diminishes as the number of copies of *SMN2* increases (Jiang et al. 2019). In our study, we evaluated the relationship between different copy numbers of the *SMN2* gene (CNV) and clinical subtypes of SMA. According to this, all of the type 1 SMA patients had 2 CNV, 81.8% of type 2 patients had 3 CNV, 53.5% of type 3 patients had ≥ 4 CNV. Also, 1 CNV was not observed in any of the patients. We observed that there was a significant relationship between *SMN2* copy number and clinical subtypes, which was consistent with the literature (Calucho et al. 2018). Although it is known that the *SMN2* copy number affects the clinical status of SMA patients, it is thought that there are other factors that cause clinical heterogeneity. In this context, SMN locus genes need to be investigated in detail. SMA phenotype has also thought to been associated with *NAIP*, *GTF2H2(p44)* and *SERF1(H4F5)* gene variants located close to the SMN locus (Liu et al. 2016). According to the literature, the SMN locus genes have been related to the severity of SMA (Jiang et al. 2019).

In a study conducted in 2015, it was determined that there was a significant difference between individuals with and without SMA when *NAIP* copy numbers were compared (Fang et al. 2015). Similarly, in another study, remarkable differences were found between SMA patients and controls from the point of *NAIP* copy numbers (Tran et al. 2008). As for our study, we found that there was a significant difference between SMA patients and the healthy control group in terms of *NAIP* deletion. This seems compatible with the literature. However, we were unable to detect a relationship between our molecular findings and clinical features of the patients.

In a Japanese study, it was reported that the NAIP deletion is strongly associated to the clinical severity of SMA and is a prognostic factor of SMA (Akutsu et al. 2002). In a case study, it was emphasized that the *NAIP* gene deletion could indicate the SMA pathology up to the central nervous system (Maeda et al. 2019). Large deletions spanning both the SMN and NAIP genes were detected in numerous of type I SMA patients in the Indian investigation. Furthermore, it has been demonstrated that minor mutations in the SMN1 gene, although not impairing NAIP, can nevertheless result in a severe phenotype (Kesari et al. 2005). The *NAIP* gene, noted as a phenotype modifier is thought to play a significant role in SMA pathogenesis. But its role in pathogenesis has not been fully clear up yet (Ahn, Eun-ji, Yum, Mi-Sun 2016). In 2020, it was studied in 40 SMA patients in Yunnan Province, China, and it was demonstrated synergistic modification effects of the *SMN2* and *NAIP* genes on the SMA phenotype (Zhang et al. 2020). In addition, in a study conducted in Iran in 2019 with pedigree and STR analysis, it was reported that *NAIP* deletion also increases the risk of carriage (Sharifi et al. 2019). *NAIP* gene deletion analysis was performed in twenty-four SMA patients in a Malaysian research to better understand the pathophysiology of the disease, and it was reported that deletion of the NAIP gene was related to disease severity. Furthermore, *NAIP* gene deletions are particularly common in type 1 SMA patients (Watihayati et al. 2007). In another study conducted in Malaysia, *SMN2* copy number and *NAIP* deletion were investigated

in a total of 42 SMA patients. It was determined that *NAIP* deletion was detected in 50% of type 1 SMA patients, 10% of type 2 SMA patients and there was no *NAIP* deletion in type 3 SMA patients (Watihayati et al. 2009). The *SMN2* and *NAIP* copy number distributions were investigated in a research of 232 Chinese SMA patients, with the findings indicating that *SMN2* and *NAIP* copy numbers have a substantial impact on the age of onset, risk of mortality, and life expectancy (Qu et al. 2015). Contrary to the above, there are also publications in the literature reporting that there is no significant relationship between *NAIP* deletion and clinical severity of SMA disease, and that the contribution of *NAIP* deletion to the pathogenesis of SMA is controversial (Rekik et al. 2013)(Tran et al. 2008). In a recent study, *NAIP* copy number was associated with SMA type and *SMN2* copy number, but there was no data that *NAIP* copy number made an additional contribution to predict SMA phenotype (Wadman et al. 2020). The frequency of *NAIP* deletions in SMA patients has been studied in several countries throughout the world, but no data on this has yet been found in Turkey. In this study, we analyzed the *NAIP* deletion in 58 Turkish SMA patients and 40 healthy control groups. While 37% of our type 1 patients have *NAIP* deletion, 13.6% of our type 2 patients and 7.1% of our type 3 patients have *NAIP* deletion. Although *NAIP* deletion was observed at a greater rate in type 1 patients compared to other types in the scope of our study, no statistically significant difference was detected. This may be due to the insufficient number of type 1 patients and our small-scale study group.

