Case report

Compound Heterozygous Variants in FAM111A Cause Autosomal Recessive Kenny-Caffey Syndrome Type 2

Eren E et al. Hypoparathyroidism, skeletal dysplasia and FAM111A gene

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What is already known about this topic?
KCS is characterized by hypoparathyroidism, dwarfism, and dysmorphism. Osteocraniostenosis (OCS) is another syndrome similar to KCS. Autosomal dominant FAM111A mutation causes KCS2 and OCS syndrome; the perinatal and lethal forms should be kept in mind in the differential diagnosis. The FAM111A is essential for parathyroid and bone formation; it might be an essential factor in male genital development.

What does this study add?
To our knowledge, this case is the first patient with genetically confirmed KCS2 or OCS in Turkey. It is known that FAM111A mutation is transmitted by autosomal dominant manner. We present a case that showed autosomal recessive transmission. Unlike the known autosomal dominant feature of these syndromes, the cause of the patient’s phenotype may be the identified compound heterozygous mutations of the FAM111A gene. The present patient probably has OCS, which is a severe form of KCS2.

Abstract
Kenny-Caffey syndrome (KCS) is a rare autosomal recessive/dominant disease characterized by hypoparathyroidism, skeletal dysplasia, dwarfism, and dysmorphism. FAMIL11A or TBCE gene mutations are responsible for this syndrome. Osteocraniostenosis (OCS) is a lethal syndrome with similar features to KCS, and it can be a severe form of KCS type 2 that results from FAM111A gene mutation. FAMIL11A mutation is generally characterized by the autosomal dominant transition. We present a male case having compound heterozygous variants (c.976T>A and c.1714_1716del) in the FAM111A gene with an autosomal recessive inheritance pattern. Hypocalcemia developed on the second day of life. The patient and his older sister had a dysmorphic face, skeletal dysplasia, and they were diagnosed with hypoparathyroidism. Both siblings died due to septicemia. He is the first reported patient with FAM111A mutation in Turkey. The phenotype of the patient is compatible with OCS, and the detected variants may explain the disease genetically.

Keywords: hypoparathyroidism, skeletal dysplasia, osteocraniostenosis, short stature, dysmorphism, FAMIL11A gene, autosomal recessive

Introduction
Kenny-Caffey syndrome (KCS) is a rare autosomal recessive or dominant disease characterized by short stature, cortical thickening, medullary stenosis of tubular bones, delayed closure of anterior fontanel, eye abnormalities, and hypoparathyroidism (1). There are two inherent forms of KCS. While the autosomal recessive (AR) form is caused by TBCE gene mutation, the autosomal dominant (AD) form results from the FAM111A gene mutation. These gene mutations cause hypoparathyroidism, short stature, bone problems, and dysmorphic features (2,3). Although knowledge about the FAM111A gene is limited, many cases presented in the literature show that FAM111A is an essential molecule for normal bone and parathyroid gland development. In this paper, we identified and presented a patient with the FAM111A mutation using whole-exome sequencing.

