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Case Report

CDON Mutation Related to Nose Deformity with Variable Expression in Holoprosencephaly in an Iranian Family: A Case Report

Dariush D. Farhud [1,2,3,4], Parisa Varjavand [1], Marjan Zarif-Yeganeh [1,2,3]

1. *Dr. Farhud Genetic Clinic, Tehran, Iran*
2. *Research Institute of Aging, Tehran University of Medical Sciences, Tehran, Iran*
3. *Social Genetics Research Center, Nasim Institute, Tehran, Iran*
4. *Department of Basic Sciences, Iranian Academy of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: farhud@sina.tums.ac.ir

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Abstract

Holoprosencephaly, a complicated brain abnormality arising from incomplete prosencephalon cleavage, affects both the forebrain and the face. Holoprosencephaly Type 11, with variable expression or partial penetrance, is caused by *CDON* pathogenic variants associated with the disrupted Sonic Hedgehog (*SHH*)-pathway. Herein, we aimed to describe a family with genetic nose problems. After counselling and drawing pedigree in Farhud's Genetic Clinic, Tehran, Iran in 2021, DNA extraction of a proband and a few members of his family (patient and control) was conducted. Whole exome sequencing was utilized for detecting the gene and its variant in the proband with a nose deformity. The results were confirmed with Sanger sequencing. This variant was checked in other members by Sanger sequencing. Analysis of the Exome data showed a heterozygous splicing variant in the *CDON* gene (NM_016952; c.3276+1G>T) in the proband who had a nose deformity and then the results were confirmed with Sanger sequencing. Such a variant was observed in Proband's brother with a nose deformity and was not observed in Proband's cousin with no abnormal phenotype. Recent investigations, in an Iranian family, with a heterozygous splicing *CDON* mutation as a human candidate gene are discussed for the first time in relation to the likely pathogenesis of facial deformities, particularly nose deformity, in Holoprosencephaly.

Keywords: *CDON* gene; Holoprosencephaly; Exome sequencing; Nose dysmorphism

Introduction

Holoprosencephaly (HPE; MIM# 236100) comprises a clinical spectrum caused by an abnormality of the midline of the forebrain and midface. "These defects include hypotelorism, midface retrusion, median cleft, and a narrow maxillary arch with apertognathia. The cranium is usually microcephalic, and the bony defects are centered around the ethmoid bone" (1). According to the most recent estimations, HPE has a prevalence of 1.3 per 10,000 live births but an incidence of at least 1 per 250 conceptuses (2,3).

Information on the epidemiology of HPE is restricted owing to the small number of population-based studies that have been reported, as well as

the fact that small studies require a greater number of years in order to accrue enough births for a meaningful estimate (2).

Mutation in 4 significant genes in HPE patients has been identified (Sonic hedgehog or *SHH*; *ZIC2; SIX3; TGIF1*), and other genes including *TDGF1, FOXH1, TGIF1, CDON* (cell surface receptor-like protein), *NODAL*, *GAS1*, and *STIL* are the minor genes shown in the small number of cases (4,5). Many forms of HPE are associated with the disrupted Sonic Hedgehog (*SHH*)-pathway, which is regulated by a network of ligand-binding factors like *CDON* (also known as *CDO*), Boc (brother of *CDON*), and *GAS1* as a putative receptor and *PTCH1* as a primary receptor (6, 7). The first gene discovered and implicated in HPE was Sonic Hedgehog (*SHH*) (8).

Due to its ease of genetic manipulation and similarity to human development of the forebrain and face, the mouse has emerged as a model. In contrast to humans, mice with heterozygous mutations in the orthologs of the human HPE gene are very infrequently found to have HPE (8). The severity of the disease in *Gas1* and *Cdon* single mutants with microforms of HPE depends on the genetic background of the mouse model (9–11). A few cases have been associated with nongenetic factors. Maternal diabetes, alcohol intake during pregnancy, and prenatal exposure to other possible teratogens such as retinoic acid, plant alkaloids, and pharmaceutical medicines may raise the risk of HPE (12,13). Approximately 65% of HPE cases remain unsolved, implying the presence of numerous other genetic or environmental risk factors (14). An autosomal dominant, autosomal recessive inheritance or association of mutations in multiple genes has been recorded for HPE. Holoprosencephaly Type 11 (MIM 614226), with variable expression or partial penetrance, is caused by heterozygous *CDON* pathogenic variants (15).

