CASE REPORT

DOI: 10.5336/caserep.2022-89352

A Novel Homozygous Frameshift Mutation in the PLCB4 Gene Associated with Auriculocondylar Syndrome 2 and Accompanied by Mild Intellectual Disability

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ABSTRACT Auriculocondylar syndrome is a rare autosomal dominant or recessive disorder characterized by question-mark ears, a small mandibular condyle, and micrognathia. From a molecular perspective, auriculocondylar syndrome arises due to mutations in the PLCB4, GNAI3, and EDN1 genes that play roles in the endothelin signaling pathway. Here, we report a patient with findings of auriculocondylar syndrome and an additional mild intellectual disability. The patient's whole exome sequencing analyses revealed a novel homozygous frameshift mutation in the PLCB4 gene related to auriculocondylar syndrome Type 2. Our molecular studies indicated that this mutation caused a downregulation of PLCB4. This type of PLCB4 mutation has seldom been reported in auriculocondylar syndrome-2 patients and only 2 of them have had neurodevelopmental anomalies, as in our patient. We think that this study supports the possibility of intellectual disability in individuals with a homozygous truncating variant in the PLCB4 gene and contributes to the literature.

Keywords: Auriculocondylar syndrome; endothelin; intellectual disability; PLCB4

Auriculocondylar syndrome (ARCND), also known as question-mark ear syndrome or dysgnathia complex, is a craniofacial malformation syndrome with autosomal dominant or recessive hereditary patterns. ARCND is characterized by highly variable mandibular anomalies, including mild to severe micrognathia, and is often accompanied by temporomandibular joint ankylosis, and a distinctive ear malformation that consists of separation of the lobule from the external ear, giving the appearance of a question mark. Other frequently described features include prominent cheeks, cupped and posteriorly rotated ears, preauricular tags, and microstomia.[1]

Molecular genetic studies have indicated that ARCND pathogenesis involves a disruption of the endothelin signaling pathway and hinders craniofacial development.[2] Mutations in the members of the endothelin signaling pathway, including phospholipase c, beta-4 (*PLCB4*), G protein subunit alpha I3 (*GNAI3*), and endothelin 1 (*EDN1*), were associated with ARCND1 (OMIM; 602483), ARCND2 (OMIM; 614669), and ARCND3 (OMIM; 615706).[1-3]

Molecular studies have revealed that ARCND2 can occur by dominant inheritance caused by missense heterozygous mutations in the *PLCB4* gene with dominant negative effects or, more rarely, by recessive inheritance caused by bi-allelic null mutations with loss of function effects.[1,4] ARCND2, which shows autosomal recessive inheritance, has been described to date in 4 patients from 3 families, and neurodevelopmental anomalies have also been reported in 2 of these patients.[3-5]

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Peer review under responsibility of Turkiye Klinikleri Journal of Case Reports.

Received: 01 Mar 2022 ***Received in revised form:*** 25 Apr 2022 ***Accepted:*** 09 May 2022 ***Available online:*** 16 May 2022

In this report, we describe a male patient with an ARCND phenotype, accompanied by a mild intellectual disability (ID), resulting from a homozygous frameshift mutation.

CASE REPORT

A 18-year-old male patient was evaluated at Gazi University Faculty of Dentistry due to obstructive sleep apnea and pain in the temporomandibular joint. The patient, who was scheduled for orthognathic surgery, was referred to the medical genetics department for a genetic etiology evaluation. He was the 3rd living child of healthy parents who were related, and he had a history of 2 healthy sisters and a brother who had died in the postnatal 2nd month. He began walking at 18 months of age, and his ability to sit without support and speak developed within normal time frames. A physical examination revealed that his height was in the 3rd-10th centile, his weight was less than the 3rd centile, and his head circumference was the 10th centile. The patient had a triangular face, downslanting palpebral fissures, dysplastic ears, small ear lobules, anterior open bite, narrow mouth opening, and retromicrognathia. Temporal computed tomography revealed no structural anomaly in the jaw joint, but an operation was performed for mandibular hypoplasia. Mixed-type apnea, including both obstructive and central apnea, was detected in the patient. The patient had low school success and was evaluated with a borderline ID according to the Wechsler Intelligence Scale for Children-Revised (WISC-R) test. The patient also had a history of surgery due to epispadias.

