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CLINICAL AND MOLECULAR RESULTS OF SIX CASES WITH ROBERTS SYNDROME: REVIEW OF CASES FROM TURKIYE

ROBERTS SENDROMLU ALTI OLGUNUN KLİNİK VE MOLEKÜLER SONUÇLARI İLE TÜRKİYE'DEN BİLDİRİLEN OLGULARIN GÖZDEN GEÇİRİLMESİ

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ABSTRACT

Objective: Roberts syndrome is a rare autosomal recessive disease characterized by limb defects, prenatal onset growth retardation, and craniofacial anomalies. We aimed to compare the clinical and molecular findings of six cases with Roberts syndrome with the previously reported patients from Turkiye and to emphasize that a definitive diagnosis can be made in the intrauterine period with cytogenetic tests in the early period without the need to wait for molecular test results.

Materials and Methods: Six cases, diagnosed with Roberts syndrome, in our outpatient clinic of Istanbul University, Istanbul Faculty of Medicine, Medical Genetics Department between 2015-2021, were included in the study. The family history, clinical information, and cytogenetic and molecular findings of the patients were retrospectively reviewed and compared with the cases reported from Turkiye in the literature. G and C-banding techniques and Sanger sequencing of the *ESCO2* gene were performed.

Results: Pathogenic variants in homozygous in four and compound heterozygous in two patients in the *ESCO2* gene were identified. Compound heterozygous c.[417dup];[1131+1G>A] (p.[(Pro140Thrfs*8)];[(?)]) in case 1, and c.[1111dup];[760del] (p.[(Thr371Asnfs*32)];[(Thr254Leufs*13)]) in case 6, homozygous c.1131+1G>A (p.(?)) in case 2, case 3 and case 5, and homozygous c.1111dup (p.(Thr371Asnfs*32)) in case 4 were detected. The variants reported in our case series were previously asso-

ÖZET

Amaç: Roberts sendromu; ekstremite anomalileri, prenatal başlangıçlı büyüme gelişme geriliği ve kraniyofasiyal anomaliler ile karakterize nadir görülen otozomal resesif kalıtılan bir hastalıktır. Roberts sendromlu altı olgunun klinik ve moleküler bulgularını Türkiye'den daha önce bildirilen olgularla karşılaştırmayı ve moleküler test sonuçlarını beklemeye gerek kalmadan erken dönemde sitogenetik testlerle kesin tanının intrauterin dönemde yapılabileceğini vurgulamayı amaçladık.

Gereç ve Yöntem: 2015-2021 yılları arasında İstanbul Üniversitesi İstanbul Tıp Fakültesi Tıbbi Genetik Anabilim Dalı polikliniğimizde Roberts sendromu tanısı alan altı olgu çalışmaya dahil edildi. Olguların aile öyküsü, klinik bilgileri, sitogenetik ve moleküler bulguları retrospektif olarak incelendi ve literatürde Türkiye'den bildirilen olgularla karşılaştırıldı. G ve C-bantlama teknikleri ve *ESCO2* geninin Sanger dizilimi gerçekleştirildi.

Bulgular: *ESCO2* geninde dört olguda homozigot ve iki olguda bileşik heterozigot patojenik varyant tespit edildi. Olgu 1'de birleşik heterozigot c.[417dup];[1131+1G>A] (p.[(Pro140Thrfs*8)];[(?)]) ve olgu 6'da c.[1111dup];[760del](p.[(Thr371Asnfs*32)]; [(Thr254Leufs*13)]) ile olgu 2, olgu 3 ve olgu 5'te homozigot c.1131+1G>A (p.(?)), olgu 4'te homozigot c.1111dup (p.(Thr371Asnfs*32)) saptandı. Olgu serimizde bildirilen varyantlar daha önce hastalıkla ilişkilendirilmiştir. c.760del değişimi ilk kez Türkiye'den bir olguda gösterilmesi bu hastalığa neden olan toplumuzdaki genotip bilgisine katkısı olmuştur. Ayrıca li-

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ciated with the disease. The first demonstration of the c.760del in a Turkish case contributed to the genetic association of this pathogenic variants with Roberts syndrome Although all the previously reported patients were homozygous, we have detected two patients with compound heterozygous pathogenic alterations from Turkiye indicating that the disease should also be considered in families with no consanguinity.

Keywords: Roberts syndrome, *ESCO2*, tetraphocomelia

teratürde Türkiye'den daha önce bildirilmiş olgulardaki patolojik varyantların homozigot olduğu rapor edilmiştir. Bizim olgu serimizde ise ebeveynler arasında akrabalık ilişkisi olmadığı halde iki olguda birleşik heterozigotluk gösterilmesi bu sendromun akrabalık ilişkisi olmayan olgularda da rastlanılabileceğini göstermektedir.

