First Clinical Report of Two *RAB3GAP1* Pathogenic Variant in Warburg Micro Syndrome

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Abstract

Warburg micro (WARBM) syndrome is an autosomal recessive disease characterized by severe brain and eye abnormalities. Loss-of-function mutations in RAB18, RAB3GAP2, RAB3GAP1, or TBC1D20 can lead to this disease. Here, we present two unrelated WARBM syndrome patients who had an RAB3GAP1 c.559 C > T, (p.Arg187Ter) and c.520 C > T (p.Arg174Ter) homozygous state. Both patients had microcephaly, microphthalmia, microcornea, bilateral congenital cataracts, severe intellectual disability, and congenital hypotonia. Using the method of next-generation sequencing and sanger sequencing, we found two nonsense variations at the splice site in exon 7 of RAB3GAP1 in the WARBM syndrome patients. The mutations were predicted to cause the syndrome due to the early stop codon, and the patients had the WARBM1 syndrome. We present the first clinical report of two different unreported variants with RAB3GAP1 mutation in the literature.

Keywords
- RAB3GAP1
- Warburg micro syndrome
- congenital cataracts
- microcephaly
- microphthalmia

Introduction

Warburg micro (WARBM) syndrome is a recessive genetic disorder characterized by microphthalmia, microcephaly, microcornea, corpus callosum hypoplasia, congenital cataract, severe hypogonadism, and intellectual disability.[1] Loss-of-function mutations in the RAB3GAP1, RAB3GAP2 RAB18, or TBC1D20 can lead to this genetic disorder.[2,3]

The first WARBM syndrome case was reported in 1993 in Pakistan. In this report, three children of consanguineous marriage were affected with intellectual disability.[4] Those children were found to have congenital cataracts as well as microcornea, atonic pupils, and microphthalmia. The children developed postnatal-onset microcephaly, and they had cerebral malformations that comprise polymicrogyria and corpus callosum hypogenesis. In this disorder, severe developmental delay usually occurs, and commonly individuals with WARBM syndrome cannot sit without aid, talk, or walk.[5] Here, we analyzed two unrelated WARBM syndrome patients to determine the underlying genetic abnormality.

Recent studies in the literature have defined the roles of the four RABs mutated in WARBM syndrome as follows: RAB3GAP1, the catalytic subunit, forms a heterodimeric complex with RAB3GAP2 named RAB3GAP, which has a GAP activity toward RAB3 isoforms, thereby regulating Ca^{2+}-mediated exocytosis of hormones and neurotransmitters.[6] Rab proteins are small GTPases of the Ras superfamily.[7]

WARBM syndrome, a severe autosomal recessive disorder, is characterized by neuronal, ocular, and genital abnormalities. As stated in the literature, patients with WARBM syndrome have features such as microcornea, ptosis, microphthalmia, congenital cataracts, severe intellectual disability, optic atrophy, pachygyria/polymicrogyria, epilepsy, spastic cerebral palsy, hypoplasia/aplasia of the corpus callosum, cerebral atrophy, micropenis, and cryptorchidism.[2,8,9] Hypotonia has been reported in all known cases. Patients with WARBM syndrome can never develop crawling and walking skills. These patients have hypotonia and axonal peripheral neuropathy.[9]

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To date, homozygous pathogenic variants in *RAB3GAP1* gene were identified in 67 families.[10] Here, we report two new Turkish patients with WARBM syndrome. To our knowledge, this is the first clinical report of those variants with RAB3GAP1 mutation in the literature.

Case Presentation

Case 1

The first patient was a 4-year-old Turkish boy. He was born at 38 weeks' gestation at the end of an uneventful pregnancy with 3,100 g birth weight. His parents had a consanguineous marriage, and he was the first child of the family. Maternal and paternal ages were 33 and 32 years, respectively. He was admitted to the neonatal unit for hypoxia. The echocardiogram was normal. His height was 87 cm (<3p), weight was 7 kg (<3p), and head circumference (occipitofrontal circumference [OFC]) was 43 cm (<3p).

