Genetic study of a patient with congenital central hypoventilation syndrome in Iran: a case report

Reihaneh Khorasanian

Iran University of Medical Sciences

Marzieh Mojibafan (✉ marziyeh_mojibafan@yahoo.com)

Iran University of Medical Sciences

Nastaran Khosravi

Iran University of Medical Sciences

Short Report

Keywords: CCHS, PHOX2B, congenital central hypoventilation syndrome, early-onset CCHS, PARMs

Posted Date: March 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-305918/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

I. Background: Congenital central hypoventilation syndrome (CCHS) is an extremely rare genetic disorder characterized by autonomic nervous system (ANS) dysregulation caused by mutations in the *PHOX2B* gene. Here we introduce the first genetic analysis of a CCHS patient in Iran.

II. Methods and Results: Genetic analysis of the *PHOX2B* gene was performed by Sanger sequencing and interpreted using the American College of Medical Genetics and Genomics (ACMG) guideline. The results showed a heterozygous duplication in the exon 3 causing a polyalanine repeat expansion mutation (PARMs) to 27 repeats in the *PHOX2B* gene (20/27 genotype). The patient's parents were normal for this mutation.

III. Conclusions: According to the ACMG guideline, the mutation is a pathogenic one, and it was a denovo mutation in the family. The genetic study can help the family for prenatal diagnosis or pre-implantation diagnosis if the parents have gonadal mosaicism.

Introduction

Congenital central hypoventilation syndrome (CCHS) is an extremely rare genetic disorder characterized by autonomic nervous system (ANS) dysregulation and respiratory control deficiency. CCHS is manifested in the neonatal period with apnea, cyanosis, hypercapnia, and usually deteriorate during sleep(1).

This disorder is an autosomal dominant disease caused by mutation in *PHOX2B* gene(2). The paired-like homeobox2b, *PHOX2B*, gene is located on chromosome 4p12. It encodes a 314 amino acid protein that act as a transcription factor involved in the differentiation of the neural lineages of the autonomic nervous system (ANS)(1). *Phox2b* has 2 polyalanine repeat- domains (2).

Two major mutations have been identified in CCHS patients including polyalanine expansion repeat (PARM) (90%), and non-poly alanine repeat *PHOX2B* mutation (NPARM) (10%). PARM patients shows 24 to 33 alanine repeats on the affected haplotype however normal allele has 20(3). Some studies show correlation between poly alanine expansion length and severity of autonomic dysfunction(4). Different mutations have been reported in the affected individuals with CCHS and NPARMs consisting of predominantly frameshift mutations, and also non-sense, missense, missense with stop codon alteration, and partial/ whole gene deletion mutations(5).

Alanine expansions, or homeobox-distal mutations, found in the *PHOX2B* gene of CCHS patients result in a protein that is capable of binding to the right target sites in the genome, but is unable to properly do its normal regulatory function(5). poly alanine repeat mutant *phox2b* resulted in cytoplasmic aggregation whereas this effect was not seen in frameshift mutations(6). Trochete et al. suggested that mutant protein with the misfolded 33 repeats can prevent the normal protein from its usual function(7), and different studies suggested dominant negative function for Poly alanine expansion repeat mutations

(22).Homozygote patients are rarely reported; Trochet et al., described a CCHS case with homozygote PARM mutation from the asymptomatic parents, in whom both alleles had + 4 polyalanine expansion (8).

CCHS occurs with Hirschsprung disease among 20% of patients. HSCR is not prevalent in PARM (to 13 to 20% of PARMs) (9)

The present study introduces the first genetically confirmed hypoventilation syndrome patient in Iran.

Material And Methods

Case description

Preterm female infant (Gestational age: 36 weeks and five days) was delivered via cesarean section because of premature rupture of membranes (PROM). No obvious dysmorphic features were found. Her birth weight was 2560 g and the Apgar scores were 6 to 8. She was the first baby born to non-consanguineous parents. In the second day of her life, she had a seizure and positive-pressure ventilation was commenced. Additionally, she experienced recurrent episodes of apnea and cyanosis. No positive history for this phenotype was reported in the pedigree (Fig. 1).

Molecular analysis

The Proband was referred to Ali-Asghar Children Hospital one month after birth. Informed consent was received from the family patient before sampling.

DNA was extracted by salting out procedure (10). All exons and exon-intron boundaries of the *PHOX2B* gene (NM_003924.3) were sequenced. Primers are available upon request. DNA sequencing, and interpretation were performed using previously described techniques (11).