Anahtar Kelimeler: *ESCO2* geni, Roberts sendromu, tetrafokomeli

INTRODUCTION

Roberts syndrome (RBS) (OMIM #268300) is an autosomal recessive disorder, characterized by prenatal or postnatal growth retardation, extremity anomalies with bilateral symmetric tetraphocomelia/mesomelia, craniofacial anomalies with cleft palate/lip, and dysmorphic facial findings, intellectual disability, cardiac and renal anomalies. RBS is caused by biallelic mutations in the *ESCO2* (Establishment of sister chromatid cohesion N-acetyltransferase 2) gene, which encodes a protein necessary for the establishment of sister chromatid cohesion during the S phase of mitosis (1). It was first reported by Roberts in 1919 in three affected siblings with phocomelia and bilateral cleft palate/lip from a consanguineous Italian family (2). SC phocomelia (OMIM #269000) syndrome has a milder phenotype, which is allelic with RBS, described by Herrman et al. in 1969 and named this syndrome SC syndrome by using the initials of the surnames of these families (3). SC cases are also called pseudothalidomide syndrome because they resemble those exposed to thalidomide during pregnancy. RBS is the most severe form of the spectrum in which severely affected infants may be stillborn or die in the postnatal period, while individuals with SC phocomelia often represent the milder form of the spectrum (4,5). Premature centromere separation (PCS) and heterochromatin repulsion (HR) in heterochromatin regions are observed in chromosome analysis, and a diagnosis can be made by cytogenetic investigation before molecular analysis (6). The *ESCO2* gene encodes 601 amino acids and consists of 11 exons located at 8p21.1. So far, 34 variants associated with SC as reported in the HGMD (Human Genome Mutation Database) database (Professional Edition 2021.3 December 2021) composed of four missense, three nonsense, six splicing, 13 small deletion, and eight small insertion type alterations. Currently, no studies have been conducted to determine the mutation profile in patients from Turkiye. In this study, the clinical, radiological, cytogenetic, and molecular findings of six unrelated patients affected by RBS disease are presented and compared with the previously reported cases from Turkiye.

MATERIAL AND METHODS

Six cases, diagnosed with Roberts syndrome, in our outpatient clinic of Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics between 2015-2021 were included in the study. The family history, clinical information, cytogenetic and molecular findings of the patients were retrospectively reviewed. This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 29.07.2021, No: 367380). Written informed consent was obtained from all parents of the patients included in the study.

Conventional cytogenetics

For cytogenetic analysis, 72-hour phyto-haemagglutinin-induced cell cultures were performed on cells obtained from peripheral blood in cases 2-4 and 6. Long term cultures were performed on cells obtained from chorionic villus biopsy in case 1 and amniocentesis in case 5. Chromosomes were harvested according to standard techniques and staining procedures were carried out using Giemsa-Pancreatin-Leishman (GPL) and C banding (7). A minimum of 20 metaphases for each banding method were analyzed at the 500-600 band level. The metaphases were also evaluated for PCS.

Molecular genetics

Genomic DNA was extracted from peripheral blood (case 2-4), chorionic villus (case 1) and cultured amniocytes (case 5) samples of the patients and the peripheral blood of the parents by using commercial kits according to the manufacturer's instructions (High Pure PCR Template Preparation Kit, Roche). Primers were designed to cover all the coding exons and exon intron boundaries ± 20 bp. *ESCO2* gene (NM_001017420. 2) was sequenced by Sanger methods (ABI 3500). Electropherograms were analysed using SeqScape software (SeqScape Version 3.0; Applied Biosystems). The variants were analyzed in the open source data bases [dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and HGMD (http://www.hgmd.cf.ac.uk/ac/)]. The American College of Medical Genetics and Genomics' (ACMG 2015) standards were used for variant classification (8).

RESULTS

Clinical findings

The clinical, radiological, and molecular results of the cases are summarized in Table 1 and characteristic facial findings and limb malformation are shown in Figure 1.

