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Case Report
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A Turkish Patient with Spastic Paraplegia Type 4 with a De Novo Missense Mutation in the SPAST Gene

SPAST Geninde De Novo Missense Mutasyonu Olan Spastik Parapleji Tip 4'lü Bir Türk Hasta

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Abstract: Spastic paraplegia type 4 is a common type of autosomal-dominant pure hereditary spastic paraplegia that is brought on by variations in the SPAST gene. In this investigation, the SPAST genotype and clinical phenotype of a Turkish SPG4 patient were analyzed in an effort to provide additional genetic evidence for the pathophysiology of HSP. The clinical data of the proband and his family members were collected. After complete genomic DNA was isolated from peripheral blood, whole-exome sequencing technology was used to identify genes and analyze the pathogenicity of variants. Variants suspected of being pathogenic were found. Within this family, Sanger sequencing was used for verification. The sequencing of SPAST revealed a de novo missense c.1496G > A (p.Arg499His) and missense MEFV c.2177T>C (p.Val726Ala) variants. The parents and paternal relatives did not have the SPAST mutation. De novo variants of the c.1496G > A mutation in SPAST can arise at notably high frequencies. We discussed the case of a Turkish patient and examined the clinical characteristics of patients with the p.Arg499His variation in SPAST that have been documented in the literature. There is growing evidence that the p.Arg499His missense mutation in SPAST may be linked to early-onset HSP. The majority of pathogenic mutations were found in the protein's AAA domain, according to analysis of SPAST sequences; this may be closely related to the pathophysiology of SPG4. The results of this investigation may broaden the range of therapeutic applications for the p.Arg499His mutation in SPAST and offer a chance to investigate the genotype-phenotype relationship of SPG4 in more detail.
Keywords: Spastic Paraplegia Type 4, SPAST Gene, Missense Mutation

Informed Consent: Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images. Ethical approval was not required for this study in accordance with local/ national guidelines.

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Özet: Otozomal dominant kalıtsal spastik paraplejilerin yaygın bir formu, SPAST genindeki mutasyonlardan kaynaklanan spastik parapleji tip 4'tür. Bu araştırmada, HSP'nin patofizyolojisi için ek genetik kanıt sağlamak amacıyla bir Türk SPG4 hastasının SPAST genotipi ve klinik fenotipi analiz edildi. Vaka Sunumu: Probandın ve aile üyelerinin klinik verileri toplandı. Periferik kandan tüm genomik DNA izole edildikten sonra, genleri tanımlamak ve mutasyonların patojenitesini analiz etmek için tüm ekzom dizileme teknolojisi kullanıldı. Şüpheli patojenik mutasyonlar belirlendi. Bu aile için doğrulama Sanger dizilemesi ile gerçekleştirildi. SPAST gen dizilemesi sonucu de novo missense c.1496G > A (p.R499H) ve missense MEFV c.2177T>C (p.Val726Ala) mutasyonları belirlendi. SPAST mutasyonu ebeveynlerde ve akrabalarda yoktu. c.1496G > A mutasyonu SPAST'ta belirgin derecede yüksek oranlarda de novo varyant olarak ortaya çıktığı bilinmektedir. SPAST'ta p.Arg499His mutasyonu olan literatürde bildirilen hastaların klinik özelliklerini inceledik. Elde edilen kanıtlar, erken başlangıçlı HSP ile SPAST'taki p.Arg499His anlamsız mutasyonu arasında olası bir ilişki olduğunu göstermektedir. SPAST dizi analizi, patojenik mutasyonların çoğunun proteinin AAA kodunda meydana geldiğini ve bunun SPG4 patogenezi ile yakın bir ilişkide olabileceğini ortaya koymuştur. Bu araştırmanın sonuçları, SPAST'taki p.Arg499His mutasyonu için terapötik uygulama yelpazesini genişletebilir ve SPG4'ün genotip-fenotip ilişkisini daha ayrıntılı olarak araştırma şansı sunabilir.
Anahtar Kelimeler: Spastik Parapleji Tip 4, SPAST Geni, Missense Mutasyon

