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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Clinical, Radiological, and Molecular Findings in Cases with TRAPPopathies

TRAPPopatili Olgularda Klinik, Radyolojik ve Moleküler Bulgular

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ABSTRACT

Objective: Pathologies occurring in Transport Protein Particles (TRAPP) involved in vesicular traffic are rare diseases called TRAPPopathies. The aim of this study was to present a case series of TRAPPopathies, to describe the clinical and molecular findings, and additionally to review our cases together with other cases reported from Turkiye.

Materials and Methods: Patients with neurological findings such as microcephaly, epilepsy, muscular dystrophy, and intellectual disability who were referred to Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Genetics between March 2018 and March 2020 were reviewed for this study. Patients with pathogenic variants in genes with TRAPP complex family with known phenotype or not yet associated any human disease were included in the study. Clinical, radiological, and molecular findings obtained by whole exome sequences of cases were re-evaluated.

Results: Molecular analysis revealed homozygous c.454+3A>G p.(?) variant in *TRAPPC4* (NM_016146.5) gene in Case 1 with neuromotor retardation, intractable seizures, postnatal microcephaly, and cerebral-cerebellar atrophy, homozygous novel c.57C>G p.(Try19Ter) variant in *TRAPPC6B* (NM_001079537.1) in Case 2 with epilepsy, postnatal microcephaly, severe neuromotor retardation, and autism, and homozygous c.2938G>A p.(Gly980Arg) variant in *TRAPPC11* (NM_021942.5) gene in Case 3 with muscular dystrophy, cataract, neuromotor retardation, and microcephaly.

Conclusion: This study showed that newly identified genes in TRAPPopathies are responsible for microcephaly, developmental delay, epilepsy, intellectual disability, cerebral-cerebellar atrophy, and autism. Although the genes in the TRAPP family work independently of each other, the diseases in this group are called TRAPPopathies because their phenotypes overlap. The aim of our study was to discuss the clinical findings and to summarize the mutation profile of the genes in the TRAPP family in Turkiye.

Keywords: Microcephaly, rare diseases, TRAPPopathies

ÖZ

Amaç: Vezikül trafiği ile ilişkili Taşıyıcı Protein Parçacıklarında (TRAPP) meydana gelen patolojiler TRAPPopatiler olarak adlandırılan nadir hastalıklardır. Bu çalışmanın amacı, bir TRAPPopati vaka serisini sunmak, klinik ve moleküler bulguları tanımlamak ve ayrıca vakalarımızı Türkiye'den bildirilen diğer vakalarla birlikte gözden geçirmektir.

Gereç ve Yöntem: Mart 2018-Mart 2020 tarihleri arasında Bezmialem Vakıf Üniversitesi Tıp Fakültesi, Tıbbi Genetik Anabilim Dalı'na sevk edilen mikrosefali, epilepsi, kas distrofisi ve zihinsel yetersizlik gibi nörolojik bulguları olan olgular bu çalışma için gözden geçirildi. Fenotipi bilinen veya henüz herhangi bir insan hastalığı ile ilişkili olmayan TRAPP kompleks familyasına sahip genlerde patojenik varyantları olan hastalar çalışmaya dahil edildi. Klinik, radyolojik ve tüm ekzom dizilerinden elde edilen moleküler bulgular yeniden değerlendirildi.

Bulgular: Nöromotor retardasyon, nöbet, postnatal mikrosefali ve serebral-serebellar atrofili Olgu 1'de *TRAPPC4* geninde (NM_016146.5) homozigot c.454+3A>G p.(?) varyantı, epilepsi, postnatal mikrosefali, nöromotor retardasyon ve otizmi olan Olgu 2'de *TRAPPC6B* geninde (NM_001079537.1) homozigot daha önce bildirilmemiş yeni c.57C>G p.(Try19Ter) varyantı ile musküler distrofi, katarakt, nöromotor retardasyon and mikrosefalili Olgu 3'te *TRAPPC11* geninde (NM_021942.5) homozigot c.2938G>A p.(Gly980Arg) varyantı saptandı.

Sonuç: Bu çalışma, TRAPPopatilerde yeni tanımlanan genlerin mikrosefali, gelişimsel gecikme, epilepsi, zihinsel yetersizlik, serebral-serebellar atrofi ve otizm bulgularından sorumlu olduğunu göstermiştir. TRAPP ailesindeki genler birbirinden bağımsız çalışsa da bu gruptaki hastalıklara fenotipleri örtüştüğü için TRAPPopatiler adı verilir. Çalışmamızda klinik bulguların tartışılması ve Türkiye'deki TRAPP ailesindeki genlerin mutasyon profilinin özetlenmesi amaçlanmıştır.

