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Phenotypic comparison of a novel variant (p.P164R) and A founder mutation (c.748+1G>A) in Warburg Micro syndrome

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Abstract

Warburg Micro syndrome is a rare autosomal recessive disease due to pathogenic variants found most commonly in the *RAB3GAP1* gene. It is commonly seen in consanguineous marriages and is characterized by optic, neurologic, endocrinologic and some non-typical findings (cardiomyopathy, peripheral neuropathy). Here, we report two male patients from healthy consanguineous and non-consanguineous carrier parents, with homozygous variants of the *RAB3GAP1* gene, presenting with bilateral congenital cataracts, hypogonadism, hypotonia and developmental delay. The first case had a novel variant and had colpocephaly as shown in his MRI brain, which has not been previously reported in the medical literature. The second case was thought to have a founder mutation for Turkey. In conclusion, there was no phenotypical difference between the novel and founder mutations. In Turkish patients suspected to have Warburg Micro syndrome, we recommend molecular testing for the detection of a founder mutation.

Keywords: Warburg Micro syndrome, *RAB3GAP1*, novel variant, founder mutation, colpocephaly

INTRODUCTION

Warburg Micro syndrome (WARBM) is a rare genetic disorder that is related to extrauterine growth restriction, under-developed corpus callosum, and malformation of cortical development. Patients with WARBM syndrome may present with microcephaly, cognitive and motor delays, spastic diplegia, small eye and cornea, congenital cataracts, atrophy of the optic nerve, hypogonadism, and micro genitalia.[1] The prevalence of WARBM is currently unknown; there are reports from small groups of patients worldwide.[2] WARBM was first identified in a consanguineous Pakistani marriage with three affected children in 1993.[3] In 2005, WARBM1 was linked to the 2q21.3 region, in which inactivating variants in the *RAB3GAP1* gene were detected.[4] In recent years, access to molecular genetic analysis has made it possible to link WARBM phenotype with pathogenic variants. This syndrome consists of four subtypes (WARBM 1-4). These subtypes are caused by pathogenic variants in *RAB3GAP1, RAB3GAP2, RAB18*, and *TBC1D2* and are clinically indistinguishable.[5] Different variants of varioustypes (nonsense, missense, frameshift, uniparentaldisomy, andsplice site mutations) have been identified in the *RAB3GAP1* gene in WARBM patients.[6] To date, about 100 families from various ethnic groups who have been confirmed as WARBM in which mutations in *RAB3GAP1* were the most common cause (frequency 75%).[5,7]

RAB3GAP1/2 encodes the catalytic subunit of a GTPase activator protein and activates Rab18 by forming a heterodimeric complex and acting as a guanosine nucleotide exchange factor. Rab proteins are involved in membrane trafficking in the endoplasmic reticulum, axonal transport,

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autophagy, and synaptic transmission. The neuronal vacuolation and membranous inclusions and vacuoles in axons are seen in this disorder. It is still unclear whether the loss of the Rab3GAP-Rab18 complex affects only neuronal or muscle cell physiology or both concomitantly. A current article suggests that the Rab3GAP-Rab18 complex regulates autolysosomal maturation through its activity with the Vps34 Complex I. Autophagy due to the loss of the Rab3GAP-Rab18 module may lead to the development of WARBM.[8]

This report introduces two male patients with bilateral congenital cataracts, hypogonadism, hypotonia, and developmental delay with homozygous variants in the *RAB3GAP1* gene. The second case was also thought to be a founder mutation for Turkey. There was no phenotypical difference between the index cases and the other patients with founder mutation.

CASE REPORTS

Patient 1

A 19-month-old boy was referred to our pediatrics genetic clinic due to his history of developmental delay and bilateral congenital cataract surgery. There was no complication during the pregnancy, and the child was born at 39 weeks gestation by vaginal delivery with a 3100 g birth weight and 32.5 cm head circumference. The boy was the first living child of clinically healthy first-degree cousins' marriage. The father and mother were 30 and 27 years old, respectively, and their first pregnancy resulted in an abortion. Family history was also normal. Our patient was operated on due to a congenital cataract when he was 20 days old. Despite early cataract surgery, he reacted poorly to visual stimuli and followed insufficiently with both eyes. Later, he was diagnosed with glaucoma.