Here, we provide information on splicing *CDON* mutation found in a 28-year-old man with HPE inherited in an autosomal dominant detected by whole exome sequencing (WES).

Case report

A three-generational pedigree was created for the family based on their family history, which was obtained from a medical genetic counsellor in Farhud's Genetic Clinic, Tehran, Iran in 2021. The pedigree is shown a history of nose deformity in Fig. 1, and the proband is affected individual III-1, also the phenotype of II-3 is similar to the proband (Fig. 2). We have examined two other individuals in one generation with special results. His brother is affected individual III-2, and his cousin is individual III-8.

Informed written consent was obtained from all subjects participating in the study. We analyzed only three individuals. Proband was a 28-year-old Iranian man (his ethnicity is Mazani) with a severe form of nose deformity in his supra tip and columella. He had nose surgery in his childhood and lost his olfactory sense. It is unknown whether his problem is due to the surgery or genetic disorders. Clinical characteristics of autosomal dominant disorders could show striking variation from person to person, even within the same family, due to the variable expression of dominant Holoprosencephaly. A study of the family history revealed that there were nose abnormalities in many family members. The brother of the proband was a 33-year-old male. He was affected and had nasal deformity but with a less severe form. He also had syndactyly of the second and third both toes. Individual III-8 was the 33-year-old cousin of patient III-1 and showed no abnormal findings in phenotype.

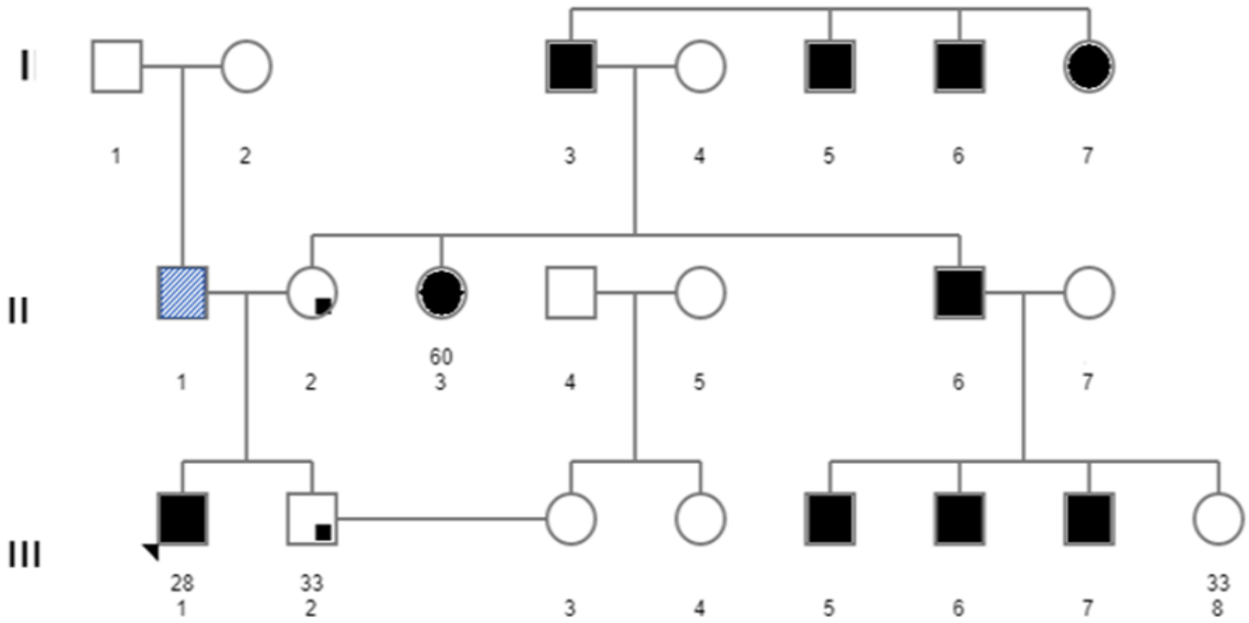

Fig. 1: The pedigree of the family with autosomal dominant inheritance in CDON. III-1, III-2, and III-8 were available for genetic testing. Affected Individuals are shaded. The phenotype of II-3 was similar to the proband (Fig.2)