Anahtar Kelimeler: Mikrosefali, TRAPPopati, nadir hastalıklar

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INTRODUCTION

The correct localization of proteins within the cell is essential for the cell to maintain its biological functions, both spatially and temporally. Recent advances in molecular genetics technologies, such as next-generation sequencing, have facilitated the identification of an increasing number of intracellular trafficking (1).

One of these diseases is a group of rare diseases called TRAPPopathies associated with TRAnsport Protein Particles (TRAPP). TRAPP proteins have been shown to be involved in membrane transport between the endoplasmic reticulum (ER) and the Golgi apparatus (1-3). The TRAPP family was first described as a multi-subunit vesicle-binding complex in yeast. Two subtypes of this protein family, which are subtypes of TRAPP-I, TRAPP-II, and TRAPP-III in yeasts, are similar to TRAPP-II and TRAPP-III identified in humans due to the difference in subunits with a common core structure. The core structure of the TRAPP complex in humans includes C1, C2, C2L, C3, C4, C5, C6, and C6B proteins. In addition to this core structure, there are C9 and C10 proteins in TRAPPII and C8, C11, C12, and C13 proteins in TRAPPIII (1-3). Neurological diseases associated with TRAPPopathies, in the order of elucidation of the disease-related phenotype in the Online Mendelian Inheritance in Man (OMIM), are autosomal recessive mental retardation 13 (MRT13), autosomal recessive limb-girdle muscular dystrophy 18 (LGMDR18), early-onset progressive encephalopathy with brain atrophy and spasticity (PEBAS), early-onset progressive encephalopathy with episodic rhabdomyolysis, neurodevelopmental disorder with microcephaly, epilepsy, and brain atrophy (NEDMEBA), intellectual disability, speech delay, facial dysmorphism, polydactyly, and neurodevelopmental disorder with epilepsy, spasticity, and brain atrophy (NEDESBA), which are caused by the genes *TRAPPC11, TRAPPC12, TRAPPC9, TRAPP6B*, *TRAPPC6A*, and *TRAPPC4*, respectively (4-6). The inheritance pattern of all identified phenotypes associated with TRAPPopathies has been autosomal recessive.

In this study, clinical, radiological, and molecular findings of three cases with pathogenic variants in genes encoding *TRAPPC4, TRAPPC6B,* and *TRAPPC11* proteins are discussed. In addition, we review the findings of our cases by comparing them with the cases of TRAPPopathies previously reported from Turkiye.

MATERIALS AND METHODS

The study included 256 patients with neurological findings (microcephaly, epilepsy, muscular dystrophy, intellectual disability) who were referred to the outpatient clinic of Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Genetics, between March 2018 and March 2020, and who underwent whole exome sequencing (WES) analysis after detailed clinical evaluation. Patients with pathogenic, likely pathogenic, and uncertain significance variants in human disease-associated TRAPPopathies genes according to the American College of Medical Genetics and Genomics (ACMG) criteria were included in the study (7).

For clinical evaluation, detailed three-generation pedigree, clinical and family history, physical and neurological examination, biochemical and metabolic analysis, and radiological findings of the cases were evaluated. DNA was extracted from whole blood using the Puregene® Blood Extraction Kit (Gentra Systems, Qiagen Inc., Mississauga, ON, Canada). WES was performed on Illumina NextSeq 500 (Illumina, San Diego, CA, USA) with the Illumina TruSeq Rapid Capture Exome Library Prep Kit (Illumina, San Diego, CA, USA). Variants were visualized in Alamut Visual Plus version v1.4,