In the examination at 19-month-old, his weight was 10,0 kg (–1.39 SDS), height 79 (-1.31 SDS), and head circumference 44 cm (–3.0 SDS). Facial features showed a significant pattern with microphthalmia, ptosis, deep-set eyes, a broad nasal bridge, and a relatively narrow mouth. In addition, he had low-set and large anteverted ears, micrognathia, and retrognathia, microcephaly when he was 3-year-old (Figure 1.1). The hearing was normal. He had a delay in motor and language development. Characteristically, our patient showed congenital hypotonia. He had no epileptic seizures. He could hold his head at five months and sit without support at 12 months of age. However, he was unable to crawl or walk. The patient can speak only two or three words and only understands basic phrases. Ophthalmological examination revealed small corneas. He also had right cryptorchidism and micropenis [according to Turkish prepubertal boys with an extended penile length of 30 mm (< -2 SDS)], his testis volume was 1 ml bilaterally, and scrotum was hypoplastic. We referred the patient to the endocrinological department for hypogonadism. The results of metabolic tests, viral screening tests for intrauterine infections, and other blood tests were normal. Skeletal system radiographs of our patients were performed since WARBM syndrome botulinum toxin treatment was required. Radiographs, as well as abdominal ultrasonography and echocardiography, were normal. Right undescended testis was detected in the scrotal ultrasound performed before one year of age, and he was operated on afterward. Brain magnetic resonance imaging (MRI) showed significant hypoplasia of the corpus callosum, mild colpocephaly, and a 6*7 mm pineal cyst detected in the pineal area (Figure 2.1). Electroencephalography (EEG) showed normal activity.

We performed a targeted (congenital hypogonadotropic hypogonadism gene panel including *RAB3GAP1*) sequencing test. The coding regions (± ten base pair intronic) of 34 genes are investigated. It revealed a novel homozygous variant, c.491C>G, in the seventh exon (p.P164R) (chr2: 135872794), in the *RAB3GAP1* gene (ENST00000442034). This variant was not found in normal population databases involving ExAC, gnomAD (https://gnomad.broadinstitute.org/), or