We performed whole exome sequencing (WES) using DNA obtained from the peripheral blood of the patient by preparing a manual library according to the manufacturer's protocol, followed by template preparation and chip loading on an Ion Chef instrument and sequencing on an Ion S5 sequencer (Thermo Fisher Scientific, Sunnyvale, CA, USA). The WES analysis including variant calling, filtering of single nucleotide variants and insertion/deletion filtering, and annotation was performed by Ion Reporter™ Software 5.10 (Thermo Fisher Scientific, Sunnyvale, CA, USA). The WES analyses revealed that the patient had homozygous frameshift indel mutation, c.175_182delGTGCTAGAinsATG (p.Val59MetfsTer24), in the *PLCB4* gene (NM_000933) and confirmed by Sanger sequencing (Figure 1). This mutation was likely pathogenic according to the guidelines for the interpretation of sequence variants of the American College of Medical Genetics (ACMG; PVS1 and PM2).[6] We also evaluated the effects of these variants on the expression levels of *PLCB4* mRNA by isolating mRNA from the patient's blood and comparing it to mRNA from a healthy individual by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). The expression of *PLCB4* was detectable, even though it was downregulated approximately two-fold compared to healthy individuals. The patient was diagnosed as ARCND2 (OMIM; 614669). In addition, WES data was also analyzed for the ID finding in the

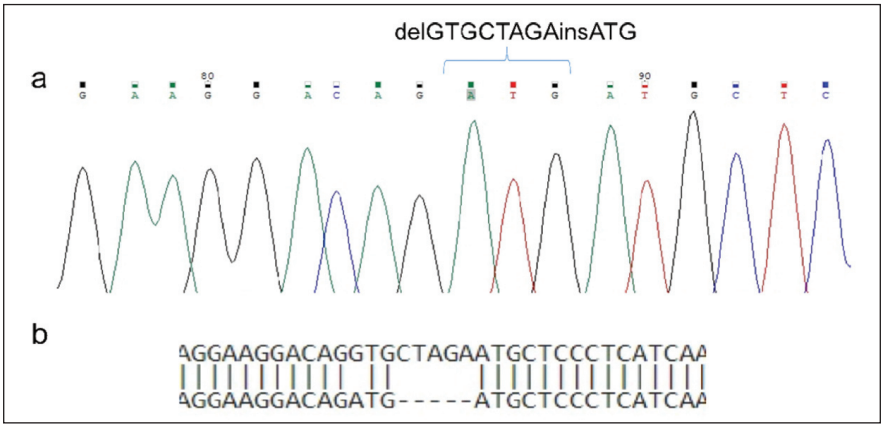

FIGURE 1: (a) Sanger sequencing view of the patient's homozygous mutation in the PLCB4 gene: c.175_182delGTGCTAGAinsATG (p.Val59MetfsTer24), **(b)** Basic Local Alignment Search Tool (BLAST) alignment view of the mutation: upper reference sequence, lower the patient's sequence.

patient, and no additional pathogenic variant that could be associated with ID was detected. Karyotype and chromosomal microarray analysis were also performed on the patient and found as normal. The informed consents were obtained from the patient and his family. Parental analysis of the *PLCB4* variant could not be performed because they did not accept it.

DISCUSSION

ARCND is a craniofacial malformation syndrome with diverse physical anomalies, including question mark ear morphology.[1] The WES analyses of our patient revealed a homozygous frameshift mutation in the *PLCB4* gene (OMIM; 614669), leading to the diagnosis of ARCND2.

ARCND2 is associated with heterozygous or homozygous mutations in the *PLCB4* gene, and most of these mutations are heterozygous missense variants with dominant inheritance.[1] These mutated regions are relatively close to the active site of the protein and missense mutations are thought to cause disease through gain of function mechanism (PMID: 25026904). A few homozygous variants have been reported to date in the *PLCB4* gene, and these are truncating variants that are expected to cause early termination of the protein (Table 1).[3-5] Heterozygous carrier parents of patients with a homozygous truncating variant are not affected, indicating that haploid insufficiency of the *PLCB4* gene does not cause ARCND2. In our patient, the mutation in the *PLCB4*

TABLE 1: Patients with homozygous null variants in the PLCB4 gene.

