J Compr Ped. 2019 November; 10(4):e86392. doi: 10.5812/compreped.86392.

Published online 2019 October 16. **Case Report**

Whole Exome Sequencing Identifies a Homozygous *PYCR1* Missense Variant in a Patient with Autosomal Recessive Cutis Laxa Type 2B: A Case Report

Ali Nikfar [1,*], Mojdeh Mansouri [2] and Gita Fatemi Abhari [3,**]

[1]Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Sciences, Zanjan, Iran
[2]Department of Genetics and Molecular Medicine, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[3]Imam Khomeini Genetic Counseling Center, Welfare Organization of Zanjan, Zanjan, Iran

[*]*Corresponding author*: Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Sciences, Zanjan, Iran. Email: ali.nikfar7@yahoo.com
[**]*Corresponding author*: Imam Khomeini Genetic Counseling Center, Welfare Organization of Zanjan, Zanjan, Iran. Email: g.fatemi.md@gmail.com

Received 2018 November 14; **Revised** 2019 July 18; **Accepted** 2019 August 31.

Abstract

Autosomal recessive cutis laxa type 2B (ARCL2B) is a rare genetic connective tissue disorder characterized by wrinkled inelastic skin, intellectual disability, growth retardation, developmental delay, skeletal abnormalities, and facial dysmorphism. Recently, *PYCR1*, encoding the pyrroline-5-carboxylate reductase 1, was reported as the first gene involved in ARCL2B. In this study, using whole exome sequencing, we identified a homozygous *PYCR1* missense mutation, c.722C>A; p.Ala241Asp, in an Iranian male patient. Our report expands the clinical spectrum of *PYCR1* mutations. Furthermore, this study shows that whole exome sequencing could serve as a viable diagnostic approach to identify the etiology of rare genetic diseases.

Keywords: Autosomal Recessive Cutis Laxa Type 2B, *PYCR1*, Whole Exome Sequencing

1. Introduction

Cutis laxa (CL) is a rare heterogeneous disorder of connective tissue characterized by loose, hypoelastic, wrinkled, sagging and redundant skin resulting in a prematurely aged appearance. It can be inherited or acquired, but inherited cases are more common. Inherited CL has considerably heterogeneous etiology and may be presented as autosomal dominant, autosomal recessive and X-linked recessive (1, 2). The autosomal recessive form of CL has most commonly been reported (3, 4). CL is separated into subtypes based on the genetic basis of the disorder. Autosomal recessive cutis laxa type 2B (ARCL2B; OMIM 612940) is caused by homozygous or compound heterozygous mutation in the pyrroline-5-carboxylate reductase 1 (*PYCR1*) gene, which is located on 17q25.3 (5, 6). Typical features of ARCL2B are wrinkled loose skin, progeroid appearance, developmental delay, growth retardation, and joint laxity (7). Patients may also have distinctive dysmorphic facial features including triangular face, high forehead, large ears, prognathism, hypotelorisms, bulbous nose, epicanthal folds, and blue sclera. Affected individuals may also suffer from skeletal anomalies, hypotonia, and variable central nervous system involvement (6, 8, 9). Using whole exome sequencing, we identified a homozygous mutation, c.722C>A, in *PYCR1* gene, and a subsequent diagnosis of ARCL2B was made.

2. Case Presentation

We reported a case on a 9-year-old boy (Figure 1) referred to our genetic counseling center due to his intellectual disability, developmental delay, and dysmorphic appearance. He was born as the first child of his consanguineous Iranian parents at 36 weeks of gestation via cesarean section. His birth weight was 2080 g (< 5th centile), length was 49 cm (25th centile), and head circumference was 30 cm (< 5th centile). No remarkable family history was reported. He could sit independently at 9 months, and was able to walk at the age of 32 months. On examination at the age of 9 years, his weight was 29.9 kg (< 5th centile), length 141 cm (90th centile), and OFC was 49.5 cm (50th centile).