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1. Introduction

The progressively developing weakness and spastic paraplegia of the lower limbs are the hallmarks of hereditary spastic paraplegia (HSP), a heterogeneous group of genetic neurodegenerative disorders. There is significant genetic and clinical variety in HSP. It is estimated that 0.1 to 9.6/100,000 people have HSP [1,2]. Autosomal dominant (AD), autosomal recessive (AR), X-linked recessive (XR), or mitochondrial inheritance are the possible modes of inheritance. Spastic paraplegia 4 (SPG4) resulting from variants in the spastin (SPAST) gene contributes for 40–45% of HSP cases in AD-HSP, with SPG3A (ATL1) and SPG31 (REEP1) accounting for roughly 10% and 6.5% of HSP cases, respectively [3,4]. The most severe pathological alteration in HSP is the bilateral corticospinal pathways in the spinal cord experiencing axonal degeneration and/or demyelination. The thoracic segment exhibits the most severe abnormalities in this regard. The clinical manifestations of HSP are varied and complex, with a high risk of missing and incorrect diagnoses. The pathogenesis of HSP is not entirely understood. When diagnosing HSP and identifying phenotypic subgroups, genetic testing is a crucial supplementary tool [5].

More than 60 genes and 80 distinct gene loci have been linked to HSP to date. The most frequently mutated gene in SPG4 among them is SPAST, which is found at 2p22.3 and has 17 exons that span over 90 kb. The 616 amino acid protein known as spastin is a member of the ATPase family, which controls the quantity, length, and vibrancy of microtubules in the cell. Thus far, 683 pathogenic variants in the SPAST gene have been documented. The most prevalent types of variants are nonsense and missense variants, which make up about 37% of instances. The two primary forms of pathogenesis are the increase of function of pathogenic spastin isoforms and the loss of spastin cleavage function [6-8].

Here, we describe a female patient whose de novo SPAST gene variation results in a clinically SPG4 phenotype.

2. Case Report

A 3-year-old girl was the youngest of three siblings born to healthy and unrelated parents. Our patient has walking and speaking delay and global developmental delay. Her 11-year old sister and 13-year-old brother were unaffected. She was born by cesarean section after an healthy pregnancy. Our patient was born in the 39th week of pregnancy with a weight of 3045 g. The patient started to stand at the 10th month, but independent walking was not observed. The anterior fontanelles closed at the 12th month. The nape hairline is low and the forehead is prominent. At 28 months, the family noticed that she was unable to crawl on her hands and knees or stand without assistance. She could not walk independently until now. Our patient also has speech delay. The patient uses single words. she did not have any cerebellar, sensory, or autonomic dysfunctions and was mentally normal. Furthermore, routine laboratory tests were normal. The motor symptoms of the patient progressed slowly, and her gait became increasingly slow and spastic over time.

As a result of the patient's clinical evaluation, karyotyping, Array comparative genomic hybridisation (a-CGH) and Whole-exome sequencing (WES) analysasseses were performed. aCGH analysis and karyotyping were performed first, followed by WES (provided aCGH profile and karyotyping had been normal). Following phytohemagglutinin-stimulated short-term lymphocyte culture, our patient's heparinized peripheral blood sample was used for chromosome analysis utilizing the Giemsa trypsin banding method.

In accordance with the manufacturer's instructions, genomic DNA was extracted from venous blood using a kit (Qiagen, Germany). The human genome CGH Agilent 180K custom array was used for the aCGH study. Every genomic coordinate is in build GRCh37/hg19.

The SPAST genotype and clinical phenotype of an SPG4 family were examined in an effort to find additional genetic evidence for the pathophysiology of HSP. The clinical data of the proband and his family members were collected. The genomic DNA of the patient and his parents was extracted from peripheral blood after obtaining written informed consent, whole-exome sequencing technology was used to identify genes and determine the pathogenicity of variants. Variants suspected of being pathogenic were found. Within this family, Sanger sequencing was used for verification.

Whole-exome sequencing

The Illumina NextSeq550 (Illumina Inc., San Diego, CA, USA) system was used to analyze a clinical

exome gene panel that contained 6699 OMIM genes. Libraries were set up in accordance with the manufacturer's guidelines. Utilizing the Qubit dsDNA BR Assay kit (Invitrogen, Carlsbad, CA), the generated libraries were subjected to quality control. The Illumina NextSeq550 (Illumina Inc., San Diego, CA, ABD) system was used to generate Fastq files. The QIAseq Targeted DNA Panel procedure (Qiagen, Hilden, Germany) was followed in the preparation of libraries encompassing the target genes. Libraries were sequenced using the Illumina NextSeq550 (Illumina Inc., San Diego, CA, USA) system after the target enrichment procedure. Variant Call Format file ordering and quality control were done using QCI analysis (Qiagen, Hilden, Germany). Using Ingenuity software (Qiagen, Hilden, Germany), a variance analysis was conducted.