Report	Ref 3	Ref 4	Ref 4	Ref 5	Present study
Patient	Case 8	Patient 1 (brother of patient 2)	Patient 2 (brother of patient 1)		
Mutation	Intragenic homozygous deletion	c.854-1G>A and c.1238+1G>C compound heterozygous	c.854-1G>A and c.1238+1G>C compound heterozygous	c.624delG (p.Lys208AsnfsTer5) homozygous	c.178_182delCTAGA (p.Leu60MetfsTer23) homozygous
Age	?	3 years	9 months	6 years	18 years
Gender	Male	Male	Male	Female	Male
Growth Weight at birth Height at birth Head circumference at birth Weight at last examination Height at last examination Head circumference at last examination	 ? ? ? ? ? ?	 2108 g (-2.95 SD) ? ? -1.0 SD -1.0 SD ?	 2474 g (-1.96 SD) ? ? ? ? ?	Failure to thrive 3rd centile 25th centile 10th centile -2 SD (at 3 years 2 months) 25th centile (at 3 years 2 months) 3rd centile (at 3 years 2 months)	 ? ? ? <3rd centile 3-10th centile 10th centile
Neurodevelopmen/ cognition/behavior	?	Autistic features	?	Mild developmental delay	Intellectual disability, borderline
Head and neck Question mark ear Skin tag Prominent cheeks Micro- and/or retrognathia Microstomia Temporomandibular joint	 + Postauricular + + + Dysplastic condyles and shallow condylar fossae	 + - + + - N/A	 + - + + - N/A	 + Postauricular + + + Hypoplastic condyle, absence of mandibular fossae	 + - + + + Joint pain, no structural defect on computed tomography
Respiratory system Mixed apnea (central apnea and obstructive sleep apnea) Laryngomalacia	 + +	 + -	 + +	 + -	 + -
Digestive system Feeding problems Constipation	 + -	 - +	 + +	 + +	 - -
Genitourinary system	Macropenis	Macropenis	Macropenis	Mild clitoral hypertrophy	History of epispadias, genital examination:N/A
Neurologic	Bulbar palsy			Hypoplasia of corpus callocum	
Other			Abdominal distention, omphalocele	Umbilical hernia	Muscular build

Ref: Reference; SD: Standard deviation.

gene were associated with the beginning of the protein, and the frameshift mutation was expected to cause premature termination. We determined the mRNA expression level of *PLCB4* from the blood where *PLCB4* mRNA expression has already been reported.[7] Our results underlined that *PLCB4* was downregulated approximately 2-fold compared to healthy individual (data not shown) and likely to cause loss of protein function. Together with the literature, these results showed that, unlike missense variants, null variants in the *PLCB4* gene cause loss of function and cause the disease when homozygous.

Craniofacial anomalies and related respiratory problems are seen in ARCND2 due to heterozygous missense variants, but mild developmental delay or autistic features have been reported in addition to these anomalies in two unrelated ARCND2 patients with homozygous null variants (Table 1).[4,5] Mild ID was identified in our patient as well, making this the third case of ARCND2 with a neurodevelopmental finding. No other pathogenic variant that could be associated with ID was detected in the patient's WES or chromosomal microarray analysis. Mild developmental delay and hypotonia have also been reported in ARCND3, another type of autosomal recessive ARCND associated with the *EDN1* gene.[8] Endothelins are vasoconstrictive peptides that are also expressed in the brain, where they affect the central nervous system and neuronal excitability.[9] *PLCB4* plays a role in the endothelin receptor pathway and is also expressed in the brain.[10] A recent study suggested that endothelin levels modulate the neuronal activity associated with experience, as decreases in endothelin signaling reduce the number of myelin sheaths formed by oligodendrocytes in knockout rodent and zebrafish models.[11] However, more studies are needed to clarify the effects of the endothelial pathway on neuronal development.

In conclusion, our findings in this patient support that homozygous loss-of-function mutations of the *PLCB4* gene can cause neurodevelopmental findings that have previously been reported in 2 patients, in addition to the ARCND phenotype.

Acknowledgement

We would like to thank the family participating in this case report on a voluntary basis.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Gülsüm Kayhan, Kübra Öztürk;* ***Design:*** *Abdullah Sezer, Emriye Ferda Perçin;* ***Control/Supervision:*** *Gülsüm Kayhan, Emriye Ferda Perçin;* ***Data Collection and/or Processing:*** *Hasan Hüseyin Kazan;* ***Analysis and/or Interpretation:*** *Gülsüm Kayhan, Abdullah Sezer,* ***Literature Review:*** *Abdullah Sezer, Gülsüm Kayhan, Hasan Hüseyin Kazan, Emriye Ferda Perçin;* ***Writing the Article:*** *Gülsüm Kayhan, Hasan Hüseyin Kazan, Kübra Öztürk, Abdullah Sezer, Emriye Ferda Perçin;* ***Materials:*** *Kübra Öztürk.*

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