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Brief Report

Clinical and Molecular Aspects of Craniofrontonasal Syndrome due to Contiguous Gene Deletion Involving *AWAT2*, *EFNB1*, *EDA*, *OTUD6A*, and *PJA1* Genes

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Abstract

Craniofrontonasal syndrome (CFNS; Online Mendelian Inheritance in Man [OMIM] 340110) is an infrequent X linked disorder characterized by specific facial features and digital abnormalities with or without visceral anomalies. There is a peculiar paradoxical difference in severity of the phenotype in heterozygous females compared to hemizygous males. Here, we present a case where the mother, with clinical features of the syndrome, had terminated her previous pregnancy as the fetus had partial agenesis of the corpus callosum. Exome sequencing of the mother revealed no pathogenic variants related to the phenotype. Chromosomal microarray revealed 1.3-Mb pathogenic heterozygous deletion in chromosome X encompassing the Xq13.1 region with five OMIM genes, including *EFNB1* gene related to craniofrontonasal syndrome. Detailed phenotyping of the parents and exact genetic etiology with molecular mechanism is important to arrive at a definitive diagnosis crucial for genetic counseling and definitive prenatal testing.

Keywords
- craniofrontonasal syndrome
- exome sequencing
- contiguous gene deletion
- *EFNB1* gene
- paradoxical inheritance

Introduction

Corpus callosum abnormalities are heterogeneous in etiology. The causes could be idiopathic, attributed to teratogen exposure, or associated with a genetic etiology. Clinical features are also heterogeneous and vary from mild behavioral problems to severe neurological deficits. Only 30 to 40% of these cases have an identifiable genetic etiology. One such rare monogenic disorder is craniofrontonasal syndrome (also named craniofrontonasal dysplasia/dysostosis), with a unique pattern of inheritance. It is phenotypically characterized by variable degrees of craniofacial dysmorphism and digital abnormalities with or without visceral anomalies, including agenesis of the corpus callosum in females.[1] Intelligence is usually normal or they can have a mild intellectual deficit.

Case Report

A nonconsanguineous couple visited the genetic department in their second pregnancy at 12 weeks of gestation (►**Fig. 1A**). Their previous fetus had partial agenesis of the corpus callosum and square shaped cavum septum pellucidum, as revealed by a detailed fetal anomaly scan (►**Fig. 1B, C**). Evaluation using fetal magnetic resonance imaging (MRI),

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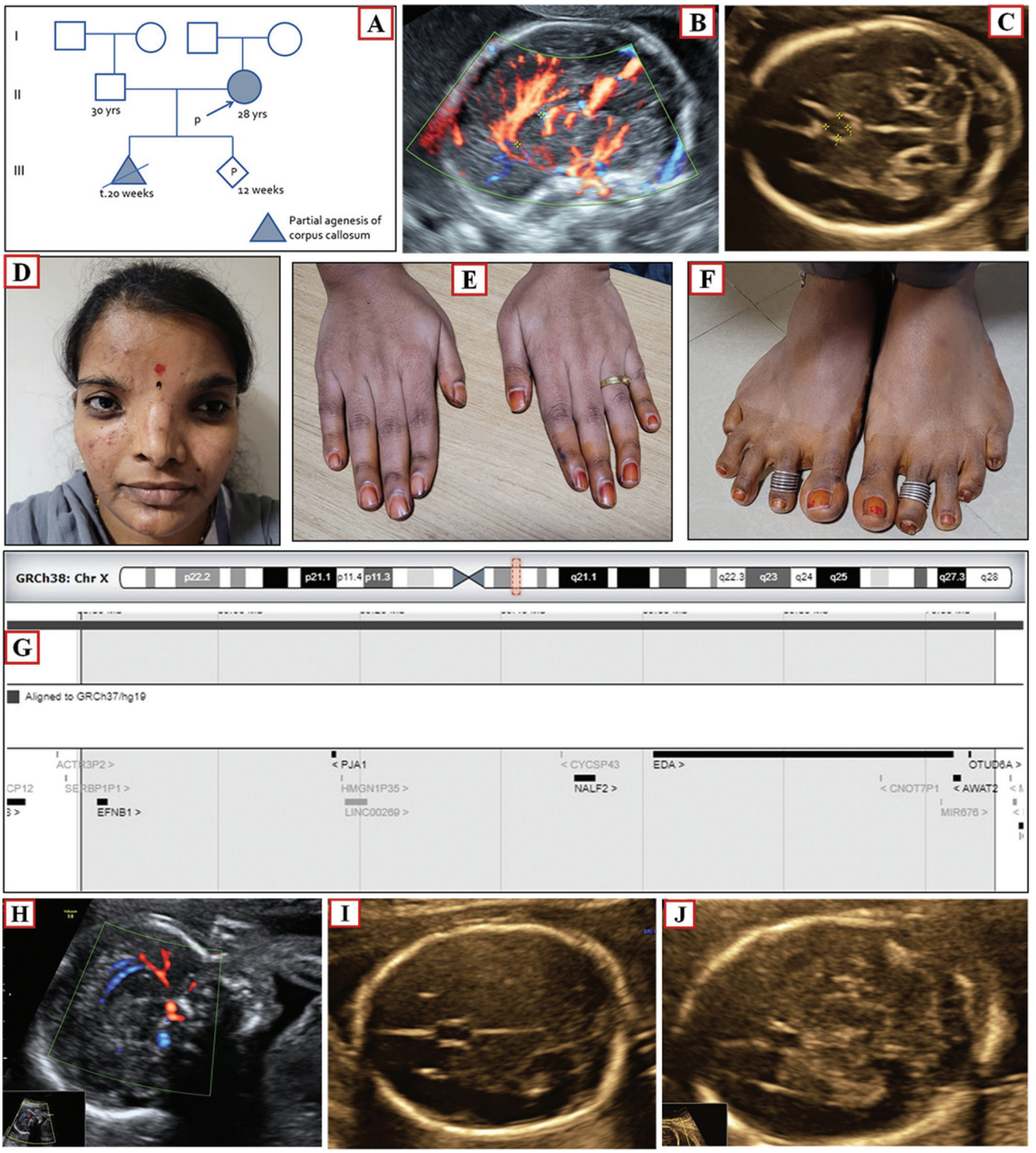