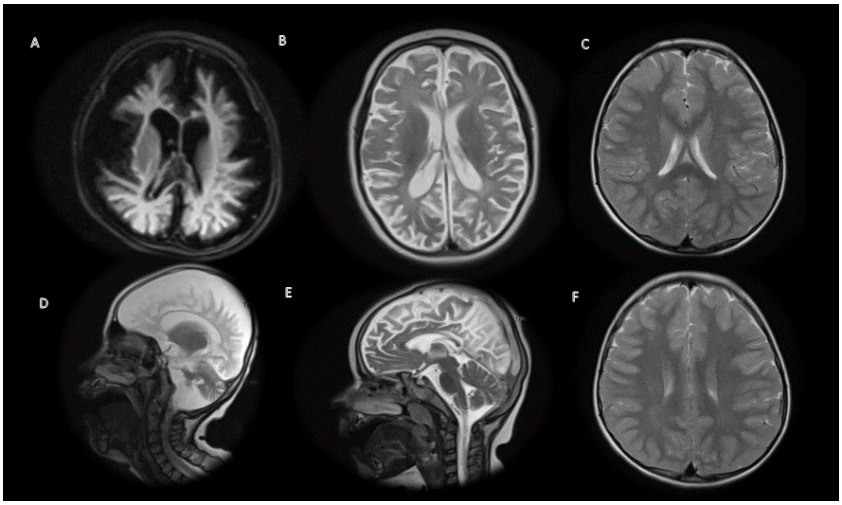

Figure 1: MRI findings of patients with variants in genes encoding for proteins within the TRAPP complex

(A, D) Axial T1-weighted and sagittal T2-weighted images showing severe cerebral and cerebellar atrophy in Case 1 with a pathogenic variant in the *TRAPPC4* gene at 3 years and 3 months of age. **(B, E)** Axial T2-weighted and sagittal T2-weighted images showing cerebral atrophy in Case 2 with a pathogenic variant in the *TRAPPC6B* gene at 2 years and 3 months of age. **(C, F)** Axial T2-weighted images showing hyperintense signal changes in the posterior portions of both lateral ventricles and subcortical regions of the occipital lobe in Case 3 with pathogenic variant in the *TRAPPC11* gene at 3 years and 2 months of age.

2021 (SOPHiA GENETICS, Lausanne, Switzerland). Variants with minor allele frequency (MAF) > 1% in the 1000 Genomes Project, Exome Aggregation Consortium (ExAC v0.3), Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org), and ESP6500 were excluded. Known and common SNPs or non-disease polymorphisms were filtered out. Variants with a frequency of less than 1% in the population were selected. Variants were validated using the Human Gene Mutation Database (HGMD) (http://www.biobase-international.com/product/hgmd), the dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and the ClinVar database (http://www.ncbi.nlm.nih.gov). The study was reviewed and approved by the Ethics Committee of Bezmialem Vakif University Faculty of Medicine (approval number: 2019/2659). Written informed consent was obtained from all parents of the patients included in the study.

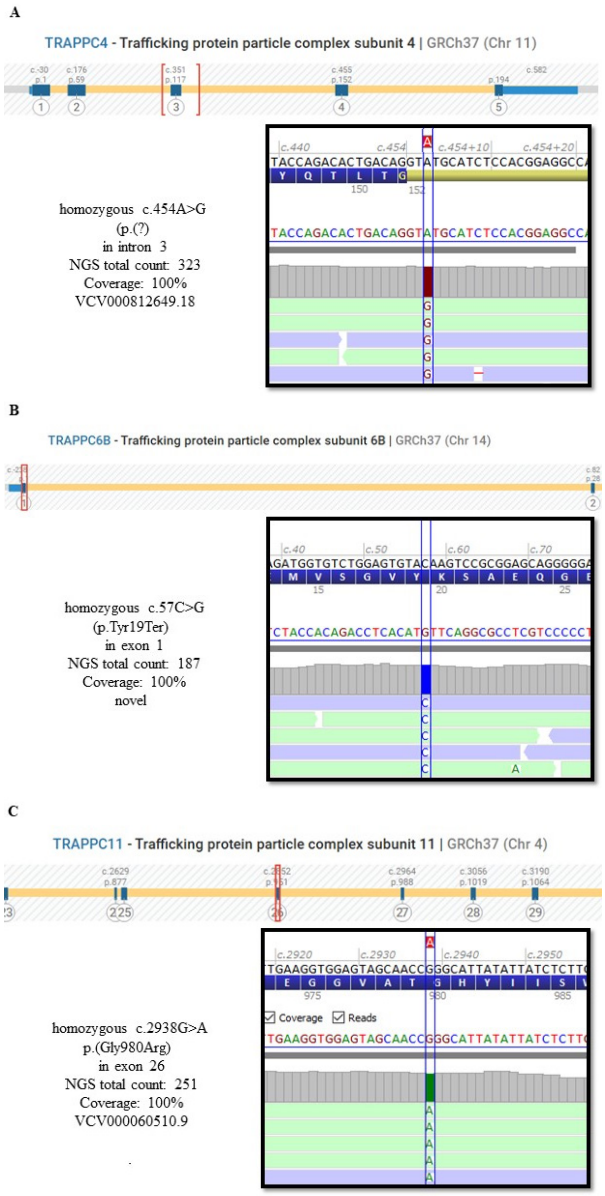

Figure 2: Schematic diagrams and BAM visualization of next generation sequencing using Alamut Visual Plus version v1.4 of *TRAPPC4*, *TRAPPC6B,* and *TRAPPC11* genes