From early life, he was severely hypotonic, and his psychomotor development was delayed. Developmental delay was noted at the age of 1 year with failure to thrive and growth retardation. He did not speak and had severe intellectual

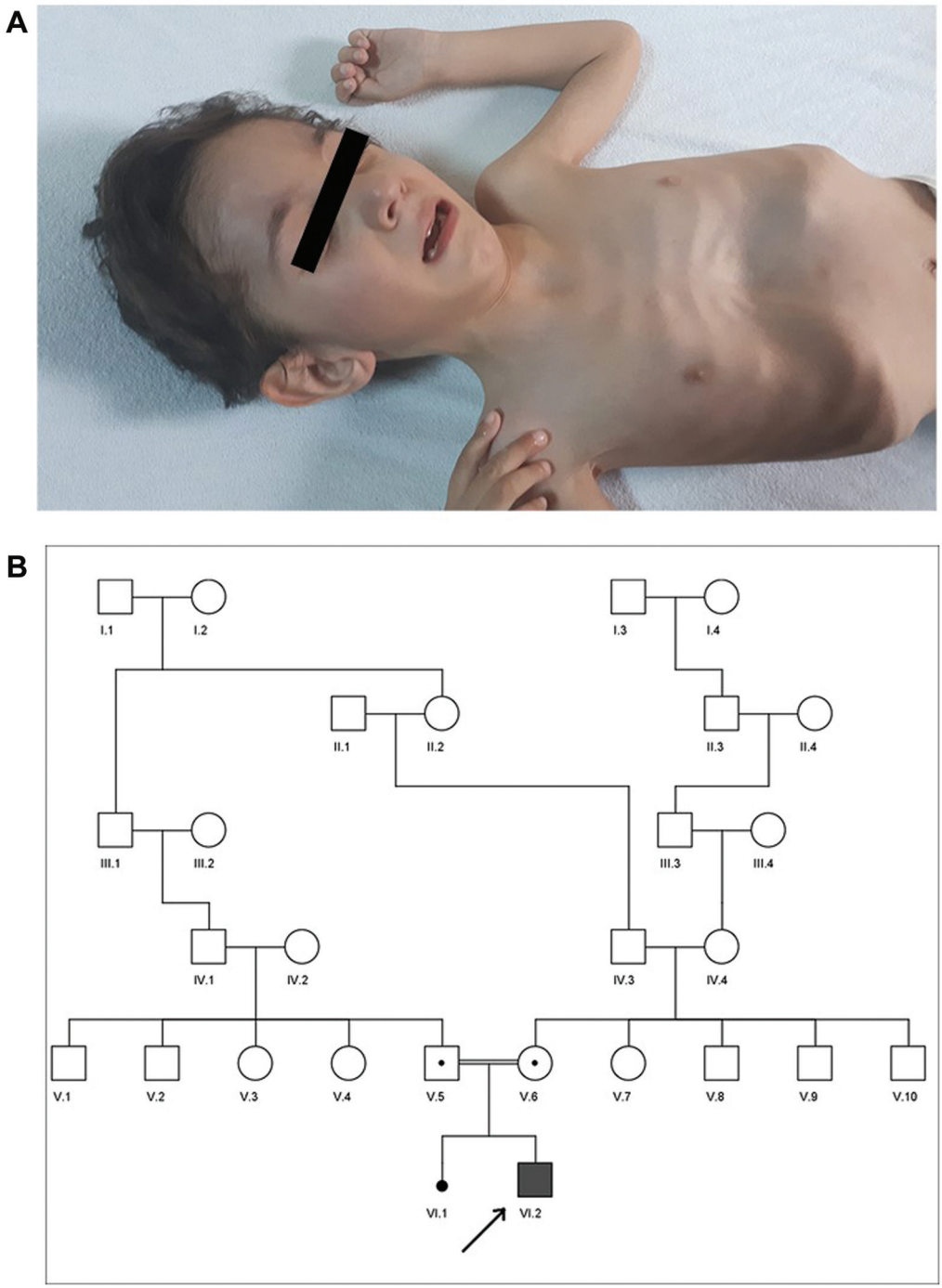

Fig. 1 (**A**) Photograph of case 1. (**B**) Pedigree of family of case 1. In family pedigrees, roman numerals indicate generation number, and arrows indicate proband.