Table 1: Clinical, radiological and molecular findings of RBS cases

Case	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Gender	♀	♀	♀	♂	♀	♂
Consanguinity **Affected siblings**	(-) (+)	1° cousin (-)	1.5° cousin (+)	1° cousin (-)	3° cousin (+)	(-) (-)
Age of diagnosed	16th GW	13 months	22 months	1.5 months	24th GW	19 months
Growth parameters Weight (at birth) Weight* Height*	MTP	1800 g (-4.56 SD) 6900 g (-2.40 SD) 52 cm (-7.7 SD)	1500 g (-4.69 SD) 4450 g (-7.1 SD) 55 cm (-8.82 SD)	2050 g (-3.48 SD) 3300 g (-2.36 SD) 48 cm (-3.02 SD)	MTP	1850 g (-4.24 SD) 7000 g (-4.28 SD) 65 cm (-5.21 SD)
Microcephaly*	nd	42 cm (-2.61 SD)	39 cm (-6.24 SD)	34 cm (-3.56 SD)	nd	45 cm (-2.37 SD)
Cleft palate/lip	-	-	Cleft lip/palate (unilateral)	-	-	Cleft lip/palate (unilateral)
Upper extremity findings	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral thumbs aplasia	bilateral radial aplasia and ulnar hypoplasia bilateral thumbs aplasia	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs
***ESCO2* gene variants in nucleotide**	c.[417dup]; [1131+1G>A]	homozygous c.1131+1G>A	homozygous c.1131+1G>A	homozygous c.1111dup	homozygous c.1131+1G>A	c.[1111dup]; [760del]

MTP: Medical Termination of Pregnancy, nd: not determined, GW: Gestation Week RBS: Roberts syndrome, *: the growth parameters at the age of diagnosed, SD: Standard Deviation, *ESCO2*: Establishment of sister chromatid cohesion N-acetyltransferase 2

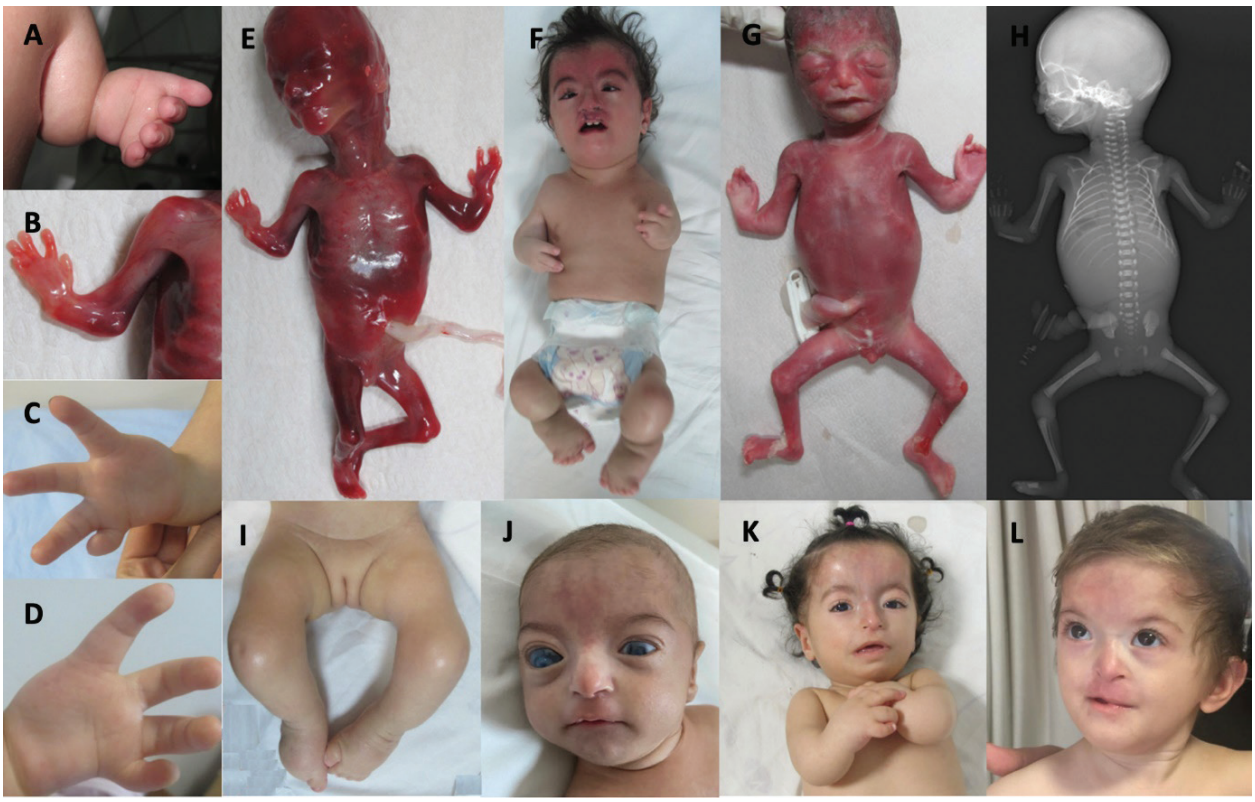

Figure 1: Clinical features of RBS cases. **A-D:** Severe mesomelic shortness and thumb a/hypoplasia in Case 3, Case 1, and Case 2. **E-G**: Bilateral cubiteal pterigium in Case 1, Case 3, and Case 5. **H:** Bilateral radial aplasia and ulnar hypoplasia in Case 5. **I:** Knee contractures in Case 3. **J-L:** Capillar malformation on the face of Case 4, Case 2, and Case 6.