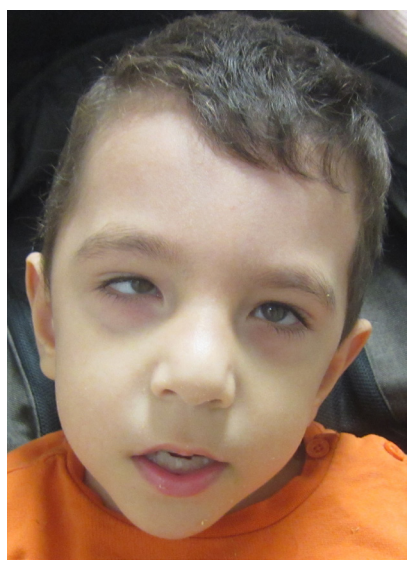

Figure 1.1 Facial dysmorphology of Patient 1

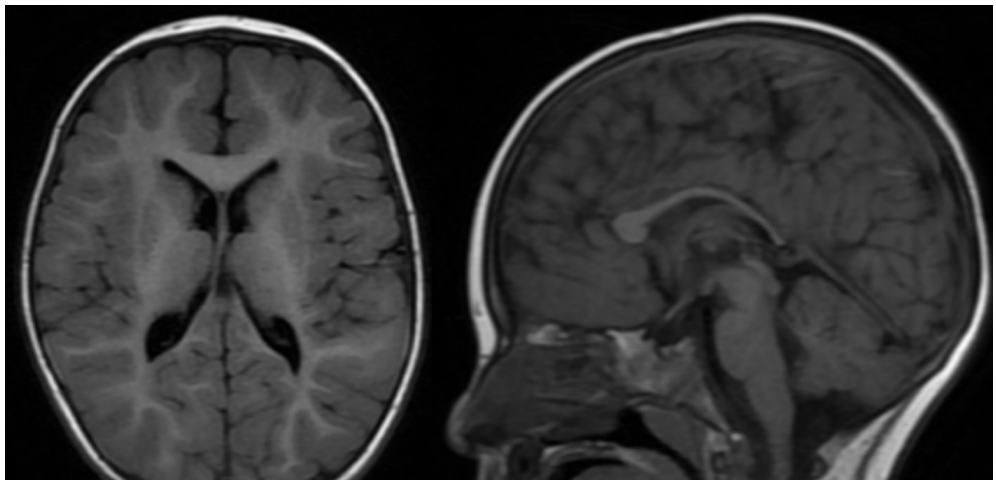

Figure 2.1 Patient 1 MRI T1 images showing hypoplastic corpus callosum and colpocephaly

Ngscloud browser (https://search.ngscloud.com/) in the Turkish population. Although functional studies have not been performed for the variant, silico analysis suggested that the variant was pathological. The programs Sorting Intolerant From Tolerant (SIFT, http://sift.bii.a-star.edu.sg/) and Mutation Taster (www.mutationtaster.org) predicted this mutation to be "damaging" and "disease-causing," respectively. Moreover, 20 of the 21 estimators identified the variant as "damaging." In addition, the ClinVar database (https://www.ncbi. nlm.nih.gov/clinvar/) and Varsome web tool (https://varsome.com/) are also used. For the pathogenicity interpretation and classification of genetic variants scoring system of ACMG (American College of Medical Genetics and Genomics) recommendations are used. Our patient was diagnosed with the WARBM associated with the *RAB3GAP1* gene found by molecular analysis. Segregation analysis by next-generation sequencing (NGS) showed that the variant was inherited from the father and mother in a heterozygous state (Figure 3).

Patient 2

The second patient presented with developmental delay and a history of congenital bilateral cataract operation when he was 11 months old. He was born to unrelated parents at 37 weeks of gestation (birth weight 2850 g). His antenatal and natal history was unremarkable. He was operated for a congenital cataract when he was 2.5 months old. The boy was born from clinically healthy unrelated parents (father 36 and mother 37 years