As a result of WES analysis, the missense NM_014946.4(SPAST):c.1496G>A (p.Arg499His) variant in our patient was identified in the dbSNP database with the number rs878854991. As a result of WES analysis (Figure 1), the patient also had missense NM_000243.3(MEFV):c.2177T>C (p.Val726Ala) was detected. NM_000243.3(MEFV):c.2177T>C (p.Val726Ala) variant was inherited from the father of the patient.

Bioinformatic analysis

The UCSC hg19 human reference genome construct was then used to align the reads. The variants were annotated using ANNOVAR (http://annovar.openbioinformatics.org/en/latest/). Excluded were common locations with population allele rates greater than 5% as reported by the 1000 Genome Project, dbSNP 13 and ExAC databases. The 2015 ACMG Standards and Guidelines were used to interpret the pathogenicity of the variations. This variant was deemed pathogenic based on the following evidence of pathogenicity, as per the norms and guidelines of the American College of Medical Genetics and Genomics: Supporting: PP2, PP3, PP5, moderate: PM1, PM2, PM5, and strong: PS2. A thorough search of ClinVar, the Human Gene Mutation Database (HGMD), and published literature was done to confirm variants The pathogenicity of the new variants found in this study was predicted using Polyphen-2, SIFT, and Mutation Taster.

Sanger sequencing

Using primer-designed software, primers were created to target the location between the upstream and downstream regions of the exon in order to identify the potentially pathogenic mutation. Sanger sequencing was then carried out to confirm the mutation in the proband and other family members. A cosegregation study was performed using the characteristics of this family to evaluate potential pathogenic mutation locations. We validated the p.Arg499His mutation in exon 13 of the SPAST gene, which was in a heterozygous state, using Sanger sequencing.

On the other hand, the patient's parents and younger sister did not have the mutation. The patient in this family carried a mutation that neither her parents nor her healthy sibling had. Her oldest sister and brother were also healthy. These observations suggest that the mutation occurred de novo in the patient.

Table 1. Clinical findings of hereditary spastic paraplegia

Patient	Nucleotide change	Amino acid change	Sex	Duration age	Age of onset	Walking achievement	Functional impairment	Spasticity	Increased reflexes	Dysarthria	MRI or intelligence	Literature
1	c.1496G>A	p.Arg499His	M	12 y	14m	No	6	3	Exaggerated muscle stretch reflexes of both the upper and lower limbs	9 y, lost ability to speak at 12 y	More pronounced hyperintense bilateral PLICs in FLAIR sequences	Ogasawara et al., (2019) (9)
2	c.1496G>A	p.Arg499His	F	36 y	<2 y	NA	6	3	NA	<6 y	Mild hyperintensities in corticospinal tracts (white arrows) in	de Souza et al.,(2016) (10)