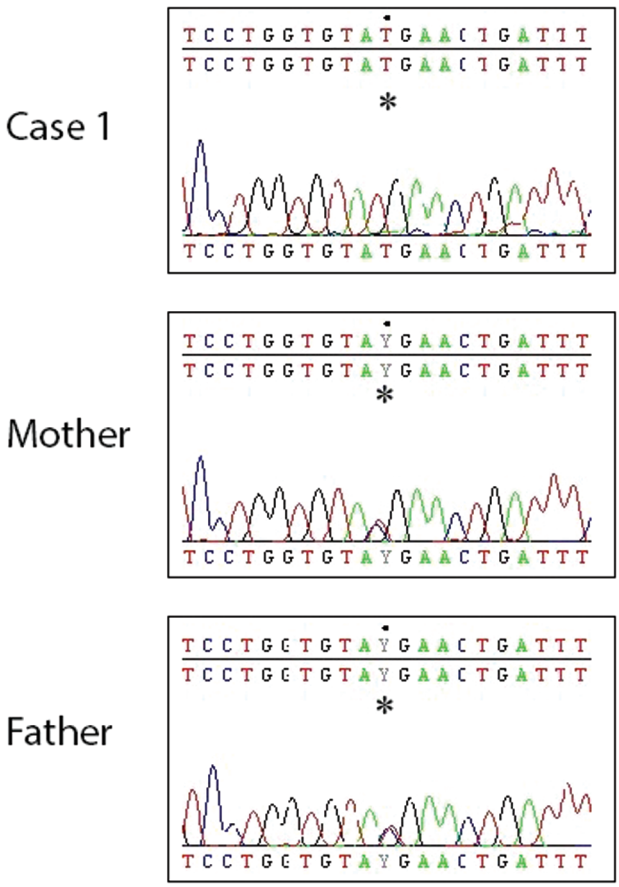

Fig. 2 Sequence electropherograms of case 1 and his family.

disability, hypotonia, and gross motor delay. He was unable to sit without support. He had a severe limb and axial hypotony with poor head control. Psychomotor retardation was remarkable. The ophthalmological examination revealed microphthalmia, pale and microcornea, atrophic optic discs, cataracts, and coloboma. The patient underwent bilateral cataract surgery. He had pectus excavatum and microcephaly.

Additional molecular analysis was performed using the sample from the proband as well as the samples of the parents. Chromosome analysis of the patient was normal. By using the method of next-generation sequencing, we diagnosed WARBM syndrome by detecting the mutation on the *RAB3GAP1* gene, which confirmed the presence of c.559C > T (p.Arg187Ter) in the homozygous state. The parents were heterozygous for the same mutation. Sanger confirmation was performed on all the individuals who had the mutation.

The patient had microcephaly, microphthalmia, high-arched palate, hypertrichosis of the forehead, bilateral cataract, and cryptorchidism. His cranial magnetic resonance imaging (MRI) showed corpus callosum hypoplasia, microphthalmia, enlarged lateral and third ventricles, and diffuse cerebral atrophy.

Hematological examination revealed normal liver, renal, and thyroid functions, also serum calcium levels were normal. A genetic evaluation which was previously performed at another center reported regular metabolic activity with 46, XX karyotype, the fluorescence in situ hybridization analysis was negative for CHARGE syndrome, and Miller–Dieker's syndrome (►**Figs. 1** and **2**).

Case 2

The second patient was a 1-year-old female infant. She was the second child of healthy consanguineous parents. Maternal and paternal ages were 22 and 31 years, respectively. The older sibling was healthy and typically developing. The pregnancy was uneventful. She was born at 39 weeks of gestation with a weight and length of 2,800 g and 47 cm, respectively. The patient weighed 7,200 g (<3p), her body length was 70 cm (10p), and OFC was 39 cm (<3p). She was referred to us due to congenital cataracts and microphthalmia. She underwent cataract surgery at 2 months of age. She had developmental delay, microcephaly, psychomotor retardation, severe intellectual disability, and gross hypotony. The patient's head control was weak. MRI of the brain showed thin corpus callosum and cerebral atrophy. She was re-evaluated at 4 years of age, at which time her weight was 8 kg (<3p), height was 85 cm (<3p), and OFC was 42 cm (<3p). Hematological examination revealed normal liver, renal, and thyroid functions, as well as normal serum calcium levels.

Chromosome analysis of the patient was normal. Sanger sequencing was performed, and the homozygous change in RAB3GAP1 c.520 C > T (p.Arg174Ter) was shown. The parents and older sibling were heterozygous for the same mutation (►**Figs. 3**,**4**,**5**).

Materials and Methods

The affected individuals from cases 1 and 2 and their parents were analyzed. Peripheral blood samples were collected after obtaining written informed consent.

