Short Communication

A novel EFNB1 mutation in a patient with craniofrontonasal syndrome and right hallux duplication

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1. Introduction

Craniofrontonasal syndrome (CFNS) was first described by Cohen in 1979, describing a 14-year-old girl with coronal craniosynostosis, hypertelorism, limitation of shoulder movement, and digital abnormalities, as a subgroup of frontonasal dysplasia (Cohen, 1979; Hogue et al., 2010; Young, 1987). CFNS shows a paradoxical inheritance because female patients are severely affected, whereas male carriers are usually mildly affected. CFNS is characterized by severe hypertelorism frequently including orbital asymmetry, depressed nasal bridge and bifid nasal tip, frontal bossing, coronal suture synostosis, large anterior fontanelle in early childhood and occasionally corpus callosum agenesis and cleft lip or palate. Frequent extracranial manifestations include asymmetry of the thoracic skeleton, pectoral muscles and breasts. Occasionally, asymmetric limbs, clinodactyly of the fingers and thumbs or halluces and cutaneous syndactyly have been described (Wieland et al., 2007). Also, affected individuals frequently have nystagmus, strabismus and other vision anomalies due to hypertelorism. Almost all patients have normal mental development. The X-linkage of CFNS was recently confirmed by the identification of mutations in the EFNB1 gene, which maps to Xq13.1, in both familial and sporadic cases. Mutations in EFNB1 have been detected in the majority of patients with familial and sporadic craniofrontonasal syndrome (Ramirez-Garcia et al., 2013; Twigg et al., 2013; Wieland et al., 2004). Until now, approximately one hundred different mutations have been reported in the HGMD.

We here present a case, with CFNS and accompanying broad and/or duplicated hallux, which is a rare finding in this syndrome, having a previously unreported mutation in the EFNB1 gene.

2. Materials and methods

Informed consent was obtained from the family. Genomic DNA was extracted from blood samples of index case and her family members according to manufacturer’s instructions (Roche, Mannheim, Germany). Sequence analysis of the EFNB1 gene was performed by PCR with the use of 5'-AGAAGAGGGAGAGAAGC-3' and 5'-AAAGCGAGGCACAAAGTTAGAAA TGAG-3' which produced a 417 bp PCR product from exon 1; primers 5'-CTGCTGCTCTTCTCTCCTC-3' and 5'-GACCTAGTAAAGTGTTC-3' which produced a 538 bp PCR product from exon 2; and primers 5'-CTCTCCCCCGACTGACTTTC-3' and 5'-AAGGGGAAGGAAACGACG-3' which produced a 684 bp PCR product from exons 3 and 4 and primers 5'-CTGAAAGAAATTGAAAGCTTG-3' and 5'-AAGCCACAAAGCATGTACG-3' which produced a 709 bp PCR product from exon 5. Primers were designed using the program PRIMER3 PLUS. PCR products were analyzed by electrophoresis on a 3% agarose gel. Mutations were detected by ABI 310 Sequence Analyzer. The sequencing data were compared with EFNB1 GenBank reference sequence (accession numbers: NM_004429.4 and NG_008887.71 and Ensembl number: ENST00000204961). De novo occurrence of the c.402 T > C heterozygous mutation in exon 2 of EFNB1 was detected in proband’s genomic DNA. PCR and sequencing of EFNB1 were used to confirm absence of mutations in parental gDNA.
3. Results

Our patient is a 7-month-old female and her parents are non-consanguineous Turkish origin. She was born after an uneventful gestation and spontaneous normal vaginal delivery at full term with normal birth parameters. At that time, her newborn examination showed brachycephaly, hypertelorism and wide anterior fontanelle and broad right hallux. When she was at 4 months, she was referred to us due to craniosynostosis and dysmorphic features from the Pediatric Neurosurgery Department. On clinical examination, the patient had brachycephaly, frontal bossing, large anterior fontanelle, widened metopic suture, hypertelorism, telecanthus, downslanting palpebral fissures, broad nasal root and bifid nasal tip with an inner canthal distance of 3.8 cm (>97th centile), interpupillary distance (IPD) of 6 cm (>97th centile) and outer canthal distance (OCD) of 9.4 cm (>97th centile) (Figs. 1a, b). Her neck was short and she had narrow sloping shoulders. On thorax examination, she had widely spaced nipples and no chest deformity. Her right hallux was broad and duplicated (Fig. 1c). The other systems were normal, as examined.

A cranial ultrasound performed at her first month showed no abnormalities. A hip ultrasound was normal and urinary system ultrasound had shown no abnormality. Radiographs showed bifid right hallux (Fig. 1d). A three-dimensional computed tomography scan (3D CT scan) revealed bilateral coronal suture synostosis, wide metopic suture and large anterior fontanelle (Fig. 1e). Adrenocorticotropic hormone (ACTH) level was 118 pg/ml (normal range: 0–100), found to be higher. She is being followed by pediatric endocrinology department. She had a 46, XX normal female karyotype.