Case Report
The male patient was born as the third child of healthy, consanguineous (3rd degree) parents at 38 weeks gestation, and skeletal dysplasia was suspected during the prenatal period. The grandfathers of the patient’s mother and father are siblings. Except for the present patient and his sister, there is no family history with a similar disease. The patient’s sister passed away due to hypocalcemia and sepsis at the age of 2 months. The patient was admitted to the hospital experiencing respiratory distress after birth. On physical examination, the child’s weight was 2770 grams (-1.4 SDS), length was 44 cm (-2.73 SDS), and head circumference was 34 cm (-0.64 SDS). Dysmorphic face (deep-set eyes, low set ear, microphthalmia, depressed nasal bridge), large anterior fontanel (5x6 cm), short
arm span, micromelia, and increased upper/lower ratio (2.2, the normal ratio is about 1.7), and micromelia were noted (Figure 1A). External genitalia was male, but micropenis (stretched penile length of 2.2 cm) and long thin bones were notable. There was no renal abnormality, and neurological examination was normal. The patient was admitted to the hospital due to respiratory distress. Hypocalcemia and hypoparathyroidism were detected [Ca 7.8 mg/dl (N: 8.5-11), P 9.3 mg/dl (N: 5.6-10.5), PTH <3 pg/ml (N: 10-65), 25OH vitamin D 37.8 mcg/l (N: 20-50)] on the second day, and oral calcium and calcitriol therapies were initiated. His calcium level was normalized two days after the start of treatment. The respiratory problems continued to the 50th day of life. Except for a bilateral fracture noticed on the 38th day, no further fracture was observed during follow-up. Calcium administration was discontinued due to hypercalcemia on the 65th day. The calcium level was not stable, and the oral calcium treatment was continued intermittently. Low-dose calcitriol therapy was continued, but this treatment was ceased due to hypercalcemia. The patient was discharged on the 76th day. In follow-up, hypoparathyroidism became evident again, and oral calcium and calcitriol therapies were commenced on the 85th day. The patient was reluctant to feed and did not gain weight. He was readmitted to the intensive care unit with septicemia at the age of 3.5 months and died from respiratory failure after 30 days. A skeletal survey showed incomplete ossification of the calvaria, short and thin ribs, hypoplastic thorax, and long thin bones (Figure 1B). He was diagnosed with KCS or Sanjad-Sakati syndrome (SSS) based on clinical and radiological findings. Karyotype analysis was 46, XY and FISH analysis for 22q11.2 deletion showed no abnormality. The patient’s clinical feature supported the initial diagnosis of SSS; however, TBCE exons Sanger sequencing revealed no abnormalities. Whole-exome sequencing (WES) was performed using peripheral blood genomic DNA from the patient and his parents. WES was performed by the Interagen Genetic Diagnosis Center in Ankara on a MiSeq sequencing platform (Illumina, San Diego, CA, USA). Detecting the potential variants of the family, data analysis and bioinformatics processing was performed after receiving the primary sequencing data. The family history of the probands identified as carrying variants in the FAM111A gene is shown in the pedigree of Figure 2. The patient pedigree demonstrates that the inheritance pattern reveals autosomal recessive inheritance. After the data analysis, we identified compound heterozygous mutations of the FAM111A gene in the patient, including a paternal heterozygous missense variant c.976T>A (p.L326I) and a maternal heterozygous in-frame deletion variant c.1714_1716del (p.Ile527del, rs779963813) (Figure 3). In this study, two variants were identified and examined in public databases (Ensembl, MutationTaster, Franklin, and Clinvar). Briefly, amino acid substitutions were identified with PROVEAN (http://provean.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://sift.jcvi.org/) tools to determine the potential consequences of missense variants on protein function. The mutation taster (http://www.mutationtaster.org/) program was used to evaluate protein stability. The transcript of the FAM111A gene is ENST00000528737.5. The c.1714_1716del mutation caused the FAM111A protein to lack only one amino acid, and the c.976T>A led to an amino acid change from leucine (Leu) to isoleucine (Ile). The SWISS-MODEL used for tertiary structure prediction showed a marked variation in the structure of c.976T>A and c.1714 1716del (Figure 4). Additionally, the p.Ile527del in the FAM111A gene was categorized as deleterious (score=-11.79) by PROVEAN Prediction and was presumed to be "disease-causing by Mutation Taster. The allele frequency of this variant is 9/251416 in the GnomAD exome database. In silico prediction tools other than Mutation taster were predicting this variant as a mild benign variant. Some other missense mutations were given very close to this mutation. Furthermore, the missense variant e.976T>A (p.L326I) in the FAM111A gene has not previously been reported in the literature, Ensembl genome databases, or Clinvar. The c.976T>A (p.Leu326Ile), a novel variant, was not found in the GnomAD exomes and GnomAD genomes database despite good coverage. This variant was classified as a variant of uncertain significance (VUS) (PM2, P62, B64). However, the results of bioinformatic prediction by PolyPhen2 and SIFT confirmed that the amino acid substitution p.L326I in protein FAM111A was possibly damaging by PolyPhen (score=0.635), tolerated by SIFT (score=0.064), and neutral by PROVEAN Prediction (score=1.05). Moreover, the variant was presumed a polymorphism by Mutation Taster; it was found in ExAC and 1000G. Additionally, e.976T>A and c.1714 1716del variants were both stated to have "splice site changes" both by Mutation Taster. However, variants were classified as VUS by the Franklin variant classification tool (https://franklin.genoox.com). As in the present case with a clinically clear picture, VUS and new/uncharacterized variants are notable because these variants can unveil unpredictable genetic and protein alterations involved in biochemical processes.