Copyright © 2019, Journal of Comprehensive Pediatrics. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

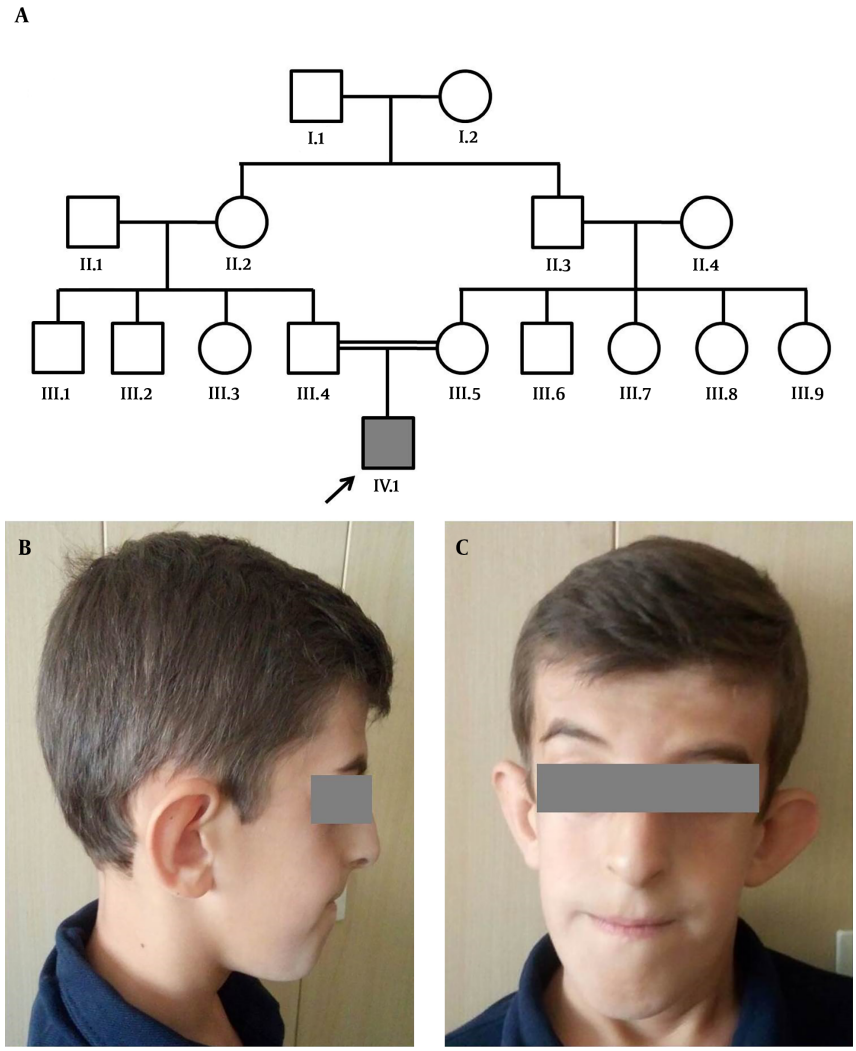

Figure 1. A, Pedigree of the family presenting autosomal recessive cutis laxa type 2B. Squares and circles represent males and females, respectively. An arrow indicates the proband. Filled symbol refers to the patient (IV.1) and clear symbols represent normal individuals. The parents are consanguineous (III.4, III.5). B and C, Facial characteristics of the patient. Note the prematurely-aged appearance, triangular-shaped face, prognathism, large ears, and bulbous nose.

Clinical findings were lax wrinkled skin, intrauterine growth retardation, joint hyperlaxity, hypotonia, facial dysmorphisms including triangular-shaped face with a prematurely-aged appearance, prognathism, large ears, bulbous nose, broad nasal bridge, down-slanting palpebral fissures, and strabismus (Figure 1B and C). The patient also suffered from intellectual disability and developmental delay. He had a learning disorder as well as a language delay. His learning difficulties required special education.

Conventional cytogenetic studies gave normal results. In addition, no pathogenic copy number variation (CNV), neither gain nor loss, was identified by array-CGH (Figure 2A). Whole exome sequencing (WES) was performed on the genomic DNA of the patient using Illumina Hiseq 4000. We found a homozygous missense mutation (c.722C>A; p.Ala241Asp) in exon six of the *PYCR1* gene (NM-006907), which was confirmed by Sanger sequencing (Figure 2B). Both parents were heterozygous carriers of the causative genetic variant. The patient's clinical phenotype, along with the *PYCR1* mutation, was consistent with ARCL2B.

