Ellis-van Creveld syndrome due to a novel EVC2 variant in a patient from Turkey

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Abstract

Here we report a Turkish child with Ellis-van Creveld syndrome whose presentation was short strature, hypodontia, narrow thorax, dysplastic nails, cardiac abnormality and polydactyly. Genetic analysis revealed novel homozygous mutation in the EVC2 gene (c.3533_3546del). Further research is needed to elucidate the pathophysiological course

INTRODUCTION

Ellis-van Creveld (OMIM #225500) syndrome, which is also known as chondroectodermal dysplasia is a infrequent autosomal recessive skeletal dysplasia which is characterized by polydactyly, ectodermal dysplasia, chondrodysplasia and congenital cardiac abnormalities. It was first identified by Richard Ellis and Simon van Creveld in three children with chondrodysplasia, polydactyly and oral abnormalities[1]. Although the exact prevalence of this disease is not known, it is estimated as 7/1,000,000 in non-Amish population[2,3]

Short stature, thoracic hypoplasia, postaxial polydactyly, abnormalities in dental structures with varying severity such as peg-shaped teeth, natal teeth and abnormalities in enamel, dysplastic nails, sparse hair and multiple frenulum can be observed in patients with Ellis-van Creveld (EvC) syndrome. In addition, approximately %60 of EvC syndrome patients have congenital heart defects which affect their prognose. The most common cardiac malformations are atrioventricular septal defect (AVSD) and single atrium. Cognitive and motor development is generally preserved[4,5] .

EvC syndrome is mostly associated with biallelic variations in two genes; *EVC* (EvC ciliary complex subunit 1, OMIM 604831) and*EVC2* (EvC ciliary complex subunit 2, OMIM 607261). Localized at 4p16.2, *EVC* and *EVC2* encode ciliar basal body proteins with 21 and 22 exons, respectively. Thus, EvC is also defined as primary ciliopathy[6]. Rarely, biallelic mutations in*DYNC2LI1* (Dynein, cytoplasmic 2, light intermediate chain 1),*GLI1* (Gli family zinc finger 1) and *WDR35* (Wd repeat-containing protein 35) have also been reported in EvC[7-9]. So far, nearly 25% of the EvC patients do not carry any genomic variation.

In this study, a novel homozygous variant, *EVC2* c.3533_3546del (p.Glu1178Glyfs*82) is identified in a patient with EvC syndrome. This variant may interfere with Hedgehog (Hh) signaling pathways thus causing abnormalities in endochondral and skeletal development[6,10].

CASE REPORT

A 10-year-old Turkish girl with short stature and polydactyly was referred to our clinic. She was the second child and was born at 40th week of pregnancy, delivered by C/S with 4,000 gr weight at birth. Birth weight

was 4,000 g (75th centile) with a length of 48 cm (25th centile) and a head circumference of 35 cm (10th centile). Apgar's score was calculated to be normal. She had neonatal jaundice on day 8 of life, and treated with phototherapy for 2–3 days. Her parents were first degree cousins. The family history revealed that the patient's sister died after birth and seemingly featured similar clinical presentation. She had a history of atrial septal defect (ASD) surgeries, when she was 1.5 years and 4 years old. The patient's developmental milestones were delayed, she started walking from the age of 4 and she started to speech from the age of 3. At 10 year of age, some dysmorphic features were detected, including high forehead, wide nasal bridge, short philtrum, disproportionate shortness of extremities, postaxial polydactyly and brachydactyly in hands and bilateral shortening of the 4^{th} and 5^{th}metatarsal bones, syndactyly between 2^{nd} and 3^{rd} toes, dystrophic hand and toe nails, hypodontia and early dental decay. She had genu valgum deformity with inability to full extension in knee and dislocated patellae, causing a limited walking distance and restriction of movements **(Fig. 1).**

The main physical findings were height 111 cm ($<3^{rd}$ centile) her weight was 19 kg ($<3^{rd}$ centile) and her head circumference was 50 cm ($<3^{rd}$ centile). Further evaluation revealed that she had previously operated ASD, mitral regurgitation, tricuspid regurgitation and dilatation in right cardiac cavities in echocardiography (ECO). Vision and hearing examination were normal. The patient's complete blood count was normal. The systemic examination was otherwise unremarkable. Cytogenetic analysis revealed a normal 46,XX karyotype. No additional abnormalities were detected. Her parents and sister were apparently healthy.

The clinical diagnosis was EvC syndrome. A signed informed consent was obtained from her family prior to genetic testing. Venous blood was sampled from the proband, and was sent to the Medical Genetic Laboratory of Haseki Education and Research Hospital in Turkey. Subsequently, genomic DNA was extracted from peripheral blood leukocytes by the help of standard protocols. Then, entire coding exons and their flanking regions of the *EVC* and *EVC2* genes were screened using targeted next-generation sequencing (MiSeq) approach.

DNA sequence analysis of the *EVC* gene was normal, while DNA sequence analysis of the complete coding region of the *EVC2* gene (NM_147127.5) showed homozygous for c.3533_3546del, p.Glu1178Glyfs*82 in exon 20 (**Figure 2** by the DECIPHER). This *EVC2* gene variation has not been reported before. Using an in-silico prediction tool, MutationTaster (mutationtaster.org), we identified this variation as a disease-causing predisposition factor. According to the American College of Medical Genetics and Genomics (ACMG) sequence variant classification guideline, the variant was classified as pathogenic. This variant was not present in healthy control population databases (gnomAD, 1000 Genomes Project) and also had not been reported in disease mutation databases (Clinvar, Human Gene Mutation Database). This is both frameshift and pathogenic variation, thereby confirming the clinical diagnosis of EVC syndrome. This is both frameshift and pathogenic variation, thereby confirming the clinical diagnosis of EVC syndrome.

