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Severe craniofrontonasal syndrome in a male patient mosaic for a novel nonsense mutation in *EFNB1*

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ABSTRACT

Craniofrontonasal syndrome (CFNS) is an X-linked disorder caused by mutations in *EFNB1*. Uncommonly and paradoxically, female patients with CFNS exhibit significantly more severe symptoms than male patients. This is explained by "cellular interference". Nevertheless, there have been a few reports of male patients severely affected with CFNS due to postzygotic mosaicism. Here, we demonstrated a male patient with severe CFNS. Whole exome sequencing showed that he harbored both wild type and nonsense mutation, c.253C > T (p.Gln85Ter), in the *EFNB1* gene. Sanger sequencing of his leukocytes, buccal swab, and hair root revealed a variable level of mosaicism. This nonsense mutation is absent in his parents and has never been previously reported. Our findings expand the mutational spectrum of *EFNB1* and substantiates that males with severely affected CFNS are mosaic.

1. Introduction

Patients with craniofrontonasal syndrome (CFNS, OMIM #304110) manifest hypertelorism, bifid nasal tip, downslanting palpebral fissures, coronal craniosynostosis, facial asymmetry, and grooved nails (Cohen, 1979). CFNS is a rare X-linked disorder caused by loss-of-function mutations in the *Ephrin B1* gene (*EFNB1*, MIM *300035) (Twigg et al., 2004; Wieland et al., 2004). Causative mutations include missense, nonsense and frameshift variants within the gene, specific exon deletions, and whole gene deletions (Twigg et al., 2006; Wieland et al., 2007). The *EFNB1* encodes a cellular transmembrane protein, Ephrin B1 (NP_004420.1), which takes part in intercellular communication (Lee et al., 2008).

Clinical manifestation of a typical X-linked disorder is generally milder in heterozygous females than that of hemizygous males. Paradoxically for CFNS, A reversed pattern of phenotypic severity is present, in which females are more severely affected (Devriendt et al., 1995). Hemizygous males with CFNS generally do not manifest dysmorphic features but present with exclusive hypertelorism and occasional cleft lip (Kapusta et al., 1992). The explanation for this odd phenomenon probably is a mechanism called cellular interference (Twigg et al., 2013). To date, only three previous studies reported eight males with severe CFNS. They were all mosaic for *EFNB1* mutations – one missense, two nonsenses, two deletions, one point mutation in the upstream open reading frame (uORF), and two extra supernumerary ring X chromosomes (Baker et al., 2010; Evers et al., 2014; Twigg et al., 2013).

Here, we report a boy with a novel nonsense mutation in *EFNB1* rendering severe clinical manifestations of CFNS, supporting the concept of cellular interference hypothesis, and expanding the mutational spectrum of *EFNB1*.

2. Clinical report

A one-year-old boy was referred to Princess Sirindhorn Craniofacial Center, King Chulalongkorn Memorial Hospital due to craniofacial dysmorphism. He was born at full term gestation after an uncomplicated pregnancy and delivery. The parents were non-consanguineous and clinically unaffected. Physical examination showed that he had plagiocephaly, frontal bossing, mild widow's peak, hypertelorism, telecanthus, orbital displacement, high-arched palate, broad nasal root and bifid nasal tip (Fig. 1a and b). CT scan of the skull showed left coronal and left squamosal suture synostosis (Fig. 1c and d). CT scan of the brain showed inferior vermian and right cerebellar