A Case1, homozygous c.454+3A>G p.(?) in the *TRAPPC4* gene

B Case 2, homozygous c.57C>G p.(Try19Ter) in the *TRAPPC6B* gene

C Case 3, homozygous c.2938G>A p.(Gly980Arg) in the *TRAPPC11* gene

RESULTS

Clinical findings

Case 1, a 5 year and 4 month-old female (G3P3), the third child of a first degree cousin marriage) was evaluated for neurodevelopmental disease. The patient, whose family history was unremarkable, had two healthy sisters aged nine and one. After the progression of oligohydramnios, which started at 34th gestational week (GW) in the antenatal period, the pregnancy was terminated by cesarean section at 37th GW with a height of 50 cm (0.87 SD), a weight of 2775 g (0.2 SD), and a head circumference (HC) of 33 cm (-0.45 SD). Her neuromotor development was normal in the first three months and the patient could smile and had eye tracking. ACTH treatment, which was started at the age of 3.5 months with the diagnosis of infantile spasm, was discontinued and valproic acid and clonazepam were started. Generalized chaotic multiple spike wave discharges were observed in EEG examination. Magnetic resonance imaging (MRI) scans showed significant atrophy in the frontotemporal region and severe cerebral-cerebellar atrophy at the age of 3 years (Figure 1 A, D). Poor eye tracking was noted after 9 months of age and the optic disc was pale in the eye examination. At 5 years and 4 months of age, she had severe progressive microcephaly [HC: 41 cm (-6.67 SD)], axial hypotonicity, and spastic tetraparesis. She had a large mouth with bitemporal narrowness, full cheeks, and thin lips.

Case 2, a 4 year and 2-month-old male patient (G2P2), the first child of a first degree cousin marriage) was evaluated for epilepsy, microcephaly, severe neuromotor developmental delay, absent speech, and autism. Family history was unremarkable and his older brother was healthy. The case was delivered at 39th GW by normal spontaneous vaginal delivery with a weight of 3000 g (0.94 SD), a height of 50 cm (-0.95 SD), and a head circumference of 32 cm (-2.32 SD). The patient had global hypotonia from birth. Generalized tonic seizures were noted at the first month. He acquired head control at 2 years of age. At the time of our evaluation at 3 years of age, he had severe microcephaly [HC: 43 cm (-4.50 SD)] axial hypotonia and brisk deep tendon reflexes in four extremities. No obvious facial dysmorphism was noted. Cerebral atrophy was detected on cranial MRI (Figure 1 B, E).

Case 3, a 3 year and 4-month-old male patient (G2P2), the first child of a second degree cousin marriage) was evaluated due to muscular dystrophy. Family history revealed no similar affected patients in the family. He had a healthy 6-year-old brother. Intrauterine growth retardation was detected in the last trimester; the case was born with a weight of 2300 g (-2.61 SD) by cesarean section at 38th GW due to fetal

Table 1: Clinical, radiological and molecular findings of cases with TRAPPopathies from Türkiye

Gene	TRAPPC4	TRAPPC6B	TRAPPC9	TRAPPC11	TRAPPC12
Developmental delay	(+)	(+)	(+)	(+)	(+)
Intellectual disability	(+)	(+)	(+)	(+)	(+)
Microcephaly	(+)	(+)	(+)	(+)/(-)	(+)/(-)
Epilepsy	(+)	(+)	(+)	(+)/(-)	(+)/(-)
Cerebral atrophy	(+)	(+)	(+)	(+)/(-)	(+)
Cerebellar atrophy	(+)	(+)	(+)	(+)/(-)	(+)
Autism	(-)	(-)	(+)	(-)	(-)
Facial dysmorphism	(+)	(-)	(-)	(-)	(-)
Muscular dystrophy	(-)	(-)	(-)	(+)	(-)
Cataract	(-)	(-)	(-)	(+)	(-)
References & Mutations					
Koeher et al. 16				c.1893+3A>G	
Van Bergen et al.5	c.454+3A>G				
Aslanger et al.17					c.1880C>T and c.679T>G
Aslanger et al.18			c.696C>G		
Olmez et al.11	c.454+3A>G				
Bolat et al.19			c.484G>T		
current study	c.454+3A>G	c.57C>G		c.2938G>A	