Fig. 1 (**A**) Pedigree of the family. (**B**) Ultrasound showing partial agenesis of the corpus callosum in the previous fetus. (**C**) Square shaped cavum septum pellucidum in the previous fetus. (**D**) Facial features in the mother: broad forehead, hypertelorism with slight up slant of eyes, and broad nasal root with bifid nasal tip. (**E**) Radial deviation of the distal phalanx of fingers with longitudinally grooved fingernails. (**F**) Broad halluces, deviation of toes, and brittle nails in the bilateral feet. (**G**) Chromosomal microarray of the mother revealed a 1.3 Mb pathogenic heterozygous deletion in the Xq13.1 region containing five Online Mendelian Inheritance in Man (OMIM) genes (*AWAT2, EFNB1, EDA, OTUD6A,* and *PJA1).* (**H**) Normal corpus callosum in the second fetus. (**I, J**) Normal structure of the cavum septum pellucidum, cerebellum, and posterior fossa in the second fetus.

autopsy, or genetic workup was not done, and no photographs of the fetus were available. As per the couple, the fetus had syndactyly of the fourth and fifth toes of one foot. There was no similarly affected family member or any other significant family history. During the phenotypic evaluation of the couple, the mother had significant facial dysmorphism in the form of brachycephaly, broad forehead, hypertelorism with a slight up slant of the eyes, and broad nasal root with bifid nasal tip (►**Fig. 1D**). She also had a hypoplastic left nipple and ulnar and radial deviation of the distal phalanx of

the fingers with longitudinally grooved fingernails (►**Fig. 1E**). Both feet were broad with broad halluces, deviation of toes, and brittle nails (►**Fig. 1F**). There was a history of plastic surgery done for the broad nasal root and hypertelorism. The karyotype of the couple, done elsewhere, was normal. Given the characteristic clinical features of brachycephaly, significant hypertelorism, broad nasal root, bifid nasal tip, longitudinally grooved fingernails in the mother, and the history of the previous fetus with corpus callosum abnormality, a provisional diagnosis of craniofrontonasal syndrome was made and genetic evaluation was done. MRI of the brain had been advised for the mother but she was not willing to go for it.