DISCUSSION

EVC is characterized by short stature, chondrodystrophy, thoracic hypoplasia, postaxial polydactyly, abnormalities in dental structures with varying severity such as peg-shaped teeth, natal teeth and abnormalities in enamel, multiple frenulum, dysplastic nails, congenital heart anomalies and sparse hair. A narrow thorax due to the shortness of ribs may result in severe postnatal respiratory distress. Heart defects are present in 60% of cases. The presence of cardiac disease is the main determinant of life expantancy. Mental and cognitive retardation is not expected in this disease[4,5]. The estimated prevalence of EvC is 1-7/1,000,000 in non-Amish population. Clinical manifestations are variable among patients and not all patients exhibit whole cardinal signs[1,11].

Disproportionate short stature, polydactyly and brachydactyly in hands, shortness of extremities, genu, bilateral shortening of 4th and 5th metatarsal bones, dystrophic nails in hand and toes, hypodontia and cardiac defects were observed in our patient. Peg shaped teeth and multiple frenulum were not observe in proband. She had dislocated patellae and genu valgum deformity, causing a limited walking distance. Clinically, her ortopedic disability progress more rapidly than what is expected. While mesomelic shortness

is commonly reported in EvC syndrome, few cases with rhizomelia were also reported[12,13]. Our patient had both distal and proximal shortness with distal limb shortness being more prominent.

EvC syndrome is mostly related to the *EVC* and *EVC2* gene mutations. The clinical presentation of EvC patients with variations in*EVC* and *EVC2* genes is indistinguishable. EVC and EVC2 play role in endochondral growth and skeletal development. EVC and EVC2 are co-localize in the EvC Zone and EVC2 is essential for the localization of EVC at the base of primary cilia[14]. They encode ciliar basal body proteins[15]. Thus, EvC is also defined as primary ciliopathy.

Various types of ciliopathies are caused by defects in cilia structure or function[6]. Among the ciliopathy diseases, while the structure of the cilia is normal in EvC, Hedgehog and Fibroblast growth factor (FGF) signaling pathways are impaired[10,14]. Reduced Hedgehog signaling and increased FGF signaling at the growth plaque was reported in Evc2 mutant mice[10]. The Hehgehog signaling starts the association of Evc2 with Smoothened (Smo). Smo-Evc2 signaling complex at the EvC zone is essential for Hh signal transmission[14,16].

Homozygous mutations in the *EVC* and *EVC2 g* enes cause the EvC syndrome while heterozygous mutations cause the Weyers acrofacial dysostosis (WAD, OMIM 193530) that shows a similar phenotype as in EvC syndrome[3,6]. Weyers acrofacial dysostosis is a milder disease compared to EvC syndrome. In general, congenital heart disease is not found in WAD patients. Although many cases with EvC syndrome have been reported up to date, few cases of Weyers acrofacial dysostosis are present in the literature. Variants in both the *EVC* and*EVC2* gene have been reported in WAD. It is usually detected in the last exon of the *EVC2* . It is suggested that the last exon of the *EVC2* gene may serve as a hotspot for WAD mutations[3] .

The EVC2 gene is located on the 4p16.2 chromosome, has 22 exons and encodes a single-pass type I transmembrane protein. Gene expression occurs in many different organs including heart, placenta, lung, liver and skeletal muscles. According to the Human Gene Mutation Database (HGMD), 82 variations have been identified so far for the *EVC2* . Majority of the mutations reported are nonsense mutation. Many EvC patients with *EVC* gene mutations were reported in Turkish population, previously. To our knowledge, there is three case of a Turkish EvC patient with *EVC2* mutation reported in the literature[17]

CONCULISION

The findings of the present case study might help broaden a novel mutation of *EVC2* gene (p.Glu1178Glyfs*82) in a Turkish patient spectrum of the disease and contribute to a further understanding of the relationship with phenotype and genotype in EvC syndrome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Haydar Bağış, Özden Öztürk and M. Özgür Çevik: Contributed to the conception and manuscript writing. Semih Bolu and Özden Öztürk: Contributed to clinical data collection and analysis.

ETHIS APPROVAL AND CONSENT TO PARTICIPATE

Not required.

PATIENT CONSENT

Written informed consent was obtained from the patient for publication of this case report.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available with the corresponding author upon request

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Figure1: Clinical features of EVC syndrome observed in patient: (a). Bilateral postaxial polydactyly of hands. Fingernails are short, hypoplastic and absent on both the sixth fingers are shown. (b). Bilateral shortening of the 4th and 5th metatarsal bones, hypoplastic fingernails are noted. (c) Genu valgum is seen (d) Patient showing hypodontia. Written consent for publication of photographs was obtained from the patient and family

Figure 2 : A detailed view of the *EVC2* region where the novel homozygous variant [c.3533_3546del (p.Glu1178Glyfs*82)] was identified in our patient. *EVC2* encodes a single-pass type 1 transmenbrane protein. The image shows an absence of the homozygous p.Glu1178Glyfs*82 variant in gnomAD. Figure image from Decipher.