distress. Birth height and HC were unknown. The patient, who was hospitalized for 3 days due to postnatal hypoglycemia, had a history of neonatal onset hypotonicity. Head control was acquired at the age of 9 months and sitting without support at the age of 2. He had been operated on for bilateral cataract at 2.5 years of age. In his physical examination at 3 years and 4 months, his height was 95 cm (-1.05 SD), his weight was 12 kg (-2.11 SD), HC was 46 cm (-2.96 SD). The patient, who had central hypotonia, could not walk independently yet. The creatine kinase level was found to be 9482 U/L (26-192). MRI showed hyperintense signal changes in the posterior portions of both lateral ventricles and subcortical regions of the occipital lobe (Figure 1 C and F).

Molecular findings

In Case 1, a homozygous c.454+3A>G p.(?) variant in the *TRAPPC4* (NM_016146.5) gene was identified. This variant was previously reported to be associated with a neurodevelopmental genetic disorder, called NEDESBA. It is considered a pathogenic/likely pathogenic variant, as it has been registered in the ClinVar database with the reference number VCV000812649.18. According to the ACMG diagnostic criteria, this variant was classified as pathogenic. Furthermore, the variant is listed as rs375776811 in the dbSNP database and was identified in 0.024% (68/281054) of individuals in the gnomAD.

In Case 2, a homozygous c.57C>G p.(Try19Ter) variant in *TRAPPC6B* (NM_001079537.1) gene was detected. This variant was a novel variant, leading to a null variant, and classified as pathogenic according to ACMG criteria. The variant was found to be extremely rare, with a frequency of 1/251392 in the gnomAD, accounting for 0.0004%. Furthermore, the variant was registered as rs1397140571 in the dbSNP database but was not reported in the ClinVar database.

In case 3, a homozygous c.2938G>A p.(Gly980Arg) in *TRAPPC11* (NM_021942.5) gene was identified. This variant was previously reported in association with autosomal recessive limb-girdle muscular dystrophy subtype 18. The variant was classified as pathogenic according the ACMG diagnostic criteria. Additionally, it has been assigned a pathogenic/likely pathogenic classification, which is supported by its registration in the ClinVar database with the reference number VCV000060510.9. This variant is also identified as rs397509417 in the dbSNP. The radiological and molecular findings of the cases are illustrated in Figures 1 and 2, respectively.

DISCUSSION

Intracellular traffic is a mechanism that allows the exchange of signals and metabolites between organelles. Diseases caused by defects in intracellular trafficking are often associated with vesicular transport, which is the main communication process between membrane traffic and organelles. Vesicular transport allows proteins in membrane-bound vesicles to move between cell compartments, including the plasma membrane. In vesicular transport, proteins are transported from one cell organelle to another via carrier vesicles. TRAPPopathies are diseases associated with pathogenic variants in genes encoding proteins that function in vesicular trafficking (1,8-10). TRAPPopathies with neurological signs associated with

the TRAPP complex are diseases that cause progressive microcephaly, cerebral-cerebellar atrophy, intellectual disability, autism, epilepsy, muscular dystrophy, and severe neuromotor developmental delay (4-6).

In this study, the clinical, radiological, and molecular findings of three cases with pathogenic variants in genes encoding TRAPPC4 and TRAPPC6B proteins in the core complex and TRAPPC11 proteins in the TRAPPCIII complex are discussed.