In a genotype-phenotype correlation study conducted in Cyprus, it was emphasized that homozygous deletion of *NAIP* and *GTF2H2* can cause a severe phenotype in SMA patients (Theodorou et al. 2015). It has been reported that the lack of *GTF2H2* was strongly related to a severe form of SMA (type I) (He et al. 2013). The copy numbers of telomeric and centromeric genes at the SMA region were examined, and it was determined that telomeric NAIP and telomeric GTF2H2 followed similar patterns (Noguchi et al. 2016). However in an another investigation being conducted to assess if any of the genes in the SMA locus connect with disease severity, it has been reported that no relation between the type of SMA and *GTF2H2* deletion in patients (Arkblad et al. 2009). Exon 10 deletion in the *GTF2H2* gene was found in 17.2% of 58 SMA patients in our study, whereas no deletion was detected in any of the 40 healthy controls. We observed a statistically significant difference between the healthy control and individuals with SMA in terms of *GTF2H2* gene deletion. However, no substantial difference was detected across the SMA subtypes.

It has been reported in the literature that there is a high correlation between HFMSE and SMA type 2 and type 3 (Glanzman et al. 2011). Similarly, in our study, a significantly higher correlation was observed between HFMSE and SMA clinical subtypes (type 2 and type 3). Also, individuals who were ambulatory and non-ambulatory compared with clinical subtypes of SMA including only type 2 and type 3, we detected statistically significant relationship.

Conclusion

To the best of our knowledge, this is the first study that demonstrates if a relationship between the clinical severity of SMA, and *NAIP* and *GTF2H2* genotype, and also exhibited *SMN1*, *SMN2*, *NAIP*, and

GTF2H2 mutation profiles in Turkish patients. Based on data collected from a single center in our country, *NAIP* and *GTF2H2* deletions have a significant association between healthy controls and SMA individuals. It was observed that the genes investigated in our cohort did not have a significant effect on clinical types. This result may be due to the inequality of the number of patients distributed into clinical types, especially type 1 SMA, and small-scale study group. Another limitation of our study is one of the contiguous modifying genes *SERF1* in the SMA-related region which thought to have a role in the severity of the disease was not analyzed.

The absence of *NAIP* and *GTF2H2* deletions in the healthy population suggests that these changes are effective in SMA patients. However, how it affects SMA patients and whether it contributes clinical severity is still unclear. So, more comprehensive studies with larger patient groups are needed.

Finally, our study is important in terms of revealing the mutation profile of the SMN locus genes in SMA patients and presenting a Turkey dataset in this context and may thought to shed light on further studies to be conducted in a more comprehensive and multi-centered manner in Turkey.

Declarations

Acknowledgments: The results reported in this article were partially presented at the Erciyes Medical Genetics Days with International Participation, 21-23 February 2019 as a poster presentation.

Funding information: Not applicable

Author Contributions: Munis Dundar developed the theory and concept of the study. Hilal Akalin designed the study. Nilgun Karasu produced the first draft and conducted the literature review. Mikail Demir interpreted the results clinically. Nilgun Karasu and Mikail Demir completed the entire manuscript. Nuriye Gokce carried out the laboratory studies. Izem Olcay Sahin performed the statistical analysis. Isa Cuce performed the HFMSE scoring of SMA patients. Pediatric clinicians Hamit Acer, Ayten Gulec, Hakan Gumus, Huseyin Per, Mehmet Canpolat and Neurology clinicians Asli Ciplakligil, Ayse Caglar Sarilar provided clinical information and data of the patients.

All the authors collected patient data, and read and approved the final manuscript.

Conflicts of interest: None of the authors have potential conflicts of interest to be disclosed.

Data availability: All data are available upon request.

The data analyzed during the study are available from the corresponding author on reasonable request.

Ethical issues: This study was approved with the decision dated Sept 8th, 2021 and numbered 569 by Erciyes University Clinical Research Ethics Committee.

Informed Consent: All participants gave written informed consent in accordance with the Declaration of Helsinki.

