

## EFNB1 Gene Analysis in Craniofrontonasal syndrome

**Disorder also known as:** CFNS; Craniofrontonasal dysplasia; CFND; Craniofrontonasal dysostosis

### **Clinical Features:**

Craniofrontonasal syndrome (CFNS) is a rare X-linked dominant disorder characterized by a more severe phenotype of multiple skeletal malformations in heterozygous females in contrast to no or mild clinical features in hemizygous males. Females typically display craniofacial asymmetry, marked hypertelorism with a central nasal groove, bifid nasal tip, coronal craniosynostosis (unilateral or bilateral), corpus callosum agenesis, thick wiry hair, and occasionally cleft lip and/or palate. Extracranial features include sloping shoulders with dysplastic clavicles, asymmetry of the thoracic skeleton and pectoral muscle, unilateral breast hypoplasia, longitudinally grooved fingernails, mild cutaneous syndactyly, and umbilical and diaphragmatic hernia. Hemizygous males have no or only mild manifestations such as hypertelorism and less frequently cleft lip and/or palate. Males who are mosaic for EFNB1 variants may present with a severe phenotype similar to female patients.

### **Genetics:**

X-linked dominant with more severe phenotype in females and under-representation of carrier males in CFNS families.

CFNS is caused by pathogenic variants in the EFNB1 gene located on chromosome Xq13.1. The EFNB1 gene encodes the transmembrane protein ephrin-B1 which, as part of Eph/ephrin transduction system, controls cell patterning of the developing skeleton, nervous system, intestine, and blood vessels. The more severe phenotype seen in females has been hypothesized to occur by the process of random X-inactivation (rather than skewed X-inactivation). As ephrins are expressed in a spatially and temporally dynamic pattern during embryogenesis, it has been proposed that in heterozygous females, ephrin-B1-expressing cells and ephrin-B1-deficient cells lead to a disturbance of cell sorting and migration and subsequent skeletal malformations particularly craniosynostosis. In hemizygous males who have a homogeneous cell population, it is thought that ephrin-B1 may be replaced by another B-class ephrin. The generally mild clinical presentation in males suggests that ephrin-B1 is functionally redundant in the majority of tissues in which it is expressed<sup>1,2,3</sup>. Regarding the reasons for the observed paucity of carrier males in CFNS families, three contributing factors have been proposed: (1) predominant paternal origin of de novo EFNB1 variants, (2) occurrence of somatic variants that are expected to occur twice as often in females, and (3) reduced reproductive fitness in affected females.

## Test Methods:

Analysis is performed by bi-directional sequencing of the coding regions (exons 1-5) and splice sites of the EFNB1 gene. In cases where (1) no small intragenic variant is identified and (2) no heterozygous positions are observed, focused array CGH analysis with exon-level resolution (ExonArrayDx) is available for detection of a partial/whole deletion of one EFNB1 allele. Variants found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method.

## Test Sensitivity:

In the largest published study, EFNB1 variants were identified in 33 out of 38 (86.8%) individuals with clinically diagnosed CFNS5. Specifically, pathogenic variants were detected in 25 of 29 (86.2%) patients with sporadic CFNS and in 8 of 9 (88.9%) families. In two additional studies with a total of 23 families, EFNB1 variants were found in all clinically affected individuals<sup>1,2</sup>. The sequencing approach used by GeneDx will identify >99% of existing small, intragenic variants in the EFNB1 gene, however it cannot detect partial or whole gene deletions in affected females. If indicated, focused array CGH analysis with exon-level resolution (ExonArrayDx) is available to detect such deletions or duplications.

## References:

1. Wieland, I. et al. Mutations of the Ephrin-B1 Gene Cause Craniofrontonasal Syndrome. *Am J Hum Genet.* 74:1209-1215, 2004.
2. Twigg, S. et al. Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *PNAS.* 101:8652-8657, 2004.
3. Wieland, I. et al. Dissecting the molecular mechanism in craniofrontonasal syndrome: differential mRNA expression of mutant EFNB1 and the cellular mosaic. *Eur J Hum Genet.* 16: 184-191, 2008.
4. Twigg, S. et al. The Origin of EFNB1 Mutations in Craniofrontonasal Syndrome: Frequent Somatic Mosaicism and Explanation of the Paucity of Carrier Males. *Am J Hum Genet.* 78:999-1010, 2006.
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7. Twigg, S. et al. Cellular interferences in craniofrontonasal syndrome: males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. *Hum Mol Genet* 22(8):1654-1662.