Fig. 1. Frontal (a) and lateral (b) views of the patient demonstrating hypertelorism, downslanting palpebral fissures and broad nasal root in frontal view (a) and brachycephaly, short upturned nose in lateral view (b). Picture (c) and radiological imaging (d) of the patient’s feet showing broad and duplicated right thumb. A three-dimensional cranial computed tomography scan imaging (e) of the patient demonstrating bilateral coronal craniosynostosis, wide metopic suture and large anterior fontanelle.
With this clinical picture, we concluded that the patient has craniofrontonasal syndrome. Sequencing of *EFNB1* showed c.402 T > C heterozygous mutation in exon 2 of *EFNB1*. There was no such mutation in the same region of her parents (Fig. 2a) and no evidence of mosaicism has been observed in the parents. Isoleucine in position 134 is highly conserved amino acid throughout the species (Fig. 2b). To our knowledge, this mutation has not been previously reported in the literature and it is not reported as a variation in the databases like HGMD, 1000 genomes and Exome Variant Server. We have shown that isoleucine to threonine substitution as a result of the de novo mutation may cause encoding of a misfolded protein (Figs. 2c/d). Although the I134 residue locates in the middle of one of the beta folds that constitute the structural core of the protein, the mutation seems to result in an abnormal loop formation as indicated by a black arrow. Detection of this mutation confirms the clinical diagnosis of CFNS in the patient.

### 4. Discussion

CFNS is an X-linked dominant disorder with typical findings of hypertelorism, coronal craniosynostosis, a bifid or cleft nasal tip, widow’s peak, and digital anomalies in affected females despite the presence of only hypertelorism in affected males usually. This disorder is caused by a loss of function mutations in *EFNB1* in approximately 87% of the cases (Twigg et al., 2004; Vasudevan et al., 2006; Wieland et al., 2004). *EFNB1* gene, localized at Xq12, is composed of five exons and it is 13.17 kb in length (Wieland et al., 2004). Broad and/or duplicated hallux is a rare finding in this syndrome with an incidence of approximately 8% of total CFNS patients (Wieacker and Wieland, 2005). The reported cases of CFNS patients with duplicated hallux and additional clinical features are summarized in Table 1. To our knowledge, all reported duplicated hallux phenotypes of CFNS patients are associated with exon 1 or 2 mutations of *EFNB1* including our case.

Fig. 2. Chromatographs of sequence analysis of *EFNB1* in proband and parents (a). A table showing the evolutionary conservation of isoleucine at 134th residue (b). A schematic diagram showing the normal (c) and altered (d) protein structure in the residue where the mutation resides, indicated with an arrow (d).
A novel de novo mutation in the 2nd exon of *EFNB1* was detected in a patient diagnosed with CFNS. The index is heterozygous for the mutation that is a T to C change at nucleotide position 402 according to the National Center for Biotechnology Informatics (NCBI) reference sequence NM_004429.4. This mutation results in substitution of isoleucine at 134th residue (NP_004420.1), which is a highly conserved residue among several species, to threonine. Isoleucine is a non-polar amino acid whereas threonine is a polar one. We suspected whether this substitution causes any conformational change in the protein structure and utilized Jmol program to compare the wild type and the mutant of *EFNB1* (Jmol). Our analysis showed that there is a conformational change (indicated with an arrow) in the residue where the mutated residue resides. To see if the function of the protein is affected from this conformational change, we used Sorting Intolerant From Tolerant (SIFT) software program, which helps in predicting the possible effects of an amino acid substitution on protein function. SIFT score is 0.01, which corresponds to a very damaging effect (possible range, 0 to 1, with scores ≤0.05 predicting damage to protein function).

We here conclude that 402 T > C, a novel mutation on *EFNB1* causes craniofrontonasal syndrome.

**Conflict of interest statement**

Authors declare no conflict of interest.

**Table 1**

Reported mutations and their localizations in *EFNB1* of CFNS patients with duplex hallux.

<table>
<thead>
<tr>
<th>Duplex hallux</th>
<th>Coronal craniosynostosis</th>
<th>Cleft Lip and/or palate</th>
<th>Corpus callosum agenesis</th>
<th>Other findings</th>
<th>Mutation</th>
<th>Exon</th>
<th>References</th>
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<tbody>
<tr>
<td>RH L</td>
<td>−</td>
<td>−</td>
<td>Developmental delay</td>
<td>30C &gt; T</td>
<td>1</td>
<td>(Twigg et al., 2006)</td>
<td></td>
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<tr>
<td>LH −</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>123C &gt; G</td>
<td>1</td>
<td>(Twigg et al., 2006)</td>
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<tr>
<td>RH and LH L</td>
<td>−</td>
<td>Parcial</td>
<td>Developmental delay</td>
<td>196C &gt; T</td>
<td>2</td>
<td>(Twigg et al., 2004)</td>
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<tr>
<td>RH R and L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>220C &gt; T</td>
<td>2</td>
<td>(Wieland et al., 2005)</td>
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<tr>
<td>LH −</td>
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<td>Parcial</td>
<td>Developmental delay</td>
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<td>(Twigg et al., 2006)</td>
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<tr>
<td>RH −</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>344A &gt; C</td>
<td>2</td>
<td>(Twigg et al., 2004)</td>
<td></td>
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<tr>
<td>RH R and L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>402 T &gt; C</td>
<td>2</td>
<td>Our patient</td>
<td></td>
</tr>
</tbody>
</table>

L:Left, R:Right, RH: Right hallux, LH: Left hallux, CL: Cleft lip, CP: Cleft palate.

**References**