Case 1, a female fetus, is the second pregnancy of a non-consanguineous couple. The family history revealed that the first pregnancy (G1) was medically terminated due to a hypoplasia of the bilateral tubular bones (humerus/radius/ulna) and a flexion deformity of the bilateral knees. The case was terminated due to a bilateral radial hypoplasia, flexion contractures in the elbows, and a ventricular septal defect (VSD) in the 15th week of the case [G2 Medical Termination of Pregnancy (MTP) 2]. In her 16th Gestation Week (GW) postmortem fetal examination; malar hypoplasia, micrognathia, bilateral low set ears, cubital pterygium, flexion contractures at the elbows, radially deviated hands, hypoplastic and proximally located thumbs, and bilateral clinodactyly were found. A Fetal radiological examination revealed bilateral radial aplasia, ulnar hypoplasia, and bilateral metacarpal and a hypoplastic phalanx structure.

Case 2, a 13-month-old female is the first child (G1P1) of a consanguineous marriage. She was born by Cesarean Section (CS) due to a breech presentation at the 40th GW, with a birth weight of 1800 g (-4.56 SD). The birth height and head circumference (HC) is unknown. The case, which was followed up in the Neonatal Intensive Care Unit (NICU) for one month, was evaluated in our outpatient clinic at 13 months old because of a small gestational age (SGA), dysmorphic findings, and extremity anomalies. Her hair was thin and sparse. She was found to have a growth deficiency and microbrachycephaly. Capillary malformation was observed on the forehead, which became more evident when crying. She had bitemporal narrowing, shallow orbit with downslanting palpebral fissures, malar hypoplasia, hypoplastic ala nasi, narrow/high palate, and micrognathia. Respiratory, cardiovascular (CVS), and gastrointestinal system (GIS) examinations were unremarkable. The patient had bilateral flexion contracture at the elbows and knees, and a marked shortening of the forearms (mesomelia). He gained head control at three months, the ability to unsupported sitting at nine months, and spelling at 13 months. Radiological examination revealed bilateral ulnar/radial hypoplasia and thumb aplasia.

Case 3, a 22-month-old girl, is the second child of a consanguineous marriage from their fourth pregnancy. She has a healthy 7-year-old brother. The couple's second and third pregnancies, which were not consulted with us, were medically terminated due to extremity anomalies (G4P2MTP2). Severe shortness was detected in all extremities in the antenatal ultrasound examinations prenatally. She was born with spontaneous vaginal delivery (SVD) at the 38th GW with a weight of 1500 g (-4.69 SD). The birth height and head circumference were unknown. She was followed up in the NICU for 90 days due to extremity anomalies. She was operated on for a unilateral cleft lip when she was 20 months old. At her 22-month-old physical examination, she had severe growth and developmental retardation and severe microbrachycephaly She had extensive capillary malformation covering the entire forehead, extending to the midline of the face, sparse hair, highly arched eyebrow, prominent eyelashes, long and downslanting palpebral fissures, hypertelorism, shallow orbits, short philtrum, hypoplastic ala nasi, malar hypoplasia, and a high palate. There was tetraphocomelia in all four extremities, in which the anterior segments were more severely affected, especially in the upper part. A single transverse line on small/hypoplastic palms and bilateral thumb aplasia were observed. She also had bilateral 40-degree knee flexion contractures and brachydactylic toes with partial syndactyly between the 4th and 5th toes. Respiratory, GIS, and CVS examinations were unremarkable. In her genital examination, labia major and minors were hypoplastic. A neurological examination revealed hypotonicity and neuromotor retardation. She has gained head control at nine months, but not yet achieved unsupported sitting.