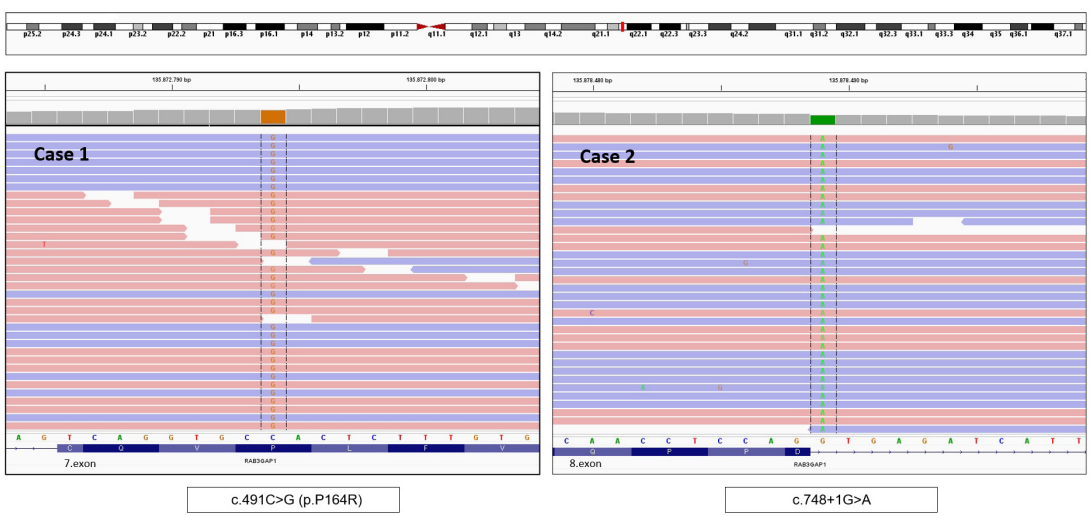

Figure 3 IGV image of Patient 1 and Patient 2

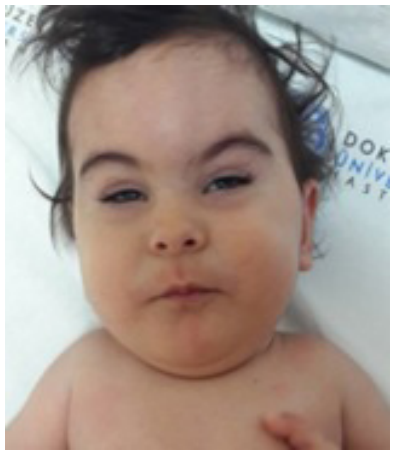

Figure 1.2 Facial dysmorphology of Patient 2

old), and he was the second child of his parents. His healthy female sibling was 2.5 years old. There was no family history of neurodevelopmental disorders. A physical examination revealed that he had no eye contact, axial hypotonia, lack of head control, deep-set eyes, ptosis, microcornea, and high palatal arch and proximal toe (Figure 1.2). In his 2-year and 3-month-old physical examination, head circumference was 46,5 cm (–2.03 SDS). Deep tendon reflexes were normal, and both lower extremities had moderate spasticity. Ophthalmological examination revealed small corneas. An auditory brainstem response and cardiological test were normal. According to Turkish prepubertal boys with an extended penile length of 25 mm (-2 SDS), he had bilateral cryptorchidism and a micropenis. His testis volume was 1 ml bilaterally, and his scrotum was hypoplastic. In the follow-up, his development improved, but neurodevelopmental retardation continued. Brain MRI revealed in T2 weighted image polymicrogyria in the insular cortex and temporal lobe and hypoplasia of the corpus callosum (Figure 2.2). Laboratory investigation (metabolic, biochemistry, and infectious) was normal except for endocrinological tests. Endocrinological tests (FSH, LH, GnRH) were scheduled in the future for hypogonadotropic hypogonadism. Chromosome analysis was 46, XY. Molecular analysis was performed on samples from the proband. NGS of the *RAB3GAP1* gene (ENST00000264158) revealed a homozygous, likely pathogenic variant in the patient: a splice donor mutation (c.748+1G>A) (chr2:135878489; rs587776651) in exon 8 (Figure 3). Segregation analysis by sanger sequencing showed that the variant was inherited from his unrelated parents, who were heterozygous for the same mutation.

DISCUSSION

The most frequent WARBM type 1 with pathogenic variants in the *RAB3GAP1* gene is a sporadic autosomal recessive genetic disorder. It

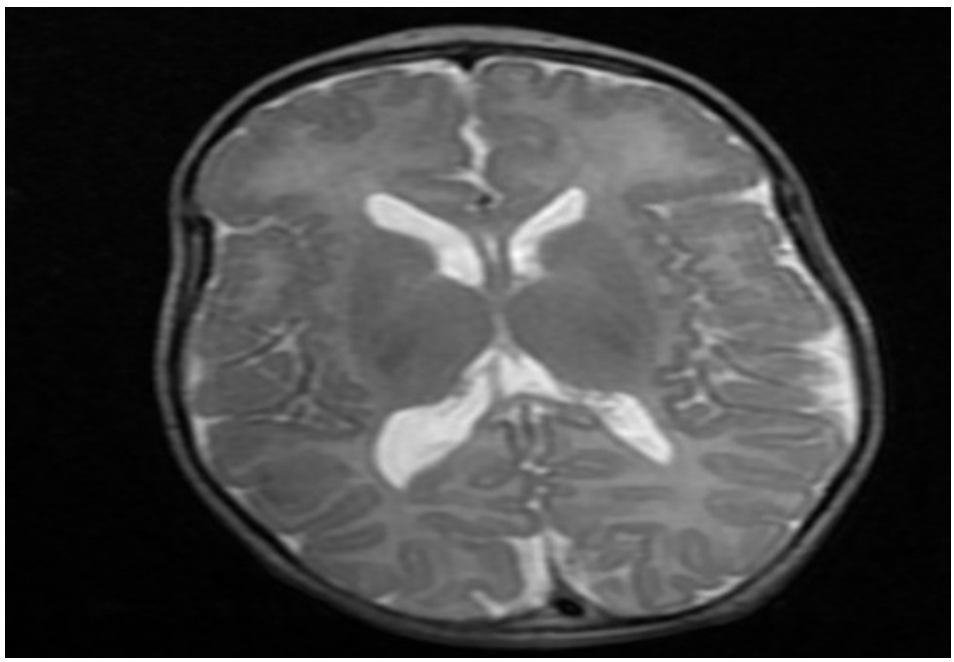

Figure 2.2 Patient 2 brain MRI revealed in T2 weighted image polymicrogyria in the insular cortex and temporal lobe.