= An affected individual with the severe form of HPE

= AN affected individual with the less severe form of HPE

= AN affected individual with Retinitis pigmentosa (RP)

Fig. 2: Photo of the affected individual with HPE. Front facial of a patient II-3, a 60-year-old female with Nose deformity

Genetic analysis

After DNA extraction from whole blood, the sample was prepared using Agilent SureSelectV6 kit and guidelines. The results were sequenced on Illumina Hiseq 4000 machine (200X). Agilent Sure Select Kit uses capture primers along with magnetic beads to target and capture exons. Two software packages, Fast QC and Qualimap, were used to generate summary statistics on the raw fastq files. The software package for quality control of input reads is Fast QC, which is used to create a summary statistic of inputs. Detected variations include single-point mutations and small indels (within 20bp). The analytical sensitivity and specificity of the NGS method used in this assay for the

detection of single point mutations and small indels are assumed to be >95%. Our scheme for variant classification followed the American College of Medical Genetics and Genomics–Association (ACMG) guidelines. Therefore, Genetic analysis, including whole exome sequencing and Sanger sequencing, was undertaken in a family affected with HPE.

WES data analysis and sanger sequencing confirmation demostrated a heterozygous splicing variant, c.3276+1G >T, in exon 17 of the *CDON* gene (Fig. 3)

According to the ACMG criteria, the identified variant was classified as Likely Pathogenic With the use of several bioinformatic tools; including (https://varsome.com/datasources/); (https://franklin.genoox.com/clinical-db/home); and (https://www.mutationtaster.org) (Table 1). A variety of web-based bioinformatics tools were used to assess rare and novel variations using the EnsEMBL SNP Effect Predictor (https://asia.ensembl.org/info/docs/tools/vep/index.html). Among them, the same maternal mutation was validated using Sanger sequencing. Patient III-2, who had the same nose anomalies, also carried this variant. Patient III-8 had no mutation in this gene, and she was intact.

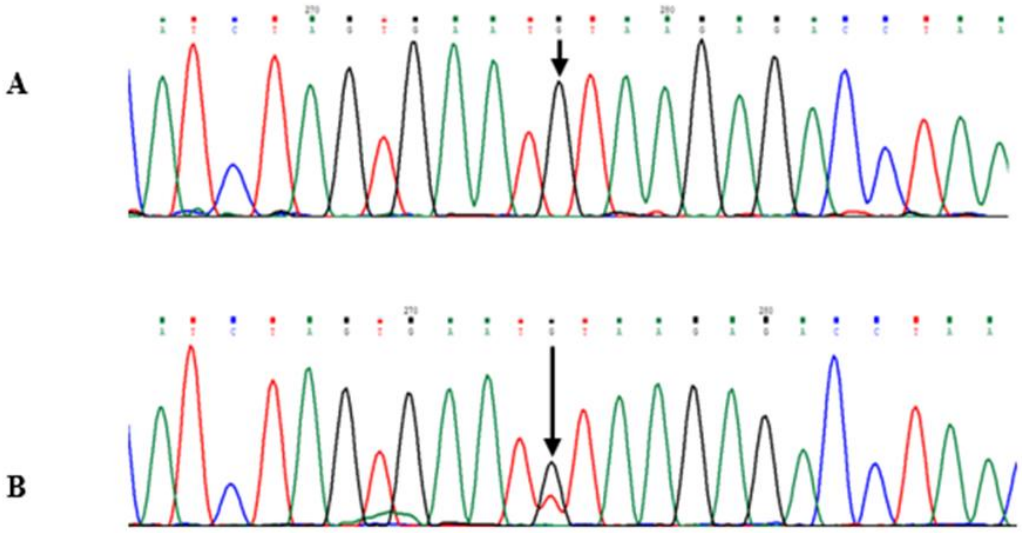

Fig. 3: **A**: Normal variant_ **B**: Mutant variant
Representative chromatograms of the heterozygous c.3276+1G>T
CDON mutation showing the affected individual III-2 (B) and individual III-8 (A)