Results

Whole exome sequencing of the mother did not reveal a pathogenic variant in the gene related to the phenotype. Because of a strong clinical suspicion of craniofrontonasal syndrome, a chromosomal microarray (CMA) was done, which revealed a 1.3 Mb pathogenic heterozygous deletion on chromosome X encompassing the Xq13.1 region in the mother (arr[GRCh37] Xq13.1(68,025,398_69,317,932)x1). There were five OMIM genes in the deleted segment of the chromosome (*AWAT2*, *EFNB1*, *EDA*, *OTUD6A*, and *PJA1*; ►**Fig. 1G**). The *EFNB1* gene is associated with the craniofrontonasal syndrome. So, the mother was heterozygous for contiguous gene deletion involving the *EFNB1* gene, leading to craniofrontonasal syndrome. *EDA* gene deletion is associated with X-linked selective tooth agenesis and hypohidrotic ectodermal dysplasia. There is a 50% risk of recurrence of craniofrontonasal syndrome in both male and female offspring, with a milder presentation in males due to paradoxical X linked inheritance and a 50% risk of recurrence of X linked hypohidrotic ectodermal dysplasia in male offspring. Females with *EDA* gene deletion are usually asymptomatic or may have milder manifestations like sparse hair, hypodontia, or underdeveloped nipples. The genes *AWAT2*, *OTUD6A*, and *PJA1* are not associated with any OMIM phenotype. The couple was counseled that there was a likely possibility for agenesis of the corpus callosum in the previous fetus due to an inherited contiguous gene deletion from the mother. In addition, the wide variability in the phenotypic spectrum of the disorder concerning visceral anomalies was also explained. The couple opted for amniocentesis. The CMA of the fetus was normal. Detailed fetal anomaly scan showed normal corpus callosum, cavum septum pellucidum, and posterior fossa (►**Fig. 1H–J**). The couple continued their pregnancy and delivered a phenotypically normal female child. The child is 6 months old and is doing well with normal developmental milestones.

Discussion

The incidence of craniofrontonasal syndrome is estimated to be 1:1,00,000 to 1:1,20,000.[1] It is an X linked disorder caused by pathogenic variants in the *EFNB1* gene, the locus of which is within the Xq13.1 region. The phenotype varies considerably among affected individuals. The most commonly reported features in females include coronal synostosis, hypertelorism, bifid nasal tip, frizzy and curly hair, longitudinal ridging, and splitting of nails. All these features were seen in our case except hair abnormality. Affected individuals can also have cleft lip and palate, rounded shoulders, webbing of the neck, pectus excavatum, asymmetric breast development, scoliosis, and digital abnormalities like cutaneous syndactyly, polydactyly, clinodactyly, broad thumb/hallux, and abnormal toes.[2] Breast asymmetry and digital abnormalities were noted in our case. Visceral abnormalities, notably of the corpus callosum, cerebellum, diaphragm, cardiac, and genitourinary system, can also be seen. Corpus callosum agenesis is seen in only 10 to 50% of cases. The previous fetus of the couple had partial agenesis of the corpus callosum. Intelligence may be normal, or individuals may present with mild intellectual disability, especially those with corpus callosum agenesis and contiguous gene deletion involving the *OPHN1* gene along with *EFNB1* deletion.[3] MRI of the brain was not done for the mother, but she had normal intelligence.

For the affected females, surgical procedures may be needed for craniosynostosis, craniofacial asymmetry, and hypertelorism based on severity. It is important to differentiate this syndrome from some of the syndromes like frontonasal dysplasia, acromelic frontonasal dysostosis, and acrofacial dysostosis, as these disorders also have a spectrum of craniofacial and limb anomalies and overlap with the clinical features of CFNS.

With the X linked dominant pattern of inheritance and CFNS in the mother, it is expected that 50% of her daughters and 50% of the sons will be affected with craniofrontonasal syndrome. But unlike other X linked dominant disorders, CFNS has a paradoxical X linked inheritance with severely affected females. Affected males usually present with a milder phenotype of hypertelorism or none at all. There is no obvious correlation between the type of mutation and expression of the phenotype.[4,5]

The *EFNB1* gene consists of five exons and encodes the ephrin B1 protein, which is a transmembrane ligand for Eph receptor tyrosine kinase. The ephrin B1 protein plays an important role in cell to cell adhesion, cell migration, and pattern formation in the development process of the embryo.[6,7] Due to random inactivation of the X chromosome, females with heterozygous mutation in the *EFNB1* gene become uniquely mosaic, and subsequently, there are patches of random expression and nonexpression of the gene. This leads to abnormal sorting of cells with abnormal boundaries between the cells. This phenomenon is termed cellular interference and is typically seen in females affected with this syndrome.[8] Dysmorphic features in CFNS appear to be due to disruption of the ephrin B1 protein within neural crest cell derived mesenchyme but not tissue specific disruption throughout neural development.[9] As of now, only two females have exhibited milder craniofacial features. On the other hand, three cases with contiguous gene duplication involving the *EFNB1* gene along with the *PJA1* and *STARD8* genes had only familial hypertelorism.[10] The probable explanation for this could be tissue specific mosaicism.