is characterized by cerebral and ocular anomalies, micro genitalia, and development delay.[9] Our patients had congenital cataracts, postnatal microcephalus, severe developmental delay, and hypogenitalia, which are the typical clinical features of the disease. Handey *et al*.[5], conducted a haplotype analysis with microsatellite markers flanking the *RAB3GAP1* and confirmed that the c.748+1G>A splicing mutation is a founder mutation in six families of Turkish origin. In the light of these data, we should remember that homozygous variants may occur not due to consanguinity but due to founder mutation.

In previous studies, *RAB3GAP1* mutations were reported to be missense, nonsense, splicing, and deletion/insertions.[6] It is rarely inherited from only one parent due to uniparental isodisomy.[10] Pathogenic mutations, predominantly frameshift mutations, nonsense mutations, and splicing mutations that cause complete loss of *RAB3GAP1*, have been demonstrated to cause the abnormal release of synaptic vesicles. The altered short-term synaptic plasticity in the hippocampus might impact WARBM cognitive phenotype.[11] The cases had missense and splicing mutations, which were expected to cause a complete loss of function in *RAB3GAP1*. Unfortunately, we could not perform a functional impairment assay for variants, a limitation of our case study.

Severe WARBM syndrome, which affects exon 24 of the gene, was previously associated with pathogenic *RAB3GAP1* variants c.2801delC and c.2865_2866insTTCT.[4,5] The c.2801delC and c.2865_2866insTTCT variants are unlikely to be subject to nonsense-mediated decay (NMD). They may not affect protein expression because they are located toward the end of the last exon. Hence, previously reported mutations might result in total loss of function without being subject to NMD. Amino acid residues encoded by the last exon of *RAB3GAP1* were proposed to be functionally essential c.2607-1G>C variant. On the contrary, one report has shown that the residual function of *RAB3GAP1*, which lacked mostly its amino acid residues encoded by the last exon, was still enough to prevent the progression of the disease to severe WARBM syndrome phenotype.[12] Although the variants of our patients were in exons 7 and 8, they did not differ significantly in terms of phenotype compared to the patients with the last exon mutation in the literature. In addition, Koparır *et al*.[12] findings suggest that the severe phenotype was characterized with deletion–insertion mutation. Spastic diplegia also generally occurs in the early years of life, with later upper limb involvement resulting in spastic quadriplegia, similar to our patients. It may be related to exon region, mutation type, or not. We will observe the severity of the disease in the future because our first case is one of the youngest patients in the literature.

WARBM cases in the literature result from consanguineous marriage.[3,5,13,14] The parents of our first patient are first-degree cousins. For the first case, this finding does not conflict with the previous studies that have suggested that consanguineous marriages increase the risk for congenital malformations and autosomal recessive disorders. In our second case, although there was no consanguineous marriage, we associated the detection of homozygous variants with the founder mutation.

Infants with TORCH infections may also be born with microcephaly and eye defects, so a thorough ophthalmologic examination and detailed cranial imaging should be performed to differentiate from the TORCH infection. Our patients were tested negative for the infection associated with bilateral congenital cataracts.

One of the aspects of this disease is a significant visual impairment with eye abnormalities, including congenital bilateral cataracts, microphthalmia, microcornea (<10 mm diameter), and small atonic pupils. Early surgery (20 days and 2,5 months, respectively) was performed in two of our patients to prevent vision loss due to bilateral cataracts. Our patients' vision problems may persist due to progressive optic atrophy and severe cortical visual impairment.