Case 1 presents with a severe neurodevelopmental disorder including severe microcephaly, cerebral-cerebellar atrophy, intractable epilepsy, spasticity, and facial dysmorphism. Notably, there was no history of perinatal asphyxia, which may be one of the most important causes of this severe condition. The patient's phenotype was attributed to a previously reported homozygous c.454+3A>G variant in intron 3 of the *TRAPPC4* gene, which was predicted to cause a truncating effect by altering a splice site and resulting in the skipping of exon 3, a frameshift, and premature termination (Leu120AspfsTer9), leading to a truncated and likely non-functional TRAPPC4 protein. The *TRAPPC4* gene, initially identified in a 2020 study by Van Bergen et al., was found to harbor a homozygous c.454+3A>G (p.?) variant in eight cases from three unrelated families of Caucasian, Turkish, and French-Canadian origin with a similar syndromic neurodevelopmental disorder, characterized by developmental delay, intellectual disability, microcephaly, and facial dysmorphism (5). In these cases, postnatal onset microcephaly (up to -7 SD), hypotonia, spasticity, failure to gain, psychomotor developmental stages, severe feeding difficulties requiring nasogastric, early onset seizures, cortical visual impairment, and/or poor visual tracking and hearing impairment with dysmorphic features including bitemporal narrowing, full cheeks, prominent nasal tip, long philtrum, wide-open mouth with thinly curved upper lip, and pointed chin were reported. Cranial imaging revealed progressive severe cerebral and cerebellar atrophy. In vitro functional studies confirm that this variant causes splicing error and show a reduction in TRAPP complexes and affecting ER/Golgi trafficking as well as autophagy formation compared to controls. Ghosh et al. reported the same c.454+3A>G (p.?) variant with similar clinical findings in 23 cases from 17 unrelated families in 2021 (6).

The c.454+3A>G variant in the *TRAPPC4* gene, albeit rare according to the gnomAD database, has been calculated to exist in a heterozygous state in at a rate of 2.4 to 5.4 of 10,000 individuals based on research laboratory data on rare diseases. The implications of the carrier frequencies of the recently identified *TRAPPC4* gene are noteworthy. Re-analyses of the exomes performed on patients manifesting the aforementioned phenotype, who had undergone exome sequencing prior to the discovery of the *TRAPPC4* gene, may potentially unveil previously undiagnosed cases affected by TRAPPC4-related disease. In the literature, the same variant was reported in a case from Turkiye in 2022 who was found to have severe cerebral-cerebellar atrophy after infantile spasm (11). So far, two missense (c.191T>C, c.278C>T), one splice (c.454+3A>G), and one gros deletion have been identified. Majethia et al. reported two novel biallelic missense variants, c.191T>C and c.278C>T, in *TRAPPC4* gene in three individuals from two Indian families, with classic clinical presentation in one family and a milder and later onset in the other (12). Case 1 is the fifth case from three different families with this variant reported from Turkiye with the same severe phenotype.

In Case 2, a clinical presentation of epilepsy, microcephaly, severe neuromotor developmental delay, absence of speech, and autism was observed. This study identified a novel c.57C>G (p.Try19Ter) variant detected in the *TRAPPC6B* gene in Case 2. This variant was interpreted as pathogenic with a high probability, as it causes the termination codon. TRAPPC6B-related disease is an autosomal recessive inherited neurodevelopmental disease characterized by autism-like stereotypical movements and absence of speech, accompanying progressive microcephaly. To date, three nonsense, four splicing, and one frameshift variants have been reported in the HGMD. The novel c.57C>G (p.Try19Ter) variant detected in Case 2 also results in a truncating protein, similar to previously reported variants in this gene. Furthermore, the phenotype associated with TRAPPC6B was consistent with the findings observed in Case 2.

Despite being a component of TRAPP complexes, which are binding complexes involved in vesicle transport, the study by Valencia et al. on patient fibroblasts did not reveal any significant endoplasmic reticulum or Golgi morphological changes, nor defects in intracellular trafficking. Functional studies of TRAPPC6B proteins have demonstrated that morpholino knockdown of the trappc6b gene in zebrafish embryos leads to reduced survival and head size, accompanied by increased apoptosis. Additionally, Trappc6b morphants exhibit a decreased seizure threshold, increased spontaneous neuronal firing, and more frequent and prolonged calcium currents, all of which are indicative of neuronal hyperexcitability (13,14).

The clinical signs observed in our case can be plausibly attributed to the newly identified variant in *TRAPPC6B* gene. However, a precise understanding of the functional characterization of its pathogenicity of this variant requires in vivo experiments as opposed to in vitro studies, as stated in previous studies.