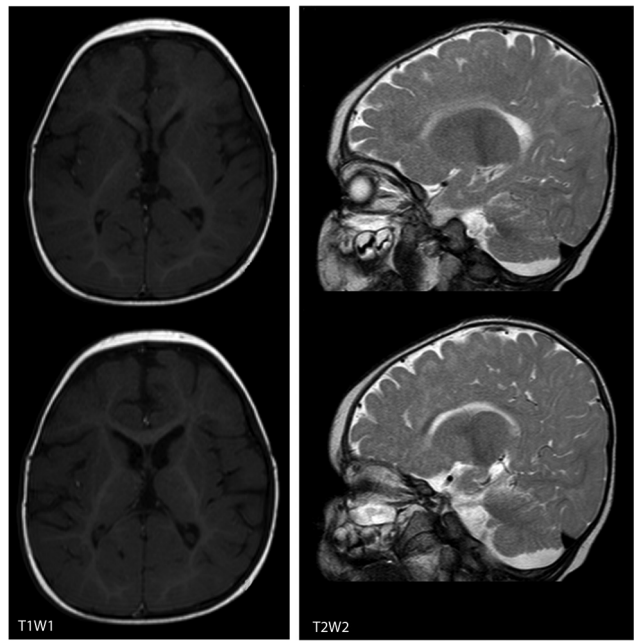

Fig. 3 Case 2, magnetic resonance imaging scans, showing thin corpus callosum and cerebral atrophy.