Polymicrogyria (PMG) of the frontal and parietal lobes and hypoplasia of the corpus callosum are typically seen on MRI. They may accountable for the convulsions of a subset of patients. We compared our two cases with the previously published MRIs from patients with mutations in *RAB3GAP1* for brain anomalies. Morris-Rosendahl *et al*.[15] reported that the brain MRI in patients with *RAB3GAP1* shows broad bilateral, predominantly frontal polymicrogyria, spreading to the Sylvian fissure and the temporal lobes. There is also expansion of Sylvian fissures, hypoplasia of corpus callosum, expansion of subdural spaces, and hypoplasia of the cerebellar and cerebellar vermis. The main MRI findings of our patients were compatible with the report. Our Patient 1 is the first case reported with a pineal cyst in the literature, we are uncertain whether it is related to the *RAB3GAP1* mutation. The colpocephaly is also unique and previously unreported in the literature. Furthermore, in

situ hybridization indicated that RAB18 mRNA was highly expressed in subventricular and ventricular zones and the cerebral cortex.[16] Wu *et al*.[16] reported that RAB18 is highly expressed in the developing cerebral cortex, and involved in neuronal migration. Thus, our patients' MRI changes may be associated with the RAB3GAP1/RAB18 complex.

Atypical findings of WARBM include pectus excavatum, pectus carinatum, congenital heart defects, cardiomyopathy, calcification in basal ganglia, peripheral neuropathy, hearing impairment, and other dysmorphic features in the limbs. In our patients, no additional findings were present. Overall, no significant difference concerning phenotypic (optic, neurologic, endocrinologic) results was found between the novel mutation and the founder mutation and between the founder mutation and other founder mutations in the literature[4,5,17,18,19,20] (Table I) in Turkish population.

WARBM is a genetic disorder with increased healthcare costs. There is no specific treatment for WARBM; only symptomatic treatment is possible. For example, our patient had undergone cataract surgery and orchidopexy for his right undescended testis and is still undergoing physical therapy. Hypogonadotropic hypogonadism leads to cryptorchidism, micropenis, labio-scrotal fusion, and hypoplastic scrotum, as in our cases. WARBM should be considered in the differential diagnosis in the presence of such clinical findings.

In conclusion, we described here homozygous variants of *RAB3GAP1* in two different Turkish families. Future progress in genetic studies will allow a better view of the genetic and clinical background for *RAB3GAP1* mutations. Turkey has a high rate of consanguineous marriage and also autosomal recessive disorders, so when a patient present with congenital cataract, microphthalmia, micropenis, and microcephaly, WARBM should be considered in the differential diagnosis. The same phenotypic findings can be observed in unrelated patients of Turkish origin with a founder mutation. In Turkish patients suspected to have WARBM, we recommend molecular testing for the detection of a founder mutation. Genetic counseling is essential for the next child, so molecular studies are highly recommended for diagnosing this rare disease and initiating supportive therapies.

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DISCLOSURE

Data availability: All data generated or analysed during this study are included in the references. Further enquiries can be directed to the corresponding author.

Ethics: Samples from the patients were obtained in accordance with the Helsinki Declaration. Ethical approval is not required for this study in accordance with local/national guidelines. The patients' parents gave their informed consent for the molecular genetic analysis, the publication of patient data, photos, and brain magnetic resonance imaging.