											T2-weighted and FLAIR sequences	
3	c.1496G>A	p.Arg499His	F	13 y	20m	No	5	LL spasticity	Lower limb hyper-reflexia	<11 y	Intellectual disability	Gillespie et al.,(2018) (11)
4	c.1496G>A	p.Arg499His	F	5 y	1 y	22 m, hold on to furniture	4	NA	Yes	Early expressive language delays	Low-lying conus medullaris with minimal thickening of the filum terminale	Gillespie et al.,(2018) (11)
5	c.1496G>A	p.Arg499His	M	11 y	1.5 y	48 m, walk alone	2	NA		No	NA	Polymeris et al.,(2016) (12)
6	c.1496G>A	p.Arg499His	M	3 y	1 y	NA	NA	Spasticity	Lower extremity hyper-reflexia, Babinski sign	No	NA	Park et al.,(2015) (13)
7	c.1495C>T	p.Arg499Cys	M	20 y	13 y	NA		1	LL increased; UL normal/yes	No	NA	Depienne et al.,(2006) (14)
8	c.1496G>A	p.Arg499His	M	>40 y	Childhood	Limited walking without aid	3	3	Not determined	NA	NA	Depienne et al.,(2006) (14)
9	c.1495C>T	p.Arg499Cys	F	60 y	Childhood	NA	3	3	Lower limb increased; upper limb normal	NA	NA	Depienne et al.,(2006) (14)
10	c.1495C>T	p.Arg499Cys	NA	63 y	Childhood	Need help for daily life	6	3	LL, UL/bilateral	NA	NA	Ribaï et al.,(2008) (15)
11	c.1495C>T	p.Arg499Cys	NA	55 y	51 y	Need help for daily life	6	1	LL, UL/bilateral	NA	NA	Ribaï et al.,(2008)
12	c.1495C>T	p.Arg499Cys	NA	53 y	Adolescence	Partially need help for daily life	2	3	LL, UL/bilateral	NA	Cortical and subcortial atrophy, nonspecific WMH	Ribaï et al.,(2008) (15)
13	c.1495C>T	p.Arg499Cys	NA	47 y	4 y	Need help for daily life	5	3	LL, UL/bilateral	NA	Cortical atrophy	Ribaï et al.,(2008) (15)
14	c.1495C>T	p.Arg499Cys	NA	45 y	5 y	None	6	3	LL, UL/bilateral	NA	NA	Ribaï et al.,(2008) (15)
15	c.1495C>T	p.Arg499Cys	NA	43 y	Birth	Need help for daily life	5	3	LL/bilateral	NA	Cortical and subcortical atrophy	Ribaï et al.,(2008) (15)
16	c.1495C>T	p.Arg499Cys	NA	39 y	Childhood	Partially need help for daily life	6	3	LL, UL/bilateral	NA	NA	Ribaï et al.,(2008) (15)
17	c.1495C>T	p.Arg499Cys	NA	29 y	Birth	Partially need help for daily life	6	3	LL, UL/bilateral	NA	Normal	Ribaï et al.,(2008) (15)
18	c.1495C>T	p.Arg499Cys	NA	27 y	1 y	Partially need help for daily life	3	2	LL/bilateral	NA	NA	Ribaï et al.,(2008) (15)
19	c.1495C>T	p.Arg49	NA	24 y	Childhoo	Need	3	2	LL/bil	NA	NA	Ribaï et

		9Cys			d	help for daily life			ateral			al.,(2008) (15)
20	c.1496G>A	p.Arg499His	F	27 y	Childhood	No	6	Yes	Lower limb increased, positive Babinski sign	Speech is slow, slurred, and the voice diminishes at 22 y	thoracic spinal cord atrophy	Ribaï et al.,(2008) (15)
21	c.1496G>A	p.Arg499His	F	3 y	28 m	Need help for daily life	NA	NA	NA	Early expressive language delays	NA	this report

LL, lower limb; UL, upper limb. Functional impairment: 0—none, 1—no functional impairment but signs at examination, 2—mild, 3—moderate, 4—walking with one cane, 5—walking with two canes, and 6—wheelchair-bounded. Gait spasticity: 0—none, 1—mild, 2—moderate, and 3—severe.