In Case 3, which had cataract, muscular dystrophy, microcephaly, and neuromotor developmental delay, a homozygous c.2938G>A (p.Gly980Arg) mutation in *TRAPPC11*gene was identified. The genetic etiopathogenesis of our case was clarified with the known c.2938G>A (p.Gly980Arg) mutation in the *TRAPPC11* gene. So far, 17 missense/nonsense, five splice, two small deletion, and one insertion type variants were identified in HGMD. Our case exhibited similar clinical features as previously reported cases from Syria, where the c.2938G>A (p.Gly980Arg) was identified (15). In the literature, Koehler reported a case from Turkiye with clinical features similar to those observed in our case, which had a homozygous c.1893+3A>G variant in *TRAPPC4* gene (16). The variants

c.1880C>T (p.Ala627Val) and c.679T>G (p.Phe227Val) in *TRAPPC12* gene and c.484G>T in *TRAPPC9* gene were reported in cases with other TRAPPopathies from Turkiye (17-19). The patients with variants in genes encoding for proteins within the TRAPP complex from Turkiye are summarized in Table 1.

Functional studies of TRAPPopathies are invaluable both to elucidate the pathogenicity of previously unreported variants and to understand the role of proteins in the TRAPP complex in vesicular trafficking. To date, function studies of TRAPPopathy genes, immunohistochemical stains in fibroblast cells obtained from cases with pathogenic variants in genes that affect proteins in TRAPP complexes and the effect of vesicular traffic between ER-Golgi organelles, and animal models such as zebrafish studies have been done. One such study, an in vivo functional characterization study on TRAPPopathies in Turkiye conducted by our research group, showed that a missense c.696C>G variant in *TRAPPC9* resulted in decreased mRNA and protein expression. Intracellular findings indicated that TRAPPC9 protein build-up around the nucleus was observed in the mutant type, while there was no specific accumulation in the control cell line. This disrupted protein pattern affected the amount of neutral lipid-carrying vesicles and their homogenous distribution, resulting in decreased levels (18).

Our study has several limitations, one of which is the small sample size in our case series. However, it should be noted that a considerable number of ultra-rare TRAPPopathy diseases have been previously reported in Turkiye. The first case associated with *TRAPPC11* and the novel variant defined in the *TRAPPC6B* gene will contribute valuable information to the mutation profile associated with TRAPPopathy diseases in our country. The discovery of new genes or variants associated with TRAPPopathies has great potential to accelerate functional characterization and therapeutic research, as well as studies of etiopathogenesis. It can also provide a candidate gene approach in cases with similar phenotypes for gene hunting in this area. Very recently, cases with neurodevelopmental disease have been reported in the literature associated with *TRAPPC10* and *TRAPPC6A* genes other than phenotypes reported in the OMIM database (20, 21). To the best of our knowledge, mutations in *TRAPPC1, TRAPPC3, TRAPPC5, TRAPPC8,* and *TRAPPC13* have not been associated with any human disease.

Conclusion: Despite the fact that the genes in the TRAPP family work independently from each other, the diseases in this group are named TRAPPopathies because their phenotypes overlap. The aim of our study is to discuss the clinical findings of TRAPPopathy cases and the etiopathogenesis they cause in vesicular traffic, and to emphasize that other genes of the TRAPP family, which are not yet associated with the disease, may be responsible for possible new phenotypes.

Ethics Committee Approval: This study was approved by the Ethics Committee of Bezmialem Vakif University Faculty of Medicine (approval number: 2019/2659).

Informed Consent: Informed consent was not obtained as it was a retrospective study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study ADA, ES, EY, BG, GY ; Data Acquisition ADA, ES, EY; Data Analysis/Interpretation ADA, ES, EY, BG, Aİ, GY; Drafting Manuscript ADA, ES ; Critical Revision of Manuscript ADA, ES, EY, BG, Aİ, GY; Final Approval and Accountability ADA, ES, EY, BG, Aİ, GY.

Conflict of Interest: Authors declared no conflict of interest.

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Hakem Değerlendirmesi: Dış bağımsız.

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REFERENCES

1. García-Cazorla A, Oyarzábal A, Saudubray JM, Martinelli D, Dionisi-Vici C. Genetic disorders of cellular trafficking. Trends Genet 2022;38(7):724-51.
2. Sacher M, Kim YG, Lavie A, Oh BH, Segev N. The TRAPP complex: insights into its architecture and function. Traffic 2008;9(12):2032–42.
3. Scrivens PJ, Noueihed B, Shahrzad N, Hul S, Brunet S, Sacher M. C4orf41 and TTC-15 are mammalian TRAPP components with a role at an early stage in ER-to-Golgi trafficking. Mol Biol Cell 2011;22(12):2083–93.
4. Sacher M, Shahrzad N, Kamel H, Milev MP. TRAPPopathies: an emerging set of disorders linked to variations in the genes encoding transport protein particle (TRAPP)-associated proteins. Traffic 2019;20(01):5–26.
5. Van Bergen NJ, Guo Y, Al-Deri N, Lipatova Z, Stanga D, Zhao S, et al. Deficiencies in vesicular transport mediated by TRAPPC4 are associated with severe syndromic intellectual disability. Brain 2020;143(1):112-30.
6. Ghosh SG, Scala M, Beetz C, Helman G, Stanley V, Yang X, et al. A relatively common homozygous TRAPPC4 splicing variant is associated with an early-infantile neurodegenerative syndrome. Eur J Hum Genet 2021;29(2):271-79.