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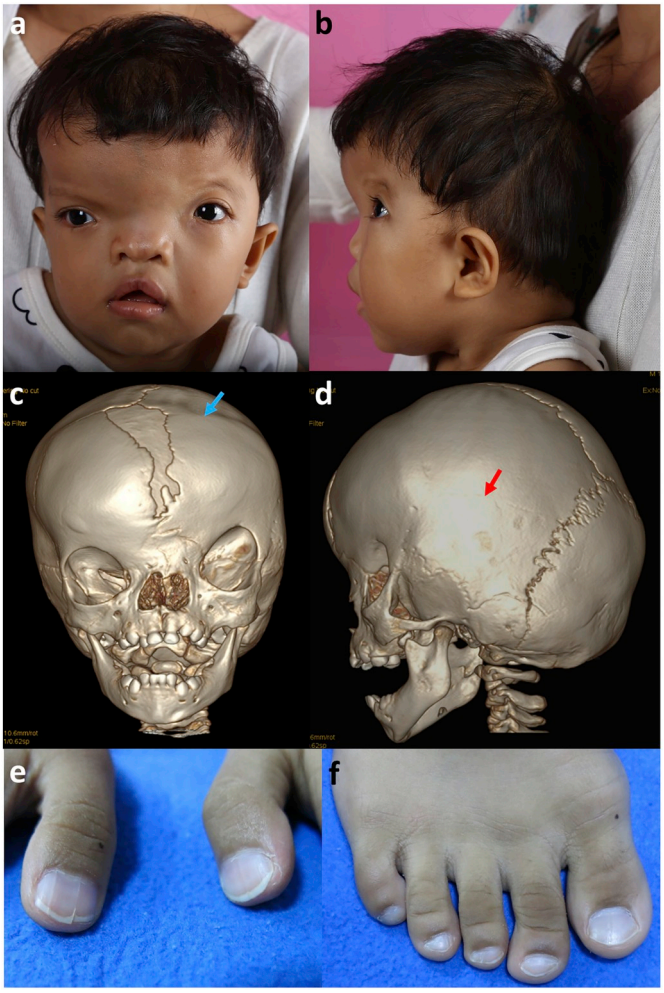

Fig. 1. Clinical features and CT imaging of the proband. (a, b) Frontal and lateral images showed plagiocephaly, frontal bossing, mild widow's peak, hypertelorism, telecanthus, downslanting palpebral fissures, broad nasal root, and bifid nasal tip. (c, d) CT scan of craniofacial bone exhibits synostosis of left coronal suture (blue arrow) and left squamosal suture (red arrow). (e, f) Vertical grooves were present on fingernails and toenails; and vertical splitting on the right thumb nail. The right fifth toe showed clinodactyly. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hypoplasia. Ultrasound of the inguinal area revealed left cryptorchidism and right hydrocele. He also had narrow sloping shoulders, sprengel deformity, scoliosis to the left side at lumbar level, longitudinally split grooved nails and clinodactyly of fifth toes (Fig. 1e and f). At the age of 4 years, his weight was 14.3 kg (3rd – 10th centile), height 105.6 cm (25th – 50th centile), and head circumference 50 cm (50th – 75th centile). He had normal development.

3. Genetic study

Genomic DNA was isolated from peripheral blood leukocytes of the patient and his parents. The DNA sample was prepared as an Illumina sequencing library. In the exome capture step, DNA was enriched using the SureSelect Human All Exon V5 Kit. The captured libraries were sequenced using Illumina HiSeq 4000. Exome data processing, variant calling, and variant annotation were performed by the previously described methods (Porntaveetus et al., 2017). Briefly, trio-whole exome sequencing (WES) analysis was undertaken and all single nucleotide variants (SNVs) and indels were filtered using the following filtering criteria; 1) located in exons or flanking introns of the annotated genes, 2) not synonymous, 3) rare with 1000G minor allele frequency of less than 1%, 4) less than 0.1% in the Genome Aggregation Database (GnomAD), 5) less than 10 alleles in 1,876 Thai exome controls, 6) (if the variant is a missense) predicted to be damaging by SIFT and Polyphen, and/or 7) related to the phenotype of the patient.

By trio-WES analysis, we identified 40 reads harboring a nonsense mutation, c.253C > T (p.Gln85Ter) in the *EFNB1* gene (NM_004429.4) and 32 reads with the wild-type C allele in the patient. The mutation c.253C > T, p.Gln85Ter (ClinVar accession: SCV001156247) was not present either in his parents, or in the dbSNP Build 153, or our in-house 1,876 Thai exomes. According to the ACMG Standards and Guidelines (Richards et al., 2015), the c.253C > T (p.Gln85Ter) was considered as a pathogenic variant (PVS1, PS2, and PM2). Sanger sequencing showed that the child was mosaic at various ratios in different tissues. Leukocytes had similar amounts of the wild-type and the mutant; while buccal swab cells had wild-type dominantly and hair root cells had mutant dominantly (Fig. 2a).

4. Discussion

Usually, males with X-linked disorders are more severely affected. Only two X-linked diseases, infantile epileptic encephalopathy (Depienne and LeGuern, 2012) and CFNS, have been shown to have milder clinical manifestations in males. Males with CFNS usually exhibit only hypertelorism and occasional cleft lips, much milder than female patients. Nonetheless, eight males with CFNS were previously reported to develop clinical presentations as severe as heterozygous females. They were all found to be mosaic for *EFNB1* mutations (Table 1) (Baker et al., 2010; Evers et al., 2014; Twigg et al., 2013).