References

1. Ahn, Eun-ji, Yum, Mi-Sun et al. (2016) Genotype-Phenotype Correlation of SMN1 and NAIP Deletions in Korean Patients with Spinal Muscular Atrophy. 13:1–5
2. Akutsu T, Nishio H, Sumino K, et al (2002) Molecular genetics of spinal muscular atrophy: Contribution of the NAIP gene to clinical severity. Kobe J Med Sci 48:25–31
3. Arkblad E, Tulinius M, Kroksmark AK, et al (2009) A population-based study of genotypic and phenotypic variability in children with spinal muscular atrophy. Acta Paediatr Int J Paediatr 98:865–872. https://doi.org/10.1111/j.1651-2227.2008.01201.x
4. Blatnik AJ, McGovern VL, Burghes AHM (2021) What genetics has told us and how it can inform future experiments for spinal muscular atrophy, a perspective. Int J Mol Sci 22:. https://doi.org/10.3390/ijms22168494
5. Calucho M, Bernal S, Alías L, et al (2018) Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. Neuromuscul Disord 28:208–215. https://doi.org/10.1016/j.nmd.2018.01.003
6. Canpolat M, Bayram AK, Bahadır O, et al (2016) Spinal musküler atrofi olgularının klinik özellikleri. Guncel Pediatr 14:18–22. https://doi.org/10.4274/jcp.95866
7. Carter TA, Bönnemann CG, Wang CH, et al (1997) A multicopy transcription-repair gene, BTF2p44, maps to the SMA region and demonstrates SMA associated deletions. Hum Mol Genet 6:229–236. https://doi.org/10.1093/hmg/6.2.229
8. D'Amico A, Mercuri E, Tiziano FD BES muscular atrophy. OJRD 2011 N 2;6:71. doi: 10. 1186/175.-1172-6-71. P 22047105; PP (2011) Spinal Muscular Atrophy. Orphanet J Rare Dis 6:1–10. https://doi.org/10.1186/1750-1172-6-71
9. Dundar M, Uzak AS, Karabulut Y (2010) Healthcare in overview of Turkey. EPMA J 1:587–594. https://doi.org/10.1007/s13167-010-0049-7
10. Fang P, Li L, Zeng J, et al (2015) Molecular characterization and copy number of SMN1, SMN2 and NAIP in Chinese patients with spinal muscular atrophy and unrelated healthy controls. BMC Musculoskelet Disord 16:1–8. https://doi.org/10.1186/s12891-015-0457-x
11. Gartland KMA, Bruschi F, Dundar M, et al (2013) Progress towards the "Golden Age" of biotechnology. Curr Opin Biotechnol 24:S6–S13. https://doi.org/10.1016/j.copbio.2013.05.011
12. Glanzman AM, O'Hagen JM, McDermott MP, et al (2011) Validation of the Expanded Hammersmith Functional Motor Scale in spinal muscular atrophy type II and III. J Child Neurol 26:1499–1507. https://doi.org/10.1177/0883073811420294
13. Goulet B, Kothary R, Parks R (2013) At the "Junction" of Spinal Muscular Atrophy Pathogenesis: The Role of Neuromuscular Junction Dysfunction in SMA Disease Progression. Curr Mol Med 13:1160–