Case 4; A 1.5-month-old male case is the first child from a consanguineous marriage. He was found to have short limbs at the 28th GW, but no further genetic counseling or invasive procedure was recommended. He was born with CS with a weight of 2050 g (-3.48 SD), a height of 47 cm (-1.28 SD), a HC of 33 cm (-1.27 SD) at the 38th GW. He was hospitalized in the NICU for one month due to postnatal respiratory distress, bilateral pes equinovarus (PEV) deformity, corneal clouding, and extremity anomalies. An eye examination revealed bilateral corneal clouding. At his 1.5-month-old physical examination, he had a growth deficiency and microcephaly. Diffuse cutis marmoratus and capillary malformation on the forehead were observed. Mild hypertelorism, proptosis, bilateral buphthalmos, which is more prominent on the right, corneal clouding, short-small nose, hypoplastic ala nasi, high/narrow palate, and micro- and retrognathia were observed. Significant mesomelic shortness in the upper extremity and bilateral hypoplastic thumbs were observed. A bilateral 90-degree elbow and 30-degree knee flexion contractures and popliteal pterygium were observed. There was bilateral clubfoot in the feet. His tonus was normal. A radiological examination revealed bilateral radius and ulna hypoplasia, aplasia of the right fibula, and hypoplasia of the left fibula.

Case 5, the second child from the second pregnancy of the consanguineous couple. The female fetus was medically terminated at the 24th GW (G2P1MTP1). He has a healthy 8-year-old sister. Nasal bone hypoplasia, absence of radius and ulna in the left upper extremity, single bone structure in the anterior segment of the right upper extremity, and a radial deviation of bilateral thumbs were detected at 19th GW. In postmortem 24th GW physical examination, the height was measured as 31 cm (25-50p),

620 g (25-50p), 22 cm (50p). There were hypertelorism, proptosis, hypoplastic ala nasi, malar hypoplasia, micrognathia, and low-set ears. Severe shortening of the forearms and radial deviation of the hands were observed bilaterally, more prominently on the left side. There was clinodactyly in the 5th fingers of the bilateral hands, and 20-degrees knee flexion contractures. A fetal X-ray examination revealed, severe mesomelic shortness in the upper extremity, radial aplasia and ulnar hypoplasia which is more prominent on the right.

Case 6; A 19-month-old male case was the second child of a non-consanguineous marriage. He has a healthy 3-year-old brother (G2P2). In the antenatal period, upper extremity shortness and PEV were observed at the 28th GW. However, invasive procedure and genetic evaluation could not be performed because the family was against medical termination. The case was born at the 38th GW by CS due to intrauterine growth retardation (IUGR), with a weight of 1850 g (-4.24 SD) and a height of 42 cm (-3.41 SD). The birth head circumference is unknown. The case was followed up in the NICU for 2 weeks due to SGA birth and congenital anomalies. He had a history of a unilateral cleft palate/lip operation at the age of 13 months. He was first evaluated by us at the age of 19 months. He had microbrachycephaly and capillary malformation extending to the forehead, glabella, nose, and philtrum. His hair was sparse and thin. He had hypertelorism, bilateral proptosis, arched brow structure, malar hypoplasia, short-small nose with hypoplastic ala nasi, cleft lip operation scar on the right part of the philtrum, micrognathia, and low-set ears. Severe mesomelia in the bilateral upper extremities and 90-degree elbow flexion contractures were observed. There were hypoplastic thumbs and bilateral PEV. Bilateral radius aplasia and ulna hypoplasia were observed in the X-Ray.

Genetic results

Cytogenetic analysis was performed on the cells obtained from the chorionic villus biopsy in case 1, amniocentesis in case 5, and the peripheral blood in cases 2-4, and 6. The karyotype were all normal [46,XX (Case 1-3 and 5) and 46,XY (Case 4, 6)]. PCS was found in all cases (Figure 2). A Sanger sequence analysis of the *ESCO2* gene revealed homozygous c.1131+1G>A variant in case 2, case 3, and case 5, and a homozygous c.1111dup variant in case 4. Two cases were compound heterozygous for the c.417dup with c.1131+1G>A, and c.1111dup with c.760del, in case 1 and case 6, respectively (Figure 3). The parents of the cases with homozygous variants were heterozygous, as expected. The mother of case 1 was heterozygous for the c.417dup, while the father was heterozygous for the c.1131+1G>A variant. The mother of case 6 was heterozygous for the c.417dup, while the father was heterozygous for the c.760del.

Figure 2: View of the C-banding of Case 3. Black arrows show characteristics premature centromere separation

DISCUSSION

RBS/SC phocomelia syndrome is currently also called a spectrum associated with the *ESCO2* gene. The diagnosis is established in a case with extremity anomalies and craniofacial findings by identification of the premature centromere separation via cytogenetic testing and/or biallelic pathogenic variants in the *ESCO2* gene by molecular testing.

