A Novel Mutation in EFNB1, Probably With a Dominant Negative Effect, Underlying Craniofrontonasal Syndrome

VORASUK SHOTELERSUK, M.D.
PICHIT SIRIWAN, M.D.
SURASAWADEE AUSAVARAT, B.SC.

Craniofrontonasal syndrome (CFNS) is an X-linked disorder whose main clinical manifestations include coronal craniosynostosis and frontonasal dysplasia. Very recently, CFNS was shown to be caused by mutations in EFNB1 encoding ephrin-B1, and 20 mutations have been described. We report a Thai woman with CFNS, in whom a novel mutation was discovered: c.685_686insG, in exon 5 of EFNB1. It is the first insertion and the most 3′ point mutation in EFNB1 reported to date. The mutation is expected to result in a truncated ephrin-B1 of 230 amino acids, composed of a nearly complete extracellular part of ephrin-B1 with no transmembrane and cytoplasmic domains. This truncated protein might become a soluble form of the ligand, which previously was shown to be able to bind to receptors, but fail to cluster and to activate them—in other words, acting as a dominant negative protein. Nonetheless, further studies to detect the protein are needed to substantiate the hypothesis.

KEY WORDS: *craniofrontonasal syndrome, EFNB1, ephrin-B1, mutation analysis*

Craniofrontonasal syndrome (CFNS; MIM 304110) is an X-linked disorder characterized by unilateral or bilateral coronal craniosynostosis, craniofacial asymmetry, hypertelorism, strabismus, bifid nasal tip, sloping shoulders with dysplastic clavicles, longitudinal splitting of the nails, and thick, wiry, and curly hair. Rare manifestations include anterior cranium bifidum, axillary pterygia, joint abnormalities, cleft lip and palate, unilateral breast hypoplasia, diaphragmatic hernia, asymmetric lower limb shortness, and agenesis of the corpus callosum (Kapusta et al., 1992; Saavedra et al., 1996; McGaughran et al., 2002). Most affected patients are females and obligate male carriers usually show no or only mild manifestations, such as hypertelorism. Very recently, CFNS was shown to be caused by mutations in the EFNB1 gene encoding ephrin-B1 and 20 mutations have been described (Twigg et al., 2004; Wieland et al., 2004). We report a Thai woman with CFNS, in which a novel mutation, possibly having a dominant negative effect, was discovered.

Dr. Shotelersuk is Head and Mr. Ausavarat is affiliated with Division of Medical Genetics and Metabolism, Department of Pediatrics, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Dr. Siriwan is Professor, Division of Plastic Surgery, Department of Surgery, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

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Address correspondence to: Dr. Vorasuk Shotelersuk, Head of Division of Medical Genetics and Metabolism, Department of Pediatrics, Sor Kor Building 11th Floor, King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand. E-mail vorasuk.s@chula.ac.th.

MATERIALS AND METHODS

Clinical Description

A 25-year-old Thai woman presented with craniofacial asymmetry, hypertelorism, strabismus, broad and bifid nasal tip, surgically repaired complete bilateral cleft lip, complete bilateral cleft palate (Fig. 1A), clinodactyly, longitudinal splitting of the nails (Fig. 1B), and thick and wiry hair. Because of her craniofacial anomalies, her parents did not send her to school. They had four children, and our patient was their second child. No other family members were affected.

Mutation Analysis

After informed consent was received, peripheral blood (3 mL) was obtained from the patient and genomic DNA was extracted by standard methods. Exons 1 to 5, which contain the entire coding region of EFNB1, were polymerase chain reaction (PCR)–amplified using primers and conditions as previously described (Twigg et al., 2004). The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH), according to the company recommendations, and sent for direct sequencing at Macrogen Inc. (Seoul, Korea). Blood samples from her parents were not available.

RESULTS

Direct sequencing analysis of the PCR products revealed that the patient was heterozygous for a guanine insertion be-

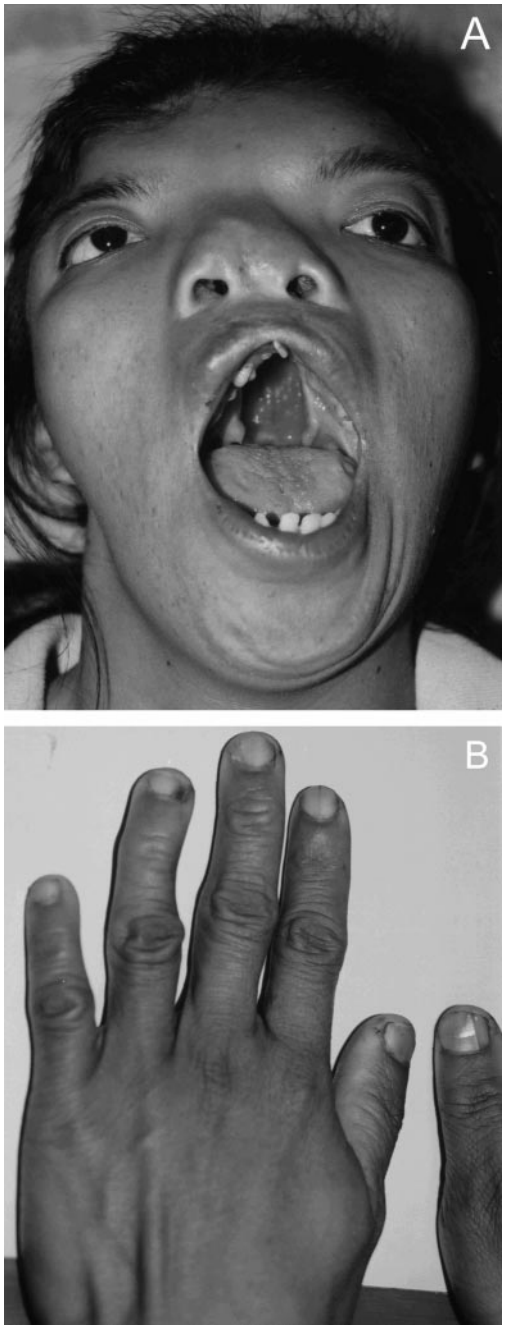

FIGURE 1 The female proband has (A) craniofacial asymmetry, hypertelorism, strabismus, bifid nasal tip, surgically repaired cleft lip, unrepaired cleft palate, and (B) longitudinal splitting of the nails.