1174. https://doi.org/10.2174/15665240113139990044

14. Hassan HA, Zaki MS, Issa MY, et al (2020) Genetic pattern of SMN1, SMN2, and NAIP genes in prognosis of SMA patients. Egypt J Med Hum Genet 21:0–6. https://doi.org/10.1186/s43042-019-0044-z
15. He J, Zhang QJ, Lin QF, et al (2013) Molecular analysis of SMN1, SMN2, NAIP, GTF2H2, and H4F5 genes in 157 Chinese patients with spinal muscular atrophy. Gene 518:325–329. https://doi.org/10.1016/j.gene.2012.12.109
16. Jiang J, Huang J, Gu J, et al (2019) Genomic analysis of a spinal muscular atrophy (SMA) discordant family identifies a novel mutation in TLL2, an activator of growth differentiation factor 8 (myostatin): A case report. BMC Med Genet 20:3–9. https://doi.org/10.1186/s12881-019-0935-3
17. Keinath MC, Prior DE, Prior TW (2021) Spinal muscular atrophy: Mutations, testing, and clinical relevance. Appl Clin Genet 14:11–25. https://doi.org/10.2147/TACG.S239603
18. Kesari A, Misra UK, Kalita J, et al (2005) Study of survival of motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) gene deletions in SMA patients. J Neurol 252:667–671. https://doi.org/10.1007/s00415-005-0714-2
19. Liang YH, Chen X ling, Yu ZS, et al (2009) Deletion analysis of SMN1 and NAIP genes in southern Chinese children with spinal muscular atrophy. J Zhejiang Univ Sci B 10:29–34. https://doi.org/10.1631/jzus.B0820125
20. Liu Z, Zhang P, He X, et al (2016) New multiplex real-time PCR approach to detect gene mutations for spinal muscular atrophy. BMC Neurol 16:1–12. https://doi.org/10.1186/s12883-016-0651-y
21. Maeda K, Chong PF, Yamashita F, et al (2019) Global Central Nervous System Atrophy in Spinal Muscular Atrophy Type 0. Ann Neurol 86:801–802. https://doi.org/10.1002/ana.25596
22. Maier JKX, Balabanian S, Coffill CR, et al (2007) Distribution of neuronal apoptosis inhibitory protein in human tissues. J Histochem Cytochem 55:911–923. https://doi.org/10.1369/jhc.6A7144.2007
23. Medrano S, Monges S, Gravina LP, et al (2016) Genotype–phenotype correlation of SMN locus genes in spinal muscular atrophy children from Argentina. Eur J Paediatr Neurol 20:910–917. https://doi.org/10.1016/j.ejpn.2016.07.017
24. Mercer EA, Korhonen L, Skoglo Y, et al (2000) NAIP interacts with hippocalcin and protects neurons against calcium-induced cell death through caspase-3-dependent and -independent pathways. 19:3597–3607
25. Noguchi Y, Onishi A, Nakamachi Y, et al (2016) Telomeric Region of the Spinal Muscular Atrophy Locus Is Susceptible to Structural Variations. Elsevier Ltd
26. Nouri N, Sedghi M, Behnam M, et al (2014) Genotype-phenotype correlation of survival motor neuron and neuronal apoptosis inhibitory protein genes in spinal muscular atrophy patients from Iran. Adv Biomed Res 3:74. https://doi.org/10.4103/2277-9175.125872
27. O'Hagen JM, Glanzman AM, McDermott MP, et al (2007) An expanded version of the Hammersmith Functional Motor Scale for SMA II and III patients. Neuromuscul Disord 17:693–697. https://doi.org/10.1016/j.nmd.2007.05.009

28. Pera MC, Coratti G, Forcina N, et al (2017) Content validity and clinical meaningfulness of the HFMSE in spinal muscular atrophy. BMC Neurol 17:1–10. https://doi.org/10.1186/s12883-017-0790-9
29. Qu YJ, Ge XS, Bai JL, et al (2015) Association of copy numbers of survival motor neuron gene 2 and neuronal apoptosis inhibitory protein gene with the natural history in a Chinese spinal muscular atrophy cohort. J Child Neurol 30:429–436. https://doi.org/10.1177/0883073814553271
30. Rekik I, Boukhris A, Ketata S, et al (2013) Deletion analysis of SMN and NAIP genes in Tunisian patients with spinal muscular atrophy. Ann Indian Acad Neurol 16:57–61. https://doi.org/10.4103/0972-2327.107704
31. Roy N, Mahadevan MS, McLean M, et al (1995) The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 80:167–178. https://doi.org/10.1016/0092-8674(95)90461-1
32. Sharifi Z, Forouzesh F, Taheri M, Zeinali S (2019) Constraints of carrier screening in spinal muscular atrophy: Co-existence of deletion and duplication in SMN1 gene and false negative MLPA result. Gene Reports 16:100440. https://doi.org/https://doi.org/10.1016/j.genrep.2019.100440
33. Theodorou L, Nicolaou P, Koutsou P, et al (2015) Genetic findings of Cypriot spinal muscular atrophy patients. Neurol Sci 36:1829–1834. https://doi.org/10.1007/s10072-015-2263-5
34. Tran VK, Sasongko TH, Hong DD, et al (2008) SMN2 and NAIP gene dosages in Vietnamese patients with spinal muscular atrophy. Pediatr Int 50:346–351. https://doi.org/10.1111/j.1442-200X.2008.02590.x
35. Verhaart IEC, Robertson A, Wilson IJ, et al (2017) Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy - A literature review. Orphanet J Rare Dis 12:1–15. https://doi.org/10.1186/s13023-017-0671-8
36. Wadman RI, Jansen MD, Stam M, et al (2020) Intragenic and structural variation in the SMN locus and clinical variability in spinal muscular atrophy. Brain Commun 2:1–13. https://doi.org/10.1093/braincomms/fcaa075
37. Watihayati MS, Fatemeh H, Marini M, et al (2009) Combination of SMN2 copy number and NAIP deletion predicts disease severity in spinal muscular atrophy. Brain Dev 31:42–45. https://doi.org/10.1016/j.braindev.2008.08.012
38. Watihayati MS, Zabidi-Hussin AMH, Tang TH, et al (2007) Deletion analyses of SMN1 and NAIP genes in Malaysian spinal muscular atrophy patients. Pediatr Int 49:11–14. https://doi.org/10.1111/j.1442-200X.2007.02302.x
39. Zhang Y, He J, Zhang Y, et al (2020) The analysis of the association between the copy numbers of survival motor neuron gene 2 and neuronal apoptosis inhibitory protein genes and the clinical phenotypes in 40 patients with spinal muscular atrophy: Observational study. Medicine (Baltimore) 99:e18809–e18809. https://doi.org/10.1097/MD.0000000000018809