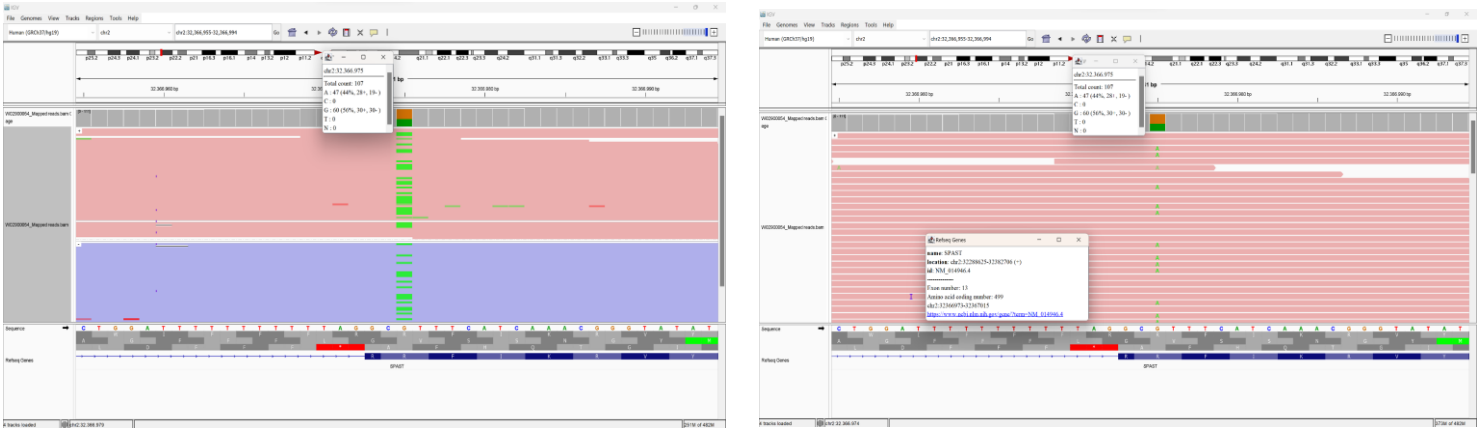

Figure 1. NM_014946.4(SPAST):c.1496G>A IGV image

3. Discussion

We detailed the clinical development of an SPG4 patient who carried a SPAST missense mutation. Numerous earlier studies have identified the p.Arg499His mutation in the SPAST gene as a disease-causing mutation [9]. This mutation is essential for microtubule-severing activity and is found in the spastin AAA ATPase cassette, which runs from amino acids 342 to 616. In patients with SPG4, more than 200 distinct variants at locations within the AAA region have been found [10]. Table 1 demonstrates that there were 20 patients with walking difficulties and lower limb spasticity who had p.Arg499His or p.Arg499Cys, an incidental variant for HSP [9,10-15]. With the exception of p.Arg499His (4/6), it starts early and affects certain people with dysarthria and mental deficiencies. In the present study, the patient also had walking and speech difficulties.

For SPAST mutations, the genotype-phenotype association has not yet been well defined. However, a growing body of evidence indicates that severe infantile-onset complex HSP is linked to the p.Arg499His mutation in SPAST [16].

Currently, HSP is thought to comprise a wide range of genetically diverse diseases[17]. Out of all HSP-SPAST cases, around 75% are hereditary, with de novo variants accounting for the other 25% of occurrences [5]. Patients with SPG4 are mostly treated for symptoms, as there is currently no cure. For impacted families, genetic counseling is crucial. HSP could also be considered and genetic testing should be done when people without a family history experience growing walking difficulties, lower limb spasticity, and other symptoms such tendon hyperreflexia.

The clinical traits and sequencing analysis findings of a patient with SPG4 were presented in the current study. The range of pathogenic variants causing SPG4 is increased by the discovery of a novel SPAST mutation, which also offers data for genetic counseling.

The current study's outcomes highlight the necessity of using molecular analysis to better identify certain variations and assess their functional significance in patients with spastic paraplegia.

In conclusion, we encountered a case of childhood-onset pure SPG4 phenotype caused by a de novo mutation in the SPAST gene in a Turkish patient. This work may increase our understanding of the variation spectrum of SPG4 and offer a clinical foundation for future investigations. This work may offer a chance to investigate the genotype-phenotype link of SPG4 in more detail and may help physicians who are doing genetic testing on patients who have difficult HSP with an early onset.

Established Facts and Novel Insights

Established Facts

· More than 60 genes and more than 80 distinct gene loci have been linked to hereditary spastic paraplegia (HSP) to date.

· The pathogenesis of HSP is not entirely understood.

· Spastic paraplegia 4 (SPG4) resulting from variants in the spastin (*SPAST*) gene contributes to 40–45% of HSP cases in AD-HSP, with SPG3A (ATL1) and SPG31 (REEP1) accounting for roughly 10% and 6.5% of HSP cases, respectively

Novel Insights

· Numerous earlier studies have identified the p.R499H mutation in the *SPAST* gene as a disease-causing mutation

· In patients with SPG4, more than 200 distinct variants at locations within the AAA region have been found

· Out of all HSP-*SPAST* cases, around 75% are hereditary, with *de novo* variants accounting for the other 25% of occurrences.

· The current study's outcomes highlight the necessity of using molecular analysis to better identify certain variations and assess their functional significance in patients with spastic paraplegia.

· In a Turkish patient, we found a case of pure SPG4 phenotype with childhood onset brought on by a de novo variation in the *SPAST* gene. This work may increase our understanding of the variation spectrum of SPG4 and offer a clinical foundation for future investigations.

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