The clinical diagnosis of RBS is considered when typical extremity and craniofacial malformations are accompanied by prenatal-onset growth deficiency and developmental retardation. Differential diagnosis includes preaxial reduction defects such as Holt-Oram and Fanconi syndrome if extremity findings are mild. Thrombocytopenia-Aplastic Radius (TAR) Syndrome is more likely if severe involvement is accompanied by thrombocytopenia. Cornelia de Lange syndrome (CdLs) should be considered, especially in the presence of SGA, if there are arched eyebrows with synophrys.

Clinical diagnosis can be distinguished according to four criteria observed in all patients; [1] symmetrical reduction defects in the extremities in which the upper extremities are affected more frequently and more severely than the lower extremities, [2] limb defect causing mesomelic shortness, which is always accompanied by a/hypoplasia of the thumb. The most frequently, and severely affected limb is the upper limb. The radius, ulna, and humerus are affected, respectively, in the upper extremity, while the fibula, tibia, and femur are affected, respectively, in the lower extremities. [3] Characteristic facial findings accompanied by microcephaly (facial capillary malformation, exophthalmos, hypertelorism, downward slanting palpebral fissures, malar hypoplasia, hypoplastic space,

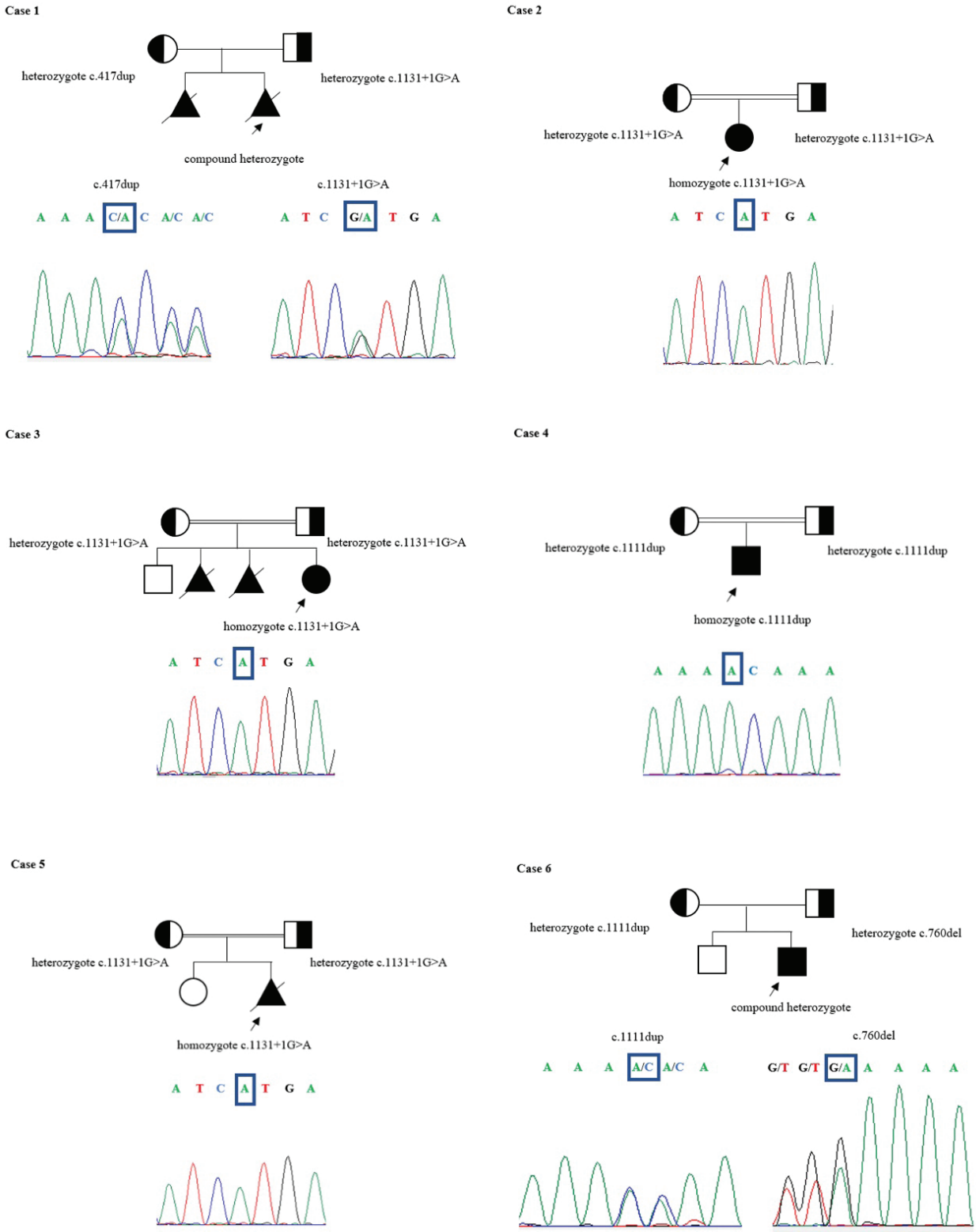

Figure 3: Molecular results of RBS cases. Sanger sequence analysis of the *ESCO2* gene revealed homozygous c.1131+1G>A mutation in case 2, case 3, and case 5, homozygous c.1111dup mutation in case 4. Two cases were compound heterozygous for the c.417dup with c.1131+1G>A, and c.1111dup with c.760del, in case 1 and case 6, respectively

cleft palate-lip, and micrognathia) [4] are consistent with the correlation between extremity malformations and craniofacial findings, and facial involvement is more pronounced in those with severe extremity defects (9).

In the study of Vega et al. in 2010, in which the clinical findings of 49 cases were compiled according to this algorithm, phocomelia with growth retardation and radial hypoplasia/aplasia and aplasia or hypoplasia of the hand and thumb in 100% of the cases, hypoplasia of the thumb in 98.7%, microcephaly in 95%, cleft lip/palate in 54.5%, corneal opacity in 36.1%, and cardiac anomalies in 25.8% (10).

Growth deficiency starts prenatally and continues in the postnatal period. All postnatally evaluated cases in our series [4/4] had SGA and the mean birth weight was -4.24 SD (minimum: -4.69 SD and maximum: -3.48 SD). In accordance with the literature, cases 2, 3, 4, and 6 diagnosed in the postnatal period had growth deficiency and microcephaly [4/4]. At the age of diagnosis, their mean weight is -4.03 SD (minimum: -7.1 SD and maximum: -2.36 SD), average height is -6.18 SD (minimum: -8.82 SD and maximum: 3.02 SD), and mean head circumferences were measured as -3.69 SD (minimum: -6.24 SD and maximum: -2.37 SD). Another finding observed in all our prenatal and postnatal cases is phocomelia [6/6]. Phocomelia is the most important feature of this syndrome that can be recognized in the prenatal period. It is a characteristic feature of shortness in all tubular bones in all four extremities where symmetrical and especially upper extremity anterior segments (mesomelia) are more severely affected. In severe cases, the upper and lower extremities are affected together. As in case 1 and case 5, diagnosed in the fetal period, the presence of especially symmetrical upper extremity forearm shortness should bring the diagnosis to mind, as in our cases in the prenatal period in the absence of craniofacial findings.

Another most common extremity finding is thumb aplasia or hypoplasia. In our series, symmetrical severe upper extremity mesomelic shortness [6/6] (bilateral radial aplasia and ulnar hypoplasia) accompanying thumb aplasia [2/6] and hypoplasia [4/6] were observed in all our cases. The relative preservation of the other fingers despite the involvement of the thumbs can be used as a clinical clue to exclude CdLs, which ranks first on the differential diagnosis list if SGA exists. Although the finding of a/hypoplasia in the thumb and radius was reported in all cases, Goh et al. reported a mildly affected adult male case in 2009 with a length of 152 cm with short tubular bones (11). Later, bilateral humeroradial synostosis without a shortening of the tubular bones was reported in a Thai and Indian case in 2020. It has been understood that phocomelia may not be present in mild cases of the spectrum associated with the *ESCO2* gene (12,13).

Cranial anomalies seen in the cases are microcephaly, brachycephaly, and craniosynostosis. Minor anomalies that cause a dysmorphic facial appearance are shallow orbits and proptosis, hypertelorism, facial capillary malformation, a wide nasal root, malar hypoplasia, and hypoplastic ala nasi. The typical dysmorphic facial appearance was observed in all [4/4] cases that were examined postnatally.

Cleft lip and/or cleft palate anomaly as a major craniofacial malformation is observed in half of the patients with RBS. The fact that a cleft palate anomaly alone was observed in less than 5% of the cases suggested that the presence of a cleft palate accompanying cleft lip anomaly is more typical for patients with RBS. It has been reported that there may be a high/narrow palate in cases where cleft lip and/or cleft palate anomalies are not observed (10). In our case series, a cleft lip anomaly was observed in case 3, a cleft palate accompanying a cleft lip anomaly in case 6, and a high/narrow palate was observed in cases 2 and 4.