7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405-24.
8. Stanga D, Zhao Q, Milev MP, Saint-Dic D, Jimenez-Mallebrera C, Sacher M. TRAPPC11 functions in autophagy by recruiting ATG2B-WIPI4/WDR45 to preautophagosomal membranes. Traffic 2019;20(5):325-45.
9. Lamb CA, Nühlen S, Judith D, Frith D, Snijders AP, Behrends C, Tooze SA. TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic. EMBO J 2016;35(3):281-301.
10. Behrends C, Sowa ME, Gygi SP, Harper JW. Network organization of the human autophagy system. Nature 2010;466(7302):68-76.
11. Olmez A, Zeybek S. Severe Microcephaly and Rapid Deterioration Due to Cortical Atrophy in Early Infancy: Consider TRAPPC4 Trappopathy. Ann Indian Acad Neurol 2022;25(4):735-8.
12. Majethia P, Do Rosario MC, Kaur P, Karanvir, Shankar R, Sharma S, et al. Further evidence of muscle involvement in neurodevelopmental disorder with epilepsy, spasticity, and brain atrophy. Ann Hum Genet 2022;86(2):94-101.
13. Kümmel D, Müller JJ, Roske Y, Misselwitz R, Büssow K, Heinemann U. The structure of the TRAPP subunit TPC6 suggests a model for a TRAPP subcomplex. EMBO Rep 2005;6(8):787-93.
14. Marin-Valencia I, Novarino G, Johansen A, Rosti B, Issa MY, Musaev D, et al. A homozygous founder mutation in TRAPPC6B associates with a neurodevelopmental disorder characterised by microcephaly, epilepsy and autistic features. J Med Genet 2018;55(1):48-54.
15. Bögershausen N, Shahrzad N, Chong JX, von Kleist-Retzow JC, Stanga D, Li Y, et al. Recessive TRAPPC11 mutations cause a disease spectrum of limb girdle muscular dystrophy and myopathy with movement disorder and intellectual disability. Am J Hum Genet 2013;93(1):181-90.
16. Koehler K, Milev MP, Prematilake K, Reschke F, Kutzner S, Jühlen R, et al. A novel *TRAPPC11* mutation in two Turkish families associated with cerebral atrophy, global retardation, scoliosis, achalasia and alacrima. J Med Genet 2017;54(3):176-85.
17. Aslanger AD, Demiral E, Sonmez-Sahin S, Guler S, Goncu B, Yucesan E, et al. Expanding Clinical Phenotype of TRAPPC12-Related Childhood Encephalopathy: Two Cases and Review of Literature. Neuropediatrics 2020;51(6):430-4.
18. Aslanger AD, Goncu B, Duzenli OF, Yucesan E, Sengenc E, Yesil G. Biallelic loss of TRAPPC9 function links vesicle trafficking pathway to autosomal recessive intellectual disability. J Hum Genet 2022;67(5):279-84.
19. Bolat H, Ünsel-Bolat G, Derin H, Şen A, Ceylaner S. Distinct Autism Spectrum Disorder Phenotype and Hand-Flapping Stereotypes: Two Siblings with Novel Homozygous Mutation in TRAPPC9 Gene and Literature Review. Mol Syndromol 2022;13(4):263-269. d
20. Rawlins LE, Almousa H, Khan S, Collins SC, Milev MP, Leslie J, et al. Biallelic variants in TRAPPC10 cause a microcephalic TRAPPopathy disorder in humans and mice. PLoS Genet 2022;18(3):e1010114.
21. Mohamoud HS, Ahmed S, Jelani M, Alrayes N, Childs K, Vadgama N, et al. A missense mutation in TRAPPC6A leads to build-up of the protein, in patients with a neurodevelopmental syndrome and dysmorphic features. Sci Rep 2018;8(1):2053.