We identified the ninth male patient having severe clinical features of CFNS. WES detected a novel pathogenic mutation, c.253C > T (p.Gln85Ter) in *EFNB1*. This is the third *EFNB1* nonsense mutation found in mosaic males. This case provides additional evidence and support to the cellular interference hypothesis. Schematic representations of all mutations observed in severely affected males with CFNS are demonstrated (Fig. 2b and c). *EFNB1* has 5 exons and encodes Ephrin B1 which is a cell surface transmembrane ligand protein for Eph receptors. Ephs play roles in cell migration, repulsion, and adhesion during neuronal, vascular, and epithelial development and maintain normal cell to cell interact via proper establishing tight junctions (Darling and Lamb, 2019; Taylor et al., 2017). Ephrin B1 comprises one main domain, Ephrin receptor-binding domain (Ephrin RBD), and 2 motifs (nuclear localization signal and PDZ-binding motifs). Most mutations in severely affected males with CFNS involve the Ephrin RBD. They are expected to cause loss-of-function of Ephrin B1 and failure to interact with signaling proteins.

Identification of mosaic *EFNB1* in severe male CFNS is beneficial for genetic counseling. Due to the postzygotic origin of the mosaic mutation, the recurrence risk for the next child is not increased and remains similar to the general population.

In conclusion, we report the ninth case of severe CFNS in male and

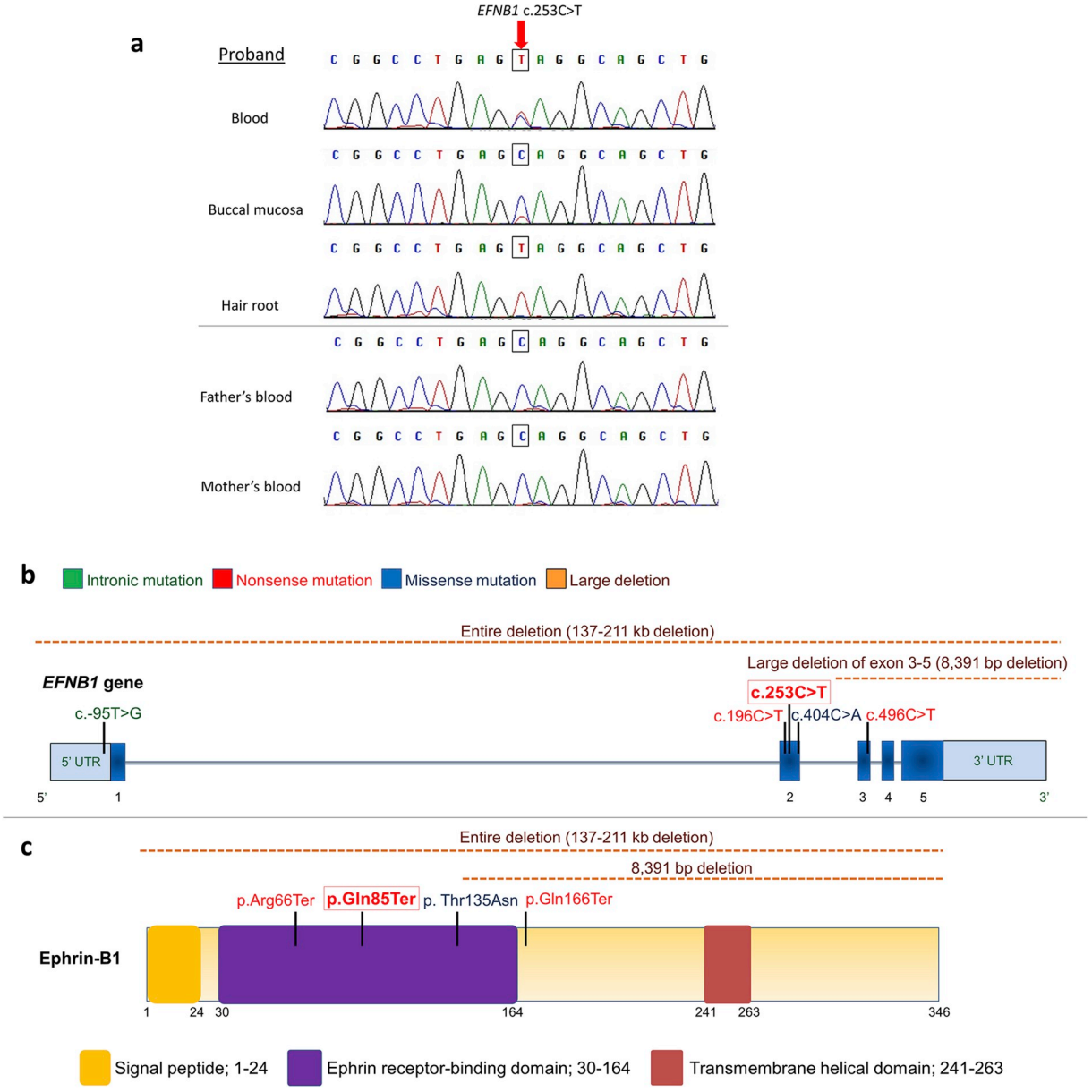

Fig. 2. Sanger sequencing and schematic diagram of *EFNB1* gene and protein. (a) Chromatograms of *EFNB1* exhibited the c.253C > T variant in the proband's blood and hair root. The c.253C was detected in the proband's buccal mucosa and parental blood. (b, c) Summary of all *EFNB1* mutations identified in the males with CFNS. The mutation identified in the proband is demonstrated in the box.

show that he is mosaic for a novel nonsense mutation in *EFNB1*, expanding its mutational spectrum.

Ethical approval

This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB 264/62) and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Varote Shotelersuk: Investigation, Writing - original draft. **Wuttichart Kamolvisit:** Methodology, Writing - original draft. **Nond Rojvachiranonda:** Investigation, Writing - review & editing. **Kanya Suphapeetiporn:** Investigation, Writing - review & editing. **Thantrira Porntaveetus:** Conceptualization, Writing - original draft, Visualization. **Vorasuk Shotelersuk:** Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Table 1