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Conflict of interest: None

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Table 1: Patients data

	No	Variant	Age, Gender	Face	Eyes	OFC at birth, Postnatal microcephaly	Hypotonia, Developmental Delay, Seizure, Contractures	Brain MRI	Genitalia	Other
Index Cases	P1	c.491C>G homozygous relative parents	19 months, M	Low-set and large anteverted ears, Micrognathia, Retrognathia, Microcephaly	Congenital cataract operation: 20 days Glaucoma+	OFC at birth NR Postnatal microcephaly+	Hypotonia + DD + Seizure – Contractures+	Hypoplasia of the corpus callosum, Mild colpocephaly, 6*7 mm pineal cyst	Cryptorchidism, Micropenis	
	P2	c.748+1G>A homozygous unrelated parents	11 months, M	Deep set eyes, High palatal arch	Congenital cataract operation: 2,5 months Microcornea Ptosis	OFC at birth NR Postnatal microcephaly+	Hypotonia + DD + Seizure – Contractures: Bilateral lower extremites had moderate spasticity.	Bilateral frontoparietal polymicrogyry, Hypoplasia of the corpus callosum	Bilateral cryptorchidism, Micropenis, Hypoplastic scrotum	Hearing: normal Proximal toe
Aligianis *et al*. (2005)[4]	P3	c.748+1G>A homozygous	NR, M	Deep set eyes, Beaked prominent root of nose, Large anteverted ears	Microcornea, Microphthalmia, Conjenital cataract, Small atonic pupils, Optic atrophy + Ptosis (left)	50-75th percentile at birth Postnatal microcephaly+	Hypotonia + DD+ Seizure – EEG normal Congenital contractures +	Delayed myelination Cerebral atrophy – particularly frontal, Hypoplasia of the corpus callosum, Pachygyria	NR	Feeding diffucilties

Yüksel *et al.* (2007)[20]	P4	c.748+1G>A homozygous relative parents	4 years, M	Deep-set eyes, Prominent ears, Thin lips, Hypertrichosis on forehead, Large philtrum, Micrognathia., Beaked nose, High arched palate	Microphthalmia, Microcornea, Ptosis, Optic atrophy NR	3th percentile at birth Postnatal microcephaly+	Hypotonia + DD+ Seizure + (Myoclonic) EEG: multiple spike Slow wave Abnormal VEP and ERG Spastic diplegia	Diffuse cerebral atrophy, Enlarged lateral and third ventricles, Dilatation of supratentorial ventricular cisterns, Hypoplasia of corpus callosum and cerebellum in both hemispheres and vermis, Myelination was compatible with age	Hypogenitalism, Cryptorchidism, Micropenis, Labioscrotal fusion, hypoplastic scrotum	Skin Hyper-extensibility, Joint hypermobility, Single palmar crease
Handley *et al.* (2013)[5]	P5-10	c.748+1G>A homozygous	NR		NR		NR	NR	NR	NR
Dursun *et al.* (2012)[17]	P11	c.748+1G>A homozygous relative parents	11 months, M	Low-set ears Large ears, Prominent ears, Prominent nasal root, Micrognathia, Broad nasal root	Congenital cataract operation: 2 months Microphthalmia, Microcornea, Ptosis, Optic atrophy (-)	NR atbirth Postnatal microcephaly+	Hypotonia + DD+ Seizure - EEG: Background activity of slow and sharp waves over the right frontotemporal localization Increased muscle tone in both legs	Hypoplasia of the corpus callosum, Cerebral atrophy	Bilateral cryptorchidism, Micropenis, Severe scrotal hypoplasia	Pectus excavatum, Polydactyly

Tasdemir *et al*. (2015)[19]	P12	c.748+1G>A homozygous unrelated parents	16 months,M	Retrognathia	Congenital cataract operation: 2 months Microphthalmia, Bilateral optic atrophy	NR at birth Postnatal microcephaly+	Hypotonia + DD+ Seizure - DTR normal	Corpus callosum atrophy Minimal diffuse cortical atrophy	Cryptorchidism Micropenis	Hammer toes on both feet Extra distal crease on the 4th finger
	P13	c.748+1G>A homozygous unrelated parents	5 months M	Retrognathia	Congenital cataract operation: 1 months Microphthalmia, Optik disk pallor	NR at birth Postnatal microcephaly-	Hypotonia + DD+ Seizure - DTR normal	Corpus callosum atrophy Minimal diffuse cortical atrophy, polymicrogyria in the frontal cortex, wide slyvian fissures	Micropenis,	Tapering finger, hammer toe Nephrolithiasis
Işık *et al*. (2019)[18]	P14	c.748+1G>A homozygous	NR	NR	NR	NR	NR	NR	NR	NR

- = not present, + = present, *NR*=Not reported

P patient, *F* female, *M* male, *DD* intellectual disability/developmental delay, *EEG* electroencephalography, *MRI* magnetic rezonans imaging