3. Discussion

We identified a homozygous *PYCR1* missense variant (c.722C>A) in an Iranian boy with intellectual disability, developmental delay, cutis laxa, and dysmorphic facial fea-

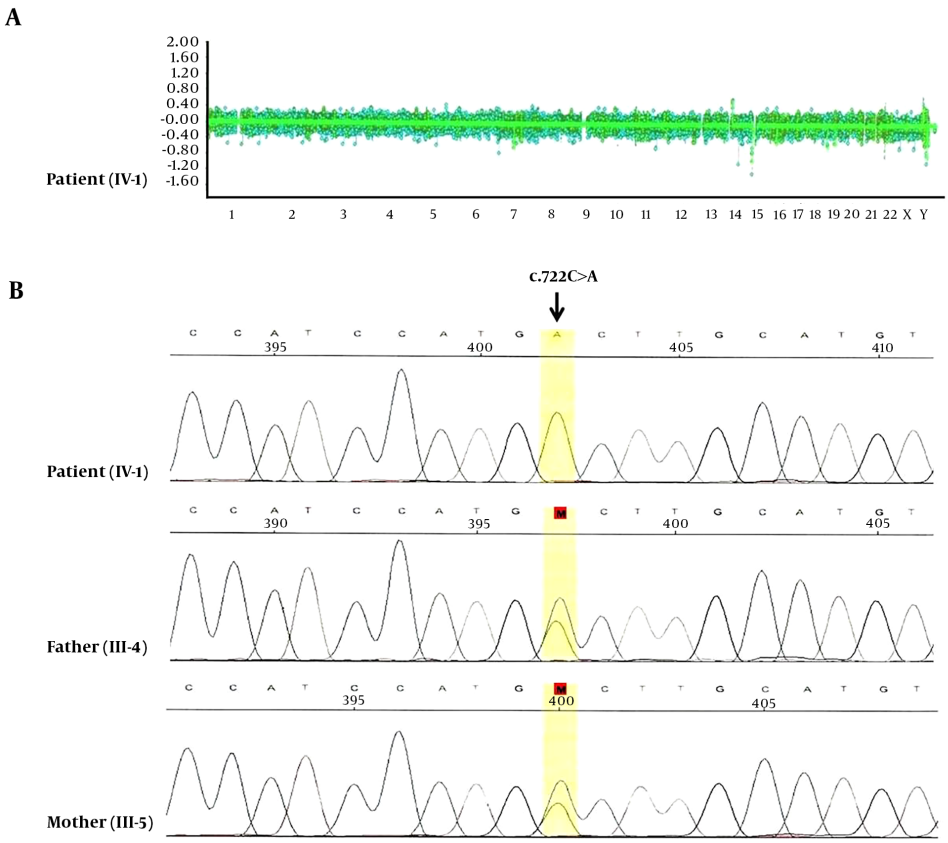

Figure 2. A, Whole genome oligo array CGH analysis. No nonpolymorphic genomic imbalance was detected. B, Sanger sequencing of the PYCR1 mutation. The pathogenic variation, c.722C>A; p.Ala241Asp, was found in homozygous state in patient and heterozygous in his parents.

tures. The mutation leads to a change in the amino acid at position 241 of the protein from alanine to aspartate. Mutations in the *PYCR1* gene can cause ARCL2B. Dimopoulou et al. reported this variant as a pathogenic variant in two Turkish patients (10). Another variant at the same position, c.722C>T; p.Ala241Val, has also been described to be associated with ARCL2B in a Brazilian patient (11).

We compared the clinical findings of our patient with previously reported patients with the same mutation (Table 1). Overall, the facial features and skin findings were similar. The patient presented here shared some clinical features with previously reported patients, such as mental retardation, developmental delay, psychomotor retardation, joint hyper laxity, and hypotonia. Although previous cases had osteopenia and suffered from microcephaly, hip dislocation, and adducted thumb, our patient lacked such phenotypes. In contrast, strabismus of the current patient was not found in the previous case.