Discussion

We present a patient having FAM111A variant that has not been previously reported as causing congenital hypoparathyroidism, dysmorphism, and skeletal dysplasia. KCS is classified into two types according to clinical features and inheritance. Whereas the TBCE gene mutations are responsible for KCS type 1 (KCS1), the FAM111A gene mutation causes KCS type 2 (KCS2). KCS1 and KCS2 are autosomal recessive syndromes characterized by hypoparathyroidism, mental retardation, facial dysmorphism, and extreme growth retardation. The family with sequence similarity 111, member A (FAM111A or KIAA1895) gene mutation causes osteocraniostenosis (OCS) or gracile bone dysplasia. Unger et al. reported five KCS and five OCS patients (2). The authors speculated that KCS2 and OCS might both be allelic disorders of differing severity. OCS is a lethal perinatal condition and was named by Verloes et al. in 1994 (5). Thomas et al. reported a lethal dysplasia in male and female siblings with severe pulmonary hypoplasia (6). These cases had pulmonary hypoplasia and required pulmonary support in the early period of their life. The patients (one which was genetically confirmed) died at 2 and 3.5 months. Most OCS patients died in the newborn period -
only one survived to the age of 21 months in Unger's series (2). Our case and his female sibling passed away due to pulmonary failure and septicemia at the age of 3.5 months and 2 months, respectively. The present case also had long, thin bones and ribs, as well as a tibial fracture. The long, thin bones may be related to hypoparathyroidism or insufficient bone development commensurate with the genetic defect's severity.

It has been postulated that FAM111A variants do not affect neural development, and therefore the affected patients would not present with developmental delay (2). In contrast, Cavolo et al. presented a patient with KCS2 having an intellectual disability and microcephaly. Our case had no neurological developmental delay and microcephaly, but the disease developed in the early prenatal period. OCS is a severe and life-threatening form, more so than KCS2, and so these cases might be labeled as OCS.

It is stated that KCS1 and SSS might be allelic disorders of differing severities (7). KCS2 and OCS may cause allelic disorders and have the overlapping phenotype. The differential diagnosis of some syndromes caused by TBCE and FAM111A mutation is shown in Table 1. KCS2 generally presents later in life, but OCS presents early. The clinical data suggest that our case has OCS because of a dysmorphic face, early-onset presentation, micromelia, long bone fracture, and disease severity.

The present case probably has OCS, which is a severe form of KCS2. OCS or KCS2 should be suspected in a hypocalcemic neonatal/infantile patient with short stature and facial dysmorphism.

**Conclusion**

There is limited knowledge about the FAM111A gene. Some genetically unconfirmed KCS patients can have the FAM111A gene mutation. FAM111A mutation is generally characterized by autosomal dominant transmission. The present patient has compound heterozygous variants (c.976T>A and c.1714_1716del) in the FAM111A gene with an autosomal recessive inheritance pattern. The present case probably has OCS, which is a severe form of KCS2. OCS or KCS2 should be suspected in a hypocalcemic neonatal/infantile patient with short stature and facial dysmorphism.

**Authorship Contributions**

Surgical and Medical Practices: Erdal Eren, Omer Tarim

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Data Collection or Processing: Erdal Eren, Havva Tezcan Unlu, Serdar Ceylaner

Analysis or Interpretation: Serdar Ceylaner, Erdal Eren, Havva Tezcan Unlu

Literature Search: Erdal Eren, Omer Tarim

Writing: Erdal Eren, Omer Tarim

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Declarations

Conflict of Interest Statement: None of the authors of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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**References**


Figure 1: A) The general appearance of the patient (a relatively large head, small eyes, and inappropriate body size are noted) and B) Skeletal radiogram of the patient (narrowing, long, thin bones, and thin ribs are noted).
Figure 2: Family pedigrees of probands found to carry c.976T>A and c.1714_1716del compound heterozygous variants. Circles are females; Squares are males. Filled symbol affected with Kenny-Caffey syndrome.

Figure 3: A schematic diagram of identified variants in FAM111A gene, A) c.976T>A and B) c.1714_1716del in the index case.
Figure 4: Figure shows the comparative 3D protein structure modeling (template:1.dua1. A) of FAM111A. The effect of c.976T>A (B), c.1714_1716del (C), c.976T>A, and c.1714_1716del together (D) variants on protein structure by the Swiss model, respectively. A wild-type, B, C, and D affected protein.
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<td>Delayed anterior fontanel closure, Macrocephaly, Prominent forehead, Hyperopia, microphthalmia, papilledema, Defective dentition, Osteosclerosis</td>
<td>Microcephaly, Micrognathia, Prominent forehead, long philtrum, Deep-set eyes, Low-set ears, posteriorly rotated ears, Delayed bone age, Patchy osteosclerosis</td>
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<td>Thickened cortex of long bones, Dense tubular bones and narrow marrow cavities</td>
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AR: autosomal recessive, AD: autosomal dominant, FAM111A: Family with Sequence Similarity 111 Member A, KCS1: Kenny-Caffey syndrome type 1, KCS2: Kenny-Caffey syndrome type 2, OCS: Osteocraniostenosis, SSS: Sanjad-Sakati Syndrome, TBCE: Tubulin-Specific Chaperone