Figures

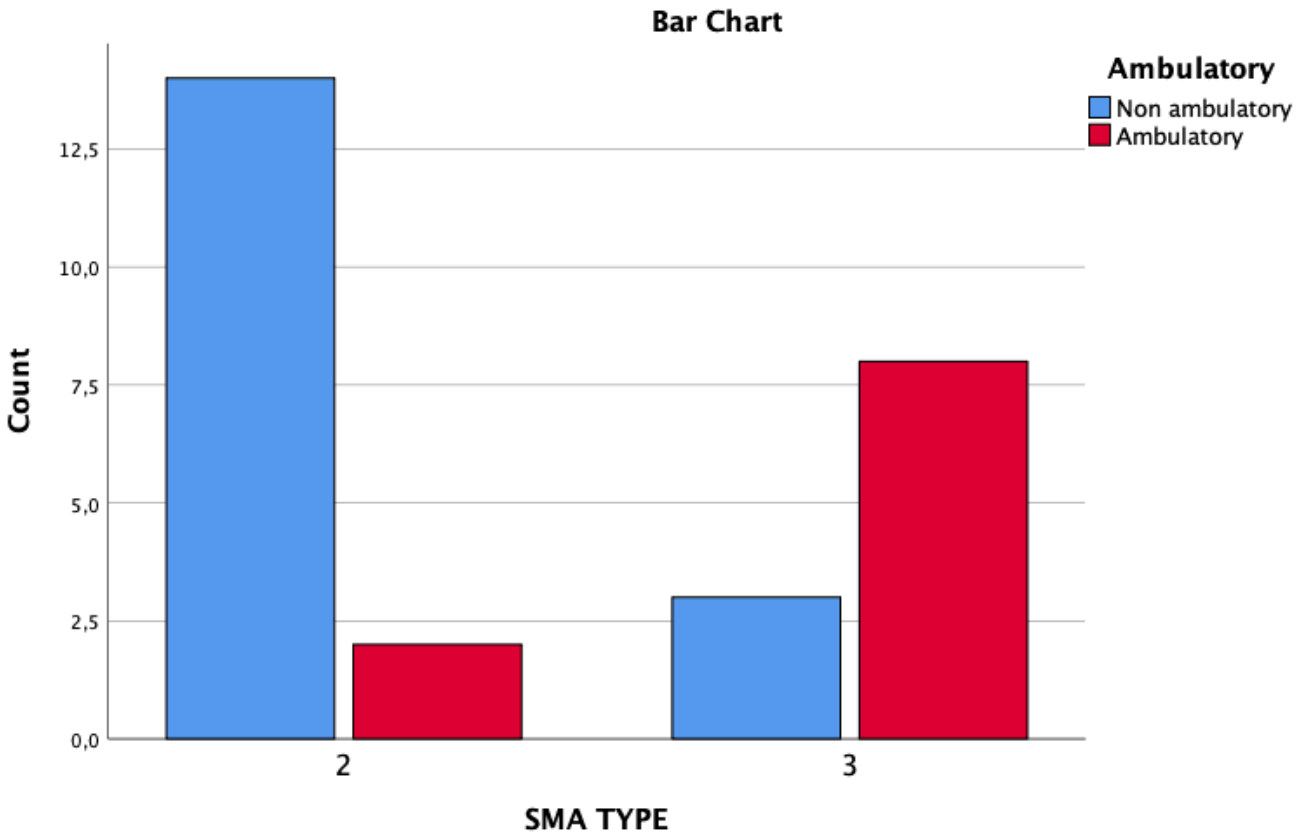

Figure 1

Association of the ambulatory and non-ambulatory status of pediatric SMA patients with clinical subtypes