Missense mutations compose the main part of mutations causing this disease. The majority of the missense mutations were located within exons four to six, which encode the most highly conserved parts of the PYCR1 protein containing many residues involved in enzymatic function (12). *PYCR1* encodes pyrroline-5-carboxylate reductase 1, a housekeeping mitochondrial enzyme, that catalyzes the last step in proline biosynthesis through NAD(P)H-dependent reduction of pyrroline-5-carboxylate (P5C) to L-proline (13).

In conclusion, this study identified a homozygous missense variant (c.722C>A; p.Ala241Asp) in a patient from a consanguineous Iranian family. *PYCR1* mutations at the homozygous state results in ARCL2B. Our findings confirmed the previous two reports for ARCL2B patients with similar mutation. These results expand our insight for the clinical phenotype of ARCL2B. Further reports are required to better understand the *PYCR1*-related phenotypes, genotype-

Table 1. Findings in the Patient of This Study Compared with Clinical Features Described in Patients with *PYCR1* Mutations at the Same Position

Author/Reference	Dimopoulou et al. (10)	Dimopoulou et al. (10)	Dimopoulou et al. (10)	Scherer et al. (11)	Our patient
Case number	Case 1	Case 2	Case 3	Case 4	Case 5
Origin	Turkey	Turkey	France	Brazil	Iran
Gender	NK	NK	NK	Male	Male
Mutations					
Status	hom	hom	het, het	hom	hom
cDNA	c.722C>A	c.722C>A	c.722C>T, c.138 + 2T>C	c.722C>T	c.722C>A
Consequence	p.Ala241Asp	p.Ala241Asp	p.Ala241Val, Splicing	p.Ala241Val	p.Ala241Asp
Exon	6	6	6, 2	6	6
Signs and symptoms					
Lax wrinkled skin	NK	+	+	+	+
IUGR	NK	+	+	NK	+
Hypotonia	+	+	+	-	+
Psychomotor retardation	+	+	+	+	+
Dysmorphic features	+	+	+	+	+
Triangular face	+	+	+	NK	+
Large ears	+	+	+	NK	+
Prominent chin	+	+	+	+	+
Postnatal growth delay	NK	-	-	+	-
Microcephaly	NK	+	-	-	-
Joint hyperlaxity	NK	+	+	+	+
Thin, translucent skin	NK	+	-	NK	+
Hip dislocation	NK	+	-	+	-
Hernias	NK	-	+	+	-
Cataract/corneal clouding	NK	-	-	-	-
Strabismus	NK	-	+	NK	+
Blue sclerae	+	+	-	NK	-
Adducted thumb	NK	-	-	+	-
Osteopenia	NK	+	NK	+	-
Wormian bones	NK	+	NK	NK	-
Late fontanel closure	NK	+	+	+	-
Corpus callosum dysgenesis	NK	-	NK	-	-
Athetoid movements	NK	-	+	-	-

Abbreviations: het, heterozygous; hom, homozygous; IUGR, intrauterine growth retardation; NK, not known.

phenotype association, pathophysiology, and epidemiology to reach a critical number of patients for prevention or therapeutic studies.

Acknowledgments

We would like to greatly thank the patient and his family for their collaboration.

Footnotes

Conflict of Interests: The authors declare no conflict of interest.

Ethical Approval: This study was approved by the Genetics Specialized Committee of Welfare Organization of Zanjan province.

Funding/Support: Funding was supported by the Welfare

Organization of Zanjan province.

Patient Consent: Informed consent was obtained from all participants.