To date, no correlation between genotype and phenotype associated with *ESCO2* variants has been established. It has been shown that there may be clinical differences even in different families carrying the same mutation or even within the same family. The difference in the severity of clinical findings in case 2, case 3 and case 5 with the same homozygous alterations in our case series is consistent with this observation.

RBS is an autosomal recessive disease. Although it is rare, it has been reported in many countries around the world of different racial and ethnic origins, although it is more common in Mediterranean countries such as Egypt and Turkiye, as well as in Germany and the American continent.

CdLs is on the differential diagnosis list for RBS. Both are known as, syndromes associated with mutations in the Cohesin complex and its regulators, a multiple subunit protein complexes known as cohesinopathies. The ESCO2 protein, responsible for RBS, acetylates the cohesion complex, contributing to the stability of the binding of the cohesion complex with DNA and holding the newly replicated sister chromatids together. Cohesin is a protein complex that mediates sister chromatid cohesion, homologous recombination, and DNA cycling. Mutations in the genes encoding the core proteins of the Cohesin complex, SMC1A, SMC3, and RAD21, and NIPBL, HDAC8, and BRD4 proteins, which are involved in the regulation and stabilization of the Cohesin complex, are responsible for CdLs (9).

In which the *ESCO2* gene was first associated with the disease, association with the 8p12-21.2 chromosome locus was established using linkage analyses in seven Co-

Table 2: *ESCO2* pathogenic variants in cases from Turkiye

Nucleotide change*	Amino acid change**	exon/intron	dbSNP	ClinVar	ACMG Classification	References of cases from Turkiye
c.417dup[1]	p.Pro140Thrfs*8	exon 3	rs80359848	VCV000021245.3	pathogenic	Vega et al. 2005, in this study
c.760del[2]	p.Thr254Leufs*13	exon 3	rs80359852	VCV000021247.10	pathogenic	in this study
c.879_880del[3]	p.Arg293Serfs*7	exon 4	rs80359857	VCV000021251.9	pathogenic	Vega et al. 2005, Gordillo et al. 2008, Vega et al. 2010
c.877dup	p.Arg293Lysfs*8	exon 4	-	-	likely pathogenic	Mengen et al. 2018
c.1111dup[4]	p.Thr371Asnfs*32	exon 6	rs80359859	VCV000021232.6	pathogenic	Vega et al. 2005, Avci et al. 2018, Sezer et al. 2019, in this study
c. 1131+1G>A	p.?	intron 6	rs80359861	VCV000021233.5	pathogenic	Gordillo et al. 2008, Doğan et al. 2014, Colombo et al. 2019, in this study
c.1461_1462del[5]	p.Arg487Serfs*19	exon 9	rs80359866	VCV000021237.2	pathogenic	Vega et al. 2005

*: DNA sequence based on GenBank number NM_001017420.2, **: Protein sequence based on GenBank number NP_001017420, c.417dup [1] [c.411_412insA veya 417_418insA], c.760del[2] [c.752del], c.879_880del[3] [877_878del], c.1111dup[4] [1104_1105insA], c.1461_1462del[5] [1457_1458del] these variants have been corrected from the original publications to conform to HGSV (*Human Genome Structural Variation*) nomenclature

lombian families and genome-wide homozygous mapping for RBS. It has been understood that it is caused by biallelic pathological variants of the gene (1).

We identified four known pathogenic variants; predicted truncating c.417dup, c. 760del, c.1111dup, and evolutionally conserved splice donor site alteration c.1131+1G>A in our study. According to ACMG guidelines, all variants are classified as pathogenic. The reported pathogenic variants detected in cases from Turkiye with RBS in the literature so far are summarized in Table 2 (14-18). The pathogenic variant c.760del, which has been reported in two cases of German and American origin in the literature, was reported in a Turkish case for the first time in our case series, which expanded the knowledge of the Turkish mutation profile (5,19). In addition, homozygous mutations were found in all previously reported Turkish cases. The determination of compound heterozygous mutations in two cases in our series shows that this disease can also occur without close consanguinity.

With the widespread use of clinical or whole-exome analyses in recent years, it is predicted that a definitive diagnosis can be made in more cases with RBS in the postnatal period. However, as seen in the family history of prenatal cases in our case series, it may be possible to medically terminate them without a diagnosis. In these cases, failure to diagnose the terminated fetus leads to the inability to provide appropriate genetic counseling to the family. It should also be kept in mind that with the increasing clinical recognition of RBS, cytogenetic diagnosis can be made without the need for further molecular analysis.

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