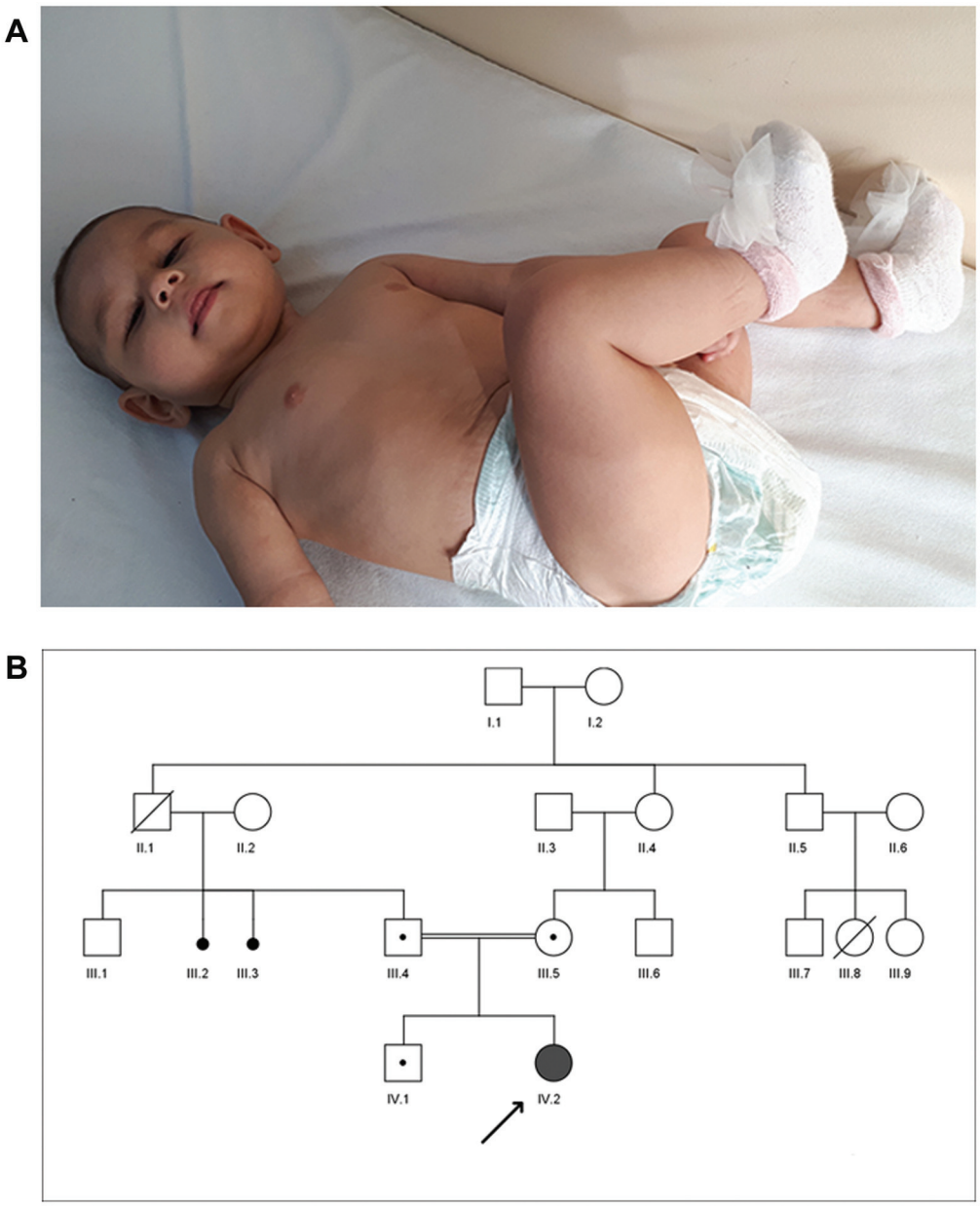

Fig. 4 (**A**) Photograph of case 2. (**B**) Pedigree of family of case 2. In family pedigrees, roman numerals indicate generation number, and arrows indicate proband.

According to the manufacturer's instructions, patients' genomic DNA was extracted from EDTA peripheral blood cells using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). The 24 coding exons of RAB3GAP1 and at least 30 bp of intronic sequence flanking the exons were amplified by polymerase chain reaction (PCR) using Taq polymerase (ThermoFisher Scientific, California, United States). The libraries were prepared using a library preparation kit (Nextera XT DNA, Illumina, San Diego, United States) and sequenced on MiSeq (Illumina). Variants were named according to NM_012233. Sequencing data were analyzed using Genomize-Seq variant analysis software and Integrative Genomics Viewer).

Each detected variant was validated by the conventional Sanger sequencing method. Sequence analyses exon 7 of RAB3GAP1 were performed by PCR with the use of following primer pairs 5′ TGTTTCCAGTTTAGTGCTTTCAA 3′ and 3′ TCAGTTGTTGCTTTAGCATGAG 5′. PCR amplicons were controlled by 2% agarose gel and then directly sequenced on ABI 3730 DNA Sequencer (ThermoFisher Scientific) and analyzed using SeqScape Software v2.7 (Life Technologies Corporation, California, United States).

Discussion

The mutations in *RAB18*, *RAB3GAP2*, *RAB3GAP1*, and *TBC1D20* genes are known to cause the WARBM syndrome in humans, which is characterized by microcornea, microphthalmia, congenital cataracts, microcephaly, corpus callosum hypoplasia, severe intellectual disability, and hypogonadism.[6,11–13]

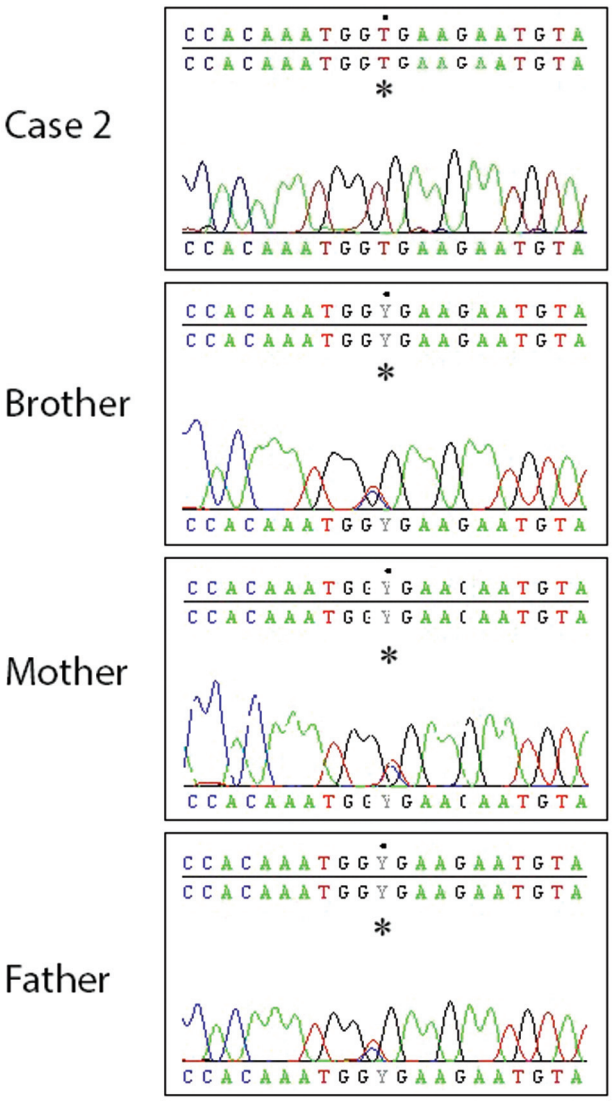

Fig. 5 Sequence electropherograms of case 2 and her family.