Clinical and molecular findings of male patients with severe CFNS and mosaic *EFNB1* mutations.

Cases		This study	Twigg et al. (2013)						Evers et al. (2014)	Baker et al. (2010)
			3269[a]	1330	4021[b]	4271	4354	3301		
EFNB1 Mutation		c.253C > T, p.Gln85Ter	c.496C > T, p.Gln116Ter	Intronic c.-95T > G	Deletion of exons 3–5	c.196C > T, p.Arg66Ter	c.404C > A, p.Thr135Asn	Deletion of the entire gene	Ring chromosome X	Ring chromosome X
Level of Mosaicism		21% - 100%[c]	27–35%	19–54%	17.4%	46–69%	15–36%	39.5%	42%	45%
Ocular hypertelorism and bifid nasal tip		+	+	+	+	+	+	+	+	+
Downslanting palpebral fissures		+	+	–	+	+	+	+	+	+
Cleft lip or palate		–	–	+	–	–	–	–	–	–
Craniosynostosis		Lt coronal and Lt squamosal suture	Rt, Lt coronal suture	Rt coronal suture	Rt, Lt coronal suture	Rt coronal suture	Lt coronal suture	Rt, Lt coronal suture	N/A	N/A
Brain anomalies	Inferior vermain and Rt cerebellar hypoplasia	Agenesis of corpus callosum	–	Agenesis of corpus callosum	Agenesis of corpus callosum	N/A	N/A	–	–	
Learning disability	–	+	+	+	+	+	–	+	+	
Grooved nails	+	+	+	+	+	+	N/A	N/A	N/A	
Dental anomalies	+	+	N/A	+	+	N/A	N/A	N/A	N/A	
Wiry hair	+	+	–	–	+	–	+	–	–	
Ptosis	–	+	–	+	+	–	–	–	+	
Sprengel deformity	+	+	–	+	–	N/A	–	N/A	N/A	
Undescended testes	+	+	–	+	+	N/A	N/A	+	+	
Other features	Scoliosis, failure to thrive	Axillary web	Small ASD, umbilical hernia, slope shoulder	Mild pectus excavatum, duplication of phalanx, polydactyly, inguinal hernia	Pectus excavatum, scoliosis, oropharynx dysphagia, VUR, hearing loss	Webbed neck	–	PDA, VSD	PDA	

+, present; -, absent; N/A, not available; Rt, right; Lt, left; PDA, patent ductus arteriosus; VSD, ventricular septal defect; VUR, vesicoureteral reflux.
[C]Buccal mucosa 21%, blood 57%, and hair root 100%.
[a] Also reported in Kapusta et al. (1992), Twigg et al. (2006), and van den Elzen et al. (2014).
[b] Also reported in Kwee and Lindhout (1983), and van den Elzen et al. (2014).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2020.103924.

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