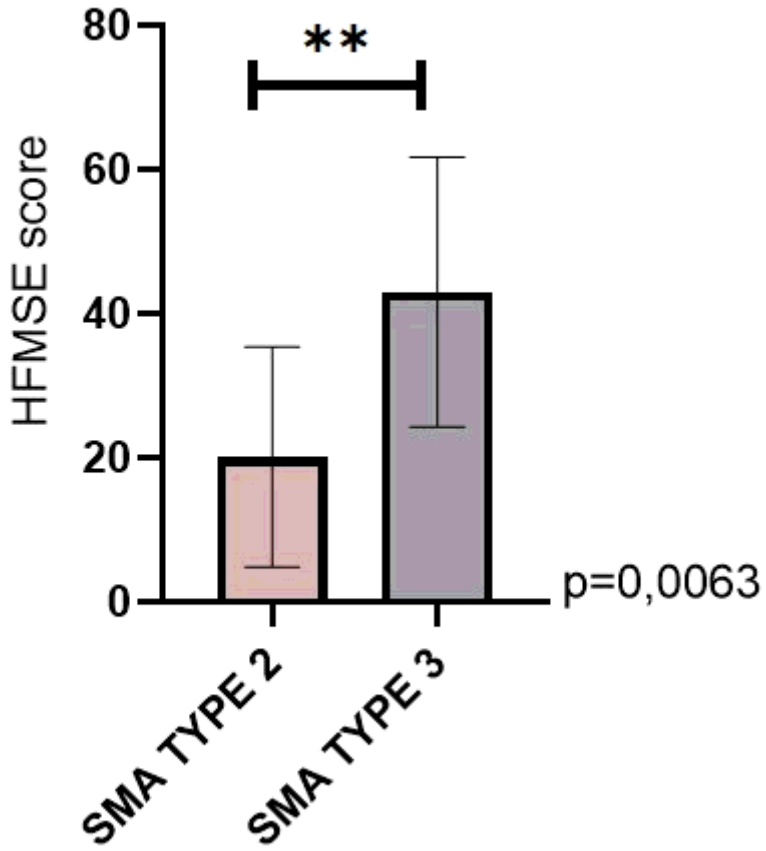

Figure 2

Association of HFMSE scores with clinical subtypes in SMA patients

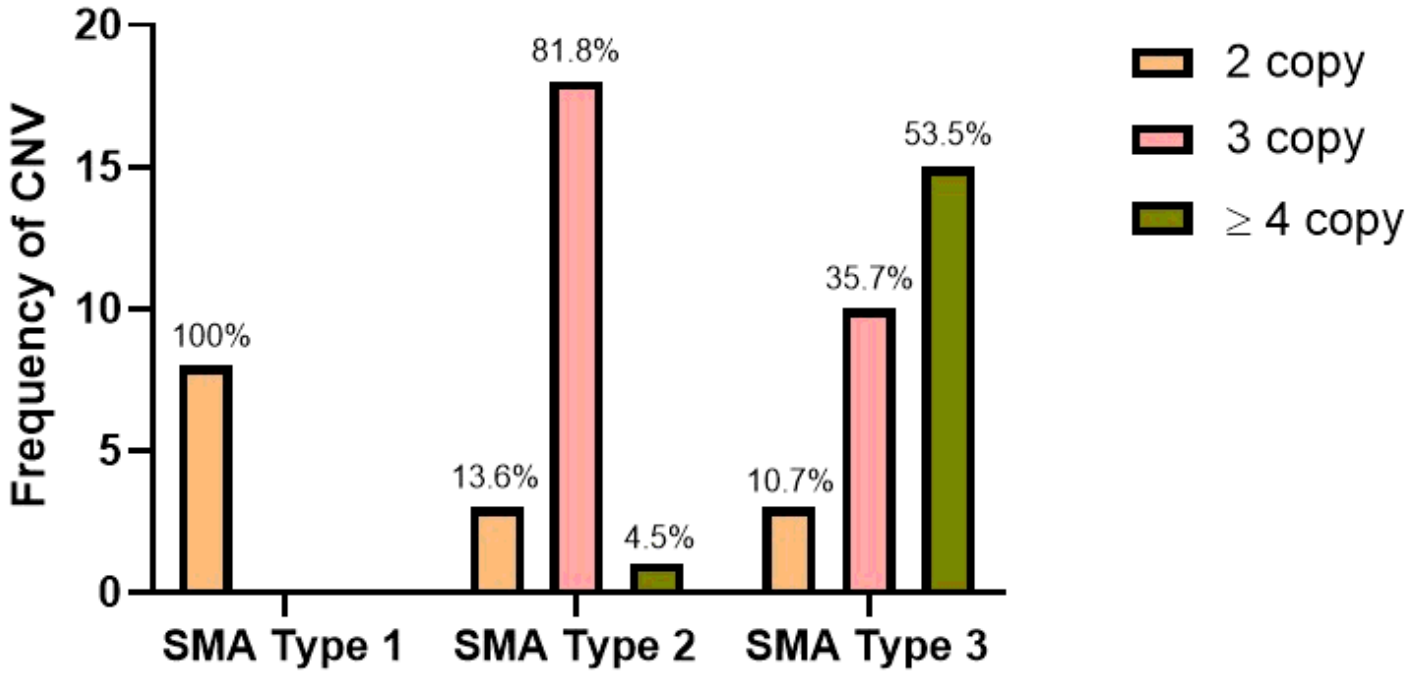

Figure 3

The percentage of individuals with various number of copies of *SMN2* gene in SMA type 1, 2, and 3.

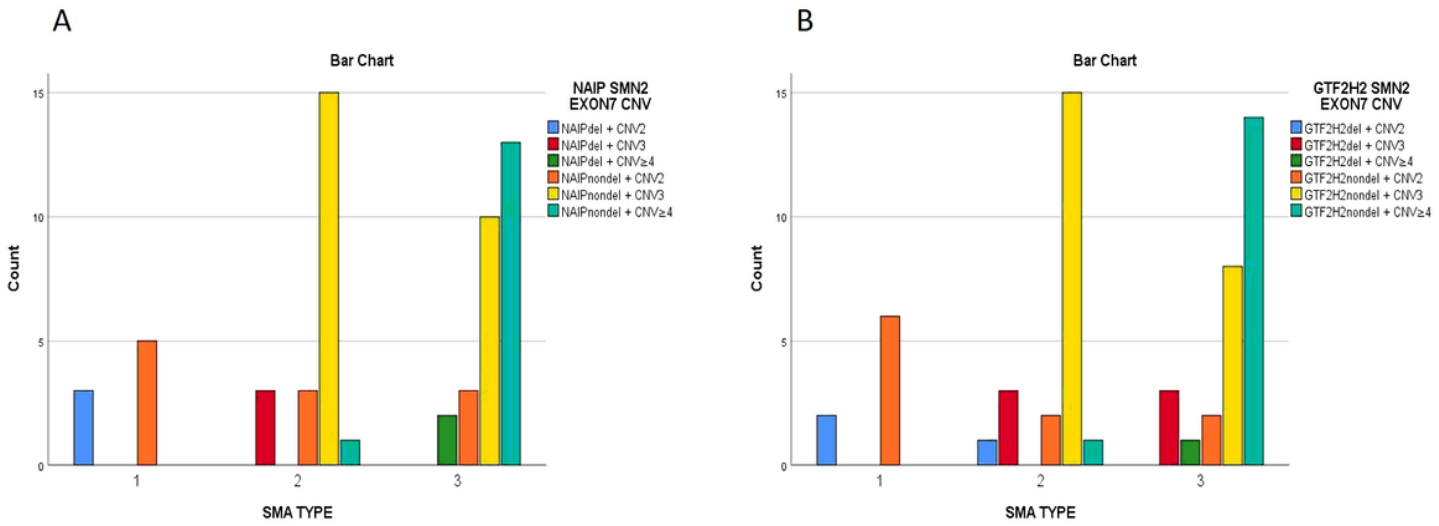

Figure 4

Association of genotype combinations with SMA subtypes. *A*. The association of *NAIP* and *SMN2* copy number combinations with SMA subtypes. *B*. The association of *GTF2H2* and *SMN2* copy number combinations with SMA subtypes. * CNV; *SMN2* copy number variant.