Likewise, our patients had microcephaly, microphthalmia, microcornea, bilateral congenital cataracts, severe intellectual disability, and congenital hypotonia. On the other hand, in this study, we identified two different unreported variants.

RAB3GAP1 and RAB3GAP2 are a RAB3GAP complex that functions as a guanine-nucleotide exchange factor (GEF) for RAB18. The RAB3GAP complex localizes to the endoplasmic reticulum (ER) and is necessary for ER targeting of Rab18. Furthermore, WARBM syndrome is caused by a deficiency in RAB18 protein. RAB3GAP1, RAB3GAP2, and TBC1D20 are each mutated, resulting in loss of the Rab18 GEF and membrane-targeting activities. As a result, both loss of Rab18 function and loss of Rab18 activation at the ER by its GEF Rab3GAP are known to cause the WARBM syndrome in humans, which is characterized by severe brain, eye, and endocrine abnormalities.[5,6,11,13,14]

The RAB3GAP1 and RAB3GAP2 proteins have been implicated in regulating synaptic vesicle exocytosis through RAB3, and the neurological deficits in WARBM syndrome individuals have been attributed to a defect in this physiological function. RAB3GAP1 is therefore important for neurodevelopmental processes and for neurons proliferation, migration, and differentiation. The RAB3GAP1 and RAB3GAP2 have been linked to the regulation of the RAB GTPase RAB3 and exocytosis of hormones and neurotransmitters at the neuronal synapse.[15–18] It has been shown in a mouse model of Rab3GAP1/2, significant plays a role in the regulation of Rab18 activity.[5,18]

It was reported that RAB3GAP1 and RAB3GAP2 are significant factors involved in autophagy, which is an intracellular, lysosome-dependent degradation process in eukaryotes, and is also involved in vesicular formation, transport, and fusion. In addition, as a vesicular trafficking protein, Rab GTPase regulates various steps of autophagy.[19,20]

The identified RAB3GAP1 nonsense mutation c.559C > T (p.Arg187Ter) (NM_012233) found at genomic position chr2:135872862 (human reference genome GRCh37) c.559C > T (p.Arg187Ter) mutation had been reported "likely pathogenic" on the ClinVar database but has no publication. This nonsense mutation is suggested as the "pathogenic" variant, based on the American College of Medical Genetics and Genomics (ACMG) guidelines regarding the interpretation of sequence variation (PVS1 + PM2 + PP5). The pathogenicity of variants was predicted by Mutation Taster (www.mutationtaster.org). The prediction scores of this mutation were evaluated by MutationTaster; the results were: mutation taster_score: 1 (disease-causing automatic), EIGEN_score: 0.8152 (pathogenic), EIGEN PC_score: 0.6856 (pathogenic), BayesDel addAF_score: 0.4986 (damaging), and BayesDel noAF_score: 0.66 (damaging).

A nonsense mutation, c.520 C > T (p.Arg174Ter) (NM_012233), found at genomic position chr2:135872823 (human reference genome GRCh37) is suggested as the "pathogenic" variant based on the ClinVar and ACMG guidelines regarding the interpretation of sequence variation (PVS1 + PM2 + PP5). The prediction scores of this mutation were evaluated by Mutation Taster; the results were: mutation taster_score: 1 (disease-causing automatic), EIGEN_score: 0.8185 (pathogenic), EIGEN PC_score: 0.6898 (pathogenic), BayesDel addAF_score: 0.5073 (damaging), and BayesDel noAF_score: 0.66 (damaging). To our knowledge, both of these mutations have no publication in the literature.

In both patients, RAB3GAP1 mutations were found at the splice site in the exon 7. These variations lead to nonsense mutations and premature termination. Segregation analysis by Sanger sequencing confirmed the affected patients to be homozygous, and their parents were heterozygous for the variant.

Conclusion

In summary, the mutations identified in this study have not been identified previously and were predicted to be disease causing by bioinformatics programs. However, mutations affect the gene's essential regions, suggesting a legitimate causal correlation between genetic alterations of the patient and clinical features. Molecular research is essential to determine the biological effects of these mutations in the

RAB3GAP1; however, all of these data propose a causal pathological correlation between our patients' clinical presentation and those genetic alterations.

Conflict of Interest
None declared.

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