tween nucleotides 685 and 686 (c.685_686insG) in exon 5 of EFNB1 (Fig. 2). The mutation is expected to result in subsequent changes of amino acids 229 and 230 and truncation at amino acid 231 because of a frameshift.

DISCUSSION

A Thai woman was identified with typical clinical features of CFNS. The syndrome was known very recently to be caused by mutations in EFNB1. The gene, localizing at Xq12, comprises 13.17 kb and 5 exons (Wieland et al., 2004). Twenty distinct mutations in the gene have been found in 23 families (Twigg et al., 2004; Wieland et al., 2004). Of these 20 mutations, 13 were missense, 3 were splicing, 2 were nonsense, and 2 were deletion mutations. Sequence analysis of the entire coding region in gDNA of our patient revealed a heterozygous c.685_686insG mutation in exon 5 of EFNB1. This mutation has not been previously reported. It is the first insertion and the most 3′ point mutation in the ENFB1 gene described to date.

The EFNB1 gene encodes ephrin-B1 of 346 amino acids (AA). Vertebrate ephrins are membrane-anchored ligands for Eph receptor tyrosine kinases (Davis et al., 1994). Ephrins are divided into two families: A and B. The A family has five members attached to the cell via a glycosylphosphatidylinositol linkage. The B family has three members (B1, B2, and B3) attached to the cell by a hydrophobic transmembrane region and a short cytoplasmic domain (Himanen and Nikolov, 2003). All 20 previously reported mutations were expected to affect the extracellular portion of the ephrin-B1, resulting in complete or partial loss of ephrin-B1 functions. Although the mutation in this patient, c.685_686insG, locates in the last exon and is the most 3′ point mutation in the EFNB1 gene reported to date, it is still 5′ to the transmembrane domain and is expected to result in a truncated ephrin-B1 of 230 AA (228 normal AA followed by two changed AA). This peptide is composed of a nearly complete extracellular part of ephrin-B1 (the extracellular part of the normal ephrin-B1 has 237 AA) with no transmembrane and cytoplasmic domains. The truncated ephrin-B1 could cause reduction of ephrin-B1 functions. Another possibility is that it could become a soluble form of the ligand and could act as a dominant negative protein. Soluble forms of ephrins have been shown to be able to bind to receptors, but fail to cluster and to activate them (Holder and Klein, 1999).

Efnb1, the murine ortholog of EFNB1, is expressed in the frontonasal neural crest and demarcates the position of the future coronal suture (Twigg et al., 2004). EFNB1 is subject to X-inactivation and has no Y counterpart. In addition, no extreme skewing in female patients with CFNS has been observed (Twigg et al., 2004; Wieland et al., 2004). Therefore, heterozygous loss-of-function EFNB1 mutations, possibly including that of this patient, result in reduction of ephrin-B1 functions in some cells and normal functions in others due to random X-inactivation. These patchy abnormalities of signaling disturb the formation of the boundary at the future coronal suture, causing craniosynostosis. Wieland and colleagues proposed the term ‘‘cellular interference’’ for this phenomenon (Wieland et al., 2004). The hemizygous loss-of-function EFNB1 mutations in male patients with CFNS result in a homogeneous cell population in which functional redundancy in the mechanisms of cranial suture determination may occur. This may explain the milder phenotype in male individuals carrying EFNB1 mutations.

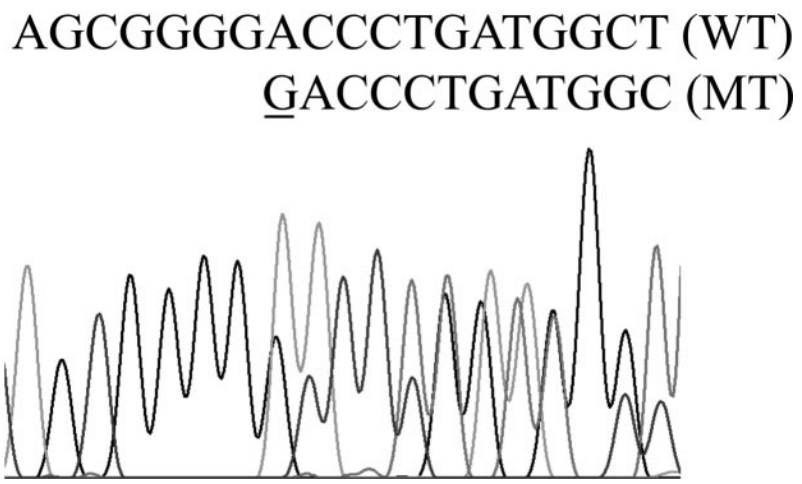

FIGURE 2 The sense sequence electropherograms in the proband. Note the wild-type sequence (WT) can be observed along with the mutant sequence (MT). The inserted G is underlined.

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