References

1. Genevieve D, Baumann C, Huber C, Faivre L, Sanlaville D, Bodemer C, et al. A novel form of syndromic cutis laxa with facial dysmorphism, cleft palate, and mental retardation. *J Med Genet*. 2004;**41**(6). e77. doi: 10.1136/jmg.2003.013573. [PubMed: 15173243]. [PubMed Central: PMC1735811].
2. Berk DR, Bentley DD, Bayliss SJ, Lind A, Urban Z. Cutis laxa: A review. *J Am Acad Dermatol*. 2012;**66**(5):842 e1–17. doi: 10.1016/j.jaad.2011.01.004. [PubMed: 22387031].
3. Andiran N, Sarikayalar F, Saraclar M, Caglar M. Autosomal recessive form of congenital cutis laxa: More than the clinical appearance. *Pediatr Dermatol*. 2002;**19**(5):412–4. doi: 10.1046/j.1525-1470.2002.00116.x. [PubMed: 12383097].
4. Morava E, Guillard M, Lefeber DJ, Wevers RA. Autosomal recessive cutis laxa syndrome revisited. *Eur J Hum Genet*. 2009;**17**(9):1099–110. doi: 10.1038/ejhg.2009.22. [PubMed: 19401719]. [PubMed Central: PMC2986595].
5. Reversade B, Escande-Beillard N, Dimopoulou A, Fischer B, Chng SC, Li Y, et al. Mutations in PYCR1 cause cutis laxa with progeroid features. *Nat Genet*. 2009;**41**(9):1016–21. doi: 10.1038/ng.413. [PubMed: 19648921].
6. Lin DS, Yeung CY, Liu HL, Ho CS, Shu CH, Chuang CK, et al. A novel mutation in PYCR1 causes an autosomal recessive cutis laxa with premature aging features in a family. *Am J Med Genet A*. 2011;**155A**(6):1285–9. doi: 10.1002/ajmg.a.33963. [PubMed: 21567914].
7. Steiner CE, Cintra ML, Marques-de-Faria AP. Cutis laxa with growth and developmental delay, wrinkly skin syndrome and gerodermia osteodysplastica: A report of two unrelated patients and a literature review. *Genet Mol Biol*. 2005;**28**(2):181–90. doi: 10.1590/s1415-47572005000200001.
8. Ritelli M, Palit A, Giacopuzzi E, Inamadar AC, Dordoni C, Mujja A, et al. Clinical and molecular characterization of a 13-year-old Indian boy with cutis laxa type 2B: Identification of two novel PYCR1 mutations by amplicon-based semiconductor exome sequencing. *J Dermatol Sci*. 2017;**88**(1):141–3. doi: 10.1016/j.jdermsci.2017.04.010. [PubMed: 28499588].
9. Guernsey DL, Jiang H, Evans SC, Ferguson M, Matsuoka M, Nightingale M, et al. Mutation in pyrroline-5-carboxylate reductase 1 gene in families with cutis laxa type 2. *Am J Hum Genet*. 2009;**85**(1):120–9. doi: 10.1016/j.ajhg.2009.06.008. [PubMed: 19576563]. [PubMed Central: PMC2706970].
10. Dimopoulou A, Fischer B, Gardeitchik T, Schroter P, Kayserili H, Schlack C, et al. Genotype-phenotype spectrum of PYCR1-related autosomal recessive cutis laxa. *Mol Genet Metab*. 2013;**110**(3):352–61. doi: 10.1016/j.ymgme.2013.08.009. [PubMed: 24035636].
11. Scherrer DZ, Baptista MB, Matos AH, Maurer-Morelli CV, Steiner CE. Mutations in PYCR1 gene in three families with autosomal recessive cutis laxa, type 2. *Eur J Med Genet*. 2013;**56**(6):336–9. doi: 10.1016/j.ejmg.2013.03.006. [PubMed: 23531708].
12. Meng Z, Lou Z, Liu Z, Li M, Zhao X, Bartlam M, et al. Crystal structure of human pyrroline-5-carboxylate reductase. *J Mol Biol*. 2006;**359**(5):1364–77. doi: 10.1016/j.jmb.2006.04.053. [PubMed: 16730026].
13. Kretz R, Bozorgmehr B, Kariminejad MH, Rohrbach M, Hausser I, Baumer A, et al. Defect in proline synthesis: Pyrroline-5-carboxylate reductase 1 deficiency leads to a complex clinical phenotype with collagen and elastin abnormalities. *J Inherit Metab Dis*. 2011;**34**(3):731–9. doi: 10.1007/s10545-011-9319-3. [PubMed: 21487760].