Table 1: The identified variant in the proband and the other affected patient

Gene/Transcript (RefSeq)	*Variant Location*	*Variant*	*Chromosome Position (GRCh37)*	*Zygosity*	*Related Phenotype*	*Inheritance Pattern*	*Variant Classification*	*Reference SNP cluster ID*
CDON: NM_016952	Exon17	c.3276+1G>T	chr11: 125850943	Het	Holoprosencephaly 11	Autosomal Dominant	Likely Pathogenic	rs374564199

Discussion

We report here the heterozygous splicing variant of *CDON* (NM_016952; c.3276+1G>T) in the individual (proband) with features of Holoprosencephaly Type 11. This was inherited from his mother, who had a nose deformity and did not show any disease symptoms. The proband's brother had the same variant and similar deformity but with a less severe form. He was available for clinical evaluation and genetic testing. The pedigree shows that three generations were affected (Fig. 1).

HPE is the craniofacial malformations involving median structures derived from the frontonasal process, including the nose, the interorbital region, nasal bones, ethmoid sinus, and premaxillary bones with the alveolar processes and the four maxillary incisors (16). Phenotypic variability and pleiotropy have been well documented in HPE (17), which is classified into three classic forms, including alobar, semi-lobar, and lobar (18). The range of midline defects, from the most severe to the least severe, is referred to as the HPE spectrum (19). In most cases of Holoprosencephaly, the foetus dies before birth, and those that do survive have severe functional disabilities, including mental retardation. The clinical features range from cyclopia and ethmocephaly in the more severe types, which seldom survive past infancy, to more modest abnormalities of the midline face structure (20).

More research demonstrates that *CDON* must engage with *SHH* ligand and other *SHH* receptor components, especially *PTCH1*, at the cell surface for adequate signaling to occur and that disruption of these interactions can result in HPE (19, 21). As a result, deficiencies in multiple *SHH* [MIM 600725]-signaling pathway components are the most well-understood causes of HPE-like abnormalities. Studies on mice have shown that the expression of *Gas1*, *Cdon*, and *Boc* may influence the expression of *Hh*. Notably, distinct Holoprosencephaly phenotypes result from the deletion of either *Gas1* or *Cdon* in mice (22). *Hh* reception is modulated by additional *Hh*-binding proteins, including the *Ihog*/*Cdo* family of immunoglobin/fibronectin-repeat-containing proteins and the *GPI*-anchored membrane-bound protein *Gas1* (9,11,23,24). *IHOGT, CDO*, and *BOC* are positively responsible for regulating *Hh* signaling according to genetic research in mice and Drosophila (9,11,23,24). Different extracerebral problems are frequently linked to *CDON* mutations. Variants in *CDON* provide a wide range of abnormal phenotypes. In addition to central nervous system abnormalities, hepatic cholestasis, biliary atresia, or dark, thick eyebrows with synophrys have been reported (23).

Previously, Cebocephaly was linked to *CDON*, another known HPE gene with substantially similar activities and structure to *BOC* (11). There was a report about a girl with a feature of Steinfeld syndrome, characterized by Holoprosencephaly, and identified heterozygous missense *CDON* mutations in the proband and her father, who had ocular hypotelorism only (25). A candidate mutation for the phenotype was discovered by the study of 58 genes. The *CDON* gene in the patient has a novel nonsense mutation (c.2764TC, Glu922Ter). Her mother, who had congenital convergent strabismus and described a very wide spectrum of HPE characteristics in connection with *CDON* mutation, also carries this gene (25).

Mutations in *CDON* have been linked to dominant inheritance (19). Contrary to the dominant pattern seen in HPE, *CDON* is the first HPE gene to be found to generate coloboma with a recessive inheritance pattern. "All three recessive coloboma alleles are likely to cause loss of function: the nonsense variant (c.622C>T p.Arg208) lies in exon 5, and the donor splice site variants affect exons 6 and 14" (26).