Hemizygous males have a complete loss of expression of the gene and subsequently, loss of function of the protein altogether. So, the phenomenon of cellular interference is not recognized in affected males, and they have a milder phenotype like hypertelorism or have normal facial features. There are a few case reports of severely affected males. A possible explanation for this can be the presence of mosaicism with wild type and mutant allele ratios similar to that seen in heterozygous females.[8]

Among the pathogenic sequence variants identified in the *EFNB1* gene, the most common are missense variants, followed by frameshift, nonsense, splice site variants, and intragenic deletions. Most of the missense variants lead to changes in amino acid residues in the extracellular domain of the ephrin protein resulting in loss of its function. This domain is important for receptor ligand interaction and cell signaling.

There are two case reports of *EFNB1* gene deletion as a part of contiguous gene deletion involving the *OPHN1*, *YIPF6*, *STARD8*, *PJA1*, and *EDA* genes. Our case also had contiguous gene deletion involving the *EDA* gene related to anhidrotic ectodermal dysplasia without the involvement of the *OPHN1* gene. This further complicates the counseling process. Females with *EFNB1* and *EDA* gene deletion will have severe manifestations of *CFNS* but will not be affected with anhidrotic ectodermal dysplasia or may have milder clinical features, whereas males have a milder phenotype of CFNS but will manifest with hypohidrotic ectodermal dysplasia unlike those who have pathogenic sequence variants in the *EFNB1* gene. The *OPHN1* gene, which is responsible for developmental delay and intellectual disability, was not involved in our case. Molecular diagnosis of CFNS is important for risk assessment and exact phenotypic presentation, and to improve the quality of management in those who wish to continue pregnancy in the absence of visceral anomalies.

Conclusion

Detailed phenotyping of the parents is critically important to arrive at a definitive diagnosis, even in the advanced genomic era. Understanding the inheritance pattern is crucial for genetic counseling and for definitive prenatal testing.

Funding
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Conflict of Interest
None declared.

References

1 Toteja N, Khera D, Sasidharan R. Craniofrontonasal dysplasia. Sudan J Paediatr 2022;22(01):119–120
2 Inoue Y, Sakamoto Y, Sugimoto M, et al. A family with craniofrontonasal syndrome: the first report of familial cases of craniofrontonasal syndrome with bilateral cleft lip and palate. Cleft Palate Craniofac J 2018;55(07):1026–1029
3 Wieland I, Weidner C, Ciccone R, et al. Contiguous gene deletions involving EFNB1, OPHN1, PJA1 and EDA in patients with craniofrontonasal syndrome. Clin Genet 2007;72(06):506–516
4 Howaldt A, Nampoothiri S, Yesodharan D, Udayakumaran S, Subash P, Kornak U. Four novel mutations in EFNB1 in Indian patients with craniofrontonasal syndrome. J Hum Genet 2019;64 (09):867–873
5 van den Elzen ME, Twigg SRF, Goos JAC, et al. Phenotypes of craniofrontonasal syndrome in patients with a pathogenic mutation in EFNB1. Eur J Hum Genet 2014;22(08):995–1001
6 Pasquale EB. Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol 2005;6(06):462–475
7 Davy A, Bush JO, Soriano P. Inhibition of gap junction communication at ectopic Eph/ephrin boundaries underlies craniofrontonasal syndrome. PLoS Biol 2006;4(10):e315
8 Twigg SRF, Babbs C, van den Elzen MEP, et al. Cellular interference in craniofrontonasal syndrome: males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. Hum Mol Genet 2013;22(08):1654–1662
9 Niethamer TK, Teng T, Franco M, Du YX, Percival CJ, Bush JO. Aberrant cell segregation in the craniofacial primordium and the emergence of facial dysmorphology in craniofrontonasal syndrome. PLoS Genet 2020;16(02):e1008300
10 Babbs C, Stewart HS, Williams LJ, et al. Duplication of the EFNB1 gene in familial hypertelorism: imbalance in ephrin-B1 expression and abnormal phenotypes in humans and mice. Hum Mutat 2011;32(08):930–938