Missense *CDON* variants in the human HPE gene were reported. The alterations result in decreased activity in *SHH*-signaling experiments, suggesting that this coreceptor plays a substantial role in HPE pathogenesis for instance this variant (c.2051C>G , p.Thr684Ser) causes aborted fetus, (2) (c.2065C>G , p.Pro689Ala) causes agenesis of the corpus callosum, hypotelorism, growth hormone deficiency, global developmental delay; dark, thick

eyebrows with synophrys, (3) (c.2071G>A , p.Val691Met) causes microcephaly and it can be fetal due to semi-lobar HPE and biliary atresia, (4) (c.2339T>A , p.Val780Glu) causes midline cyst of falx cerebri, (5) (c.2368A>G , p.Thr790Ala) causes agenesis of the corpus callosum, alobar HPE, hypotelorism, mild proptosis, median cleft lip/palate, absent columella, cryptorchidism, incomplete separation of the frontal lobes, absent pituitary, adrenal atrophy, absent corpus callosum, optic tracts with single cerebral arteryhepatic cholestasis and polysplenia, and (6) (c.2818A>C , p.Ser940Arg) causes alobar HPE findings (23).

A new heterozygous missense mutation in the *CDON* gene was related to PSIS, unilateral facial, and abducens nerve paralysis. A new *CDON* missense mutation (c.1814G > T; p. Gly605Val) is related to PSIS, and congenital cranial nerve palsy has been identified. In accordance with earlier research revealing *CDON* abnormalities, the variation exhibited autosomal dominant inheritance with partial penetrance (27). With a missing or inadequate columella, philtrum, and/or pro-labium, the nasal complex is hypoplastic. Typically, *CDON* causes the microform kind of Holoprosencephaly.

Defective development of prechordal mesoderm may cause Holoprosencephaly. This tissue gives rise to the prosencephalon and various facial features, including the ethmoid bone. The anterior craniofacial skeleton is essentially supported by the ethmoid bone. The middle and superior nasal conchae make up the majority of the superior wall of the nasal cavity, and it contributes significantly to the medial wall of the orbit. The anterior cranial base includes the cribriform plate. The nasal septum is made up in part of the perpendicular plate, which anchors the remaining cartilaginous section. The frontal, nasal, sphenoid, and vomer bones, as well as the plate, are articulated. It is clear how crucial the ethmoid bone is to the structure of the middle cranial skeleton. Multiple abnormalities in the cranial base, orbit, and nasal septum, as well as the collapse of articulating bones, occur when the ethmoid bone is absent (1).

At least 10% of all inherited disease-causing variants in humans result from single base-pair mutations in splice junctions. Typically, such mutations result in errors during the splicing process, which can result in improper intron removal and thus alter the open reading frame. In conclusion, effective protein synthesis requires the successful completion of the complex splicing operation. The splicing process consists of two steps: the identification of splicing sites at intron/exon junctions and the removal of the intron and joining of the exon ends. The majority of exon/intron boundary sequences (98.7%) contain GT (donor) and AG (acceptor) motifs at the 5' and 3' ends of the intron (Fig.4) (28).

Fig. 4: Donor (5' splice) and acceptor (3' splice) consensus sequences, as well as the branchpoint. Note the unchanging nature of the dinucleotides GT and AG (in red) at the beginning and end of the intron.(28)

The *CDON* c.3276+1G>T variant was discovered in ClinVar. The c. 3276+1G>T variant is predicted to cause abnormal splicing because the nucleotide substitution occurs in the invariant region of the splice consensus sequence and may lead to a truncated or absent protein and loss of function. Illumina has classified the significance of *CDON* gene loss of function variants as uncertain because their role in disease is currently not well-established.

Conclusion

For the first time in Iran, we suggest the heterozygous splicing variant in *CDON* leads to a phenotype including nose deformity, and the classification of this variant is Likely Pathogenic. Based on the results of this study، it is recommended that each person with mentioned this gene mutation conducts prenatal testing. Functional studies can help us gain more information about this disease.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors

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Conflict of interest

The authors declare that there is no conflict of interest.

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