Mutation analysis was performed using Mutation Surveyor software (12), and variants were analysed using different softwares such as CADD, and mutation taster. All variant interpretation was done based on the American College of Medical Genetics and Genomics (ACMG) guideline (13).

Result And Discussion

PHOX2B gene mutation analysis in this proband revealed a heterozygous duplication (c.756_776dupGGCGGCAGCGGCAGCGGCGGC, p.Ala254_Ala260dup) in the exon 3 which results in polyalanine repeat expansion mutations (PARMs) to 27 repeats in the *PHOX2B* gene (20/27 genotype) (Fig. 2A). Her parents were normal for this mutation (20/20 genotype) (Fig. 2B, 2C).

The identified mutation (rs17879189) in this study was reported as pathogenic in ClinVar database. Using different in silico analysis tools such as mutation taster and CADD also helped us to annotate the pathogenicity of this variant; Mutation Taster showed this variant is a disease causing one, and its CADD score was 23. CADD score cutoff for a variant to be deleterious is 14.

This mutation has been previously reported in 2003 (14). Over 1,000 cases have been reported worldwide till now. Incidence rate estimated one in 150,000–200,000 live births in Japan (15, 16).

The normal *PHOX2B* gene has 20 alanine repeats (20/20 genotype). More than 90% of CCHS reported cases have been heterozygous for an in-frame PARM; the normal 20-alanine tract is expanded to 24–33 alanine repeats, with resultant genotypes of 20/24–20/33 PARMs. The 20/25, 20/26, and 20/27 genotypes are the most common, although increasing numbers of even the less common mutations are being identified monthly. The remaining 10% are heterozygous for a non-polyalanine expansion repeat mutation (NPARM) in the *PHOX2B* gene. (17).

Our identified mutation, c.756_776dupGGCGGCAGCGGCAGCGGCGGC, p.Ala254_Ala260dup, is a likely pathogenic one according to the ACMG guideline because: this mutation is located in a mutational hotspot (PM1), it is absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium (PM2), multiple lines of in silico analysis support that it has a deleterious effect on the gene or gene product (PP3), patient's phenotype is highly specific for the disease (PP4), reputable sources recently report the variant is a pathogenic one (PP5).

There is significant “quantitative” correlation between the length of the polyalanine stretch and the degree of the hypoventilation severity in PARM patients (18). In general CCHS patients with 20/27–20/33 PARMs shows severe alveolar hypoventilation and require full-time ventilator support while those with shorter PARMs (20/24–20/25) have more subtle symptoms and later onset. Our patients with 20/27 PARM also were intubated shortly after her birth.

Hirschsprung was also observed in our patient, in general, it is reported in 20% of all CCHS cases (19), and affected individuals with 20/27 genotype or longer repeats are at greater risk of Hirschsprung disease (19). Cardiac arrhythmia is also described in some patients with genotypes 20/25, 20/26, and 20/27, but our patient was normal in this regard.

Since the patient's parents were normal for the mutation found in their child, the mutation could be *de novo*. Most polyalanine repeat mutations arise *de novo* in CCHS probands, although they are inherited as an autosomal dominant trait (20).

Genetic study of this syndrome has not been previously performed in Iran. The only case report was a patient suspicious of being affected by this disorder which had negative molecular result for CCHS. To our knowledge it is the first report of CCHS patient with genetic identification in Iran (21). In this paper we introduced a heterozygous PARM CCHS patient with genotype of 20/27. More investigation in these patients can help us to understand the most common mutations of *PHOX2B* gene in Iran and also predict patient's clinical symptoms and help future management of the disease. The genetic study can help the family for prenatal diagnosis or pre-implantation diagnosis if the parents have gonadal mosaicism.

Abbreviations

CCHS
Congenital central hypoventilation syndrome ;
ANS
autonomic nervous system ;
PARM
poly alanine expansion repeat mutation;
NPARM
non-poly alanine repeat mutation;
ACMG
American College of Medical Genetics and Genomics;
PROM
premature rupture of membranes

Declarations

Ethics approval

The project was approved by the ethical committee of the IUMS research committee.

Consent to participate:

Prior to sampling, genetic counseling was performed, and Informed consent from the family was obtained for participating in this project.

Consent for publication:

Prior to sampling, genetic counseling was performed, and Informed consent for publication was obtained.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest:

The authors declare that they have no competing interests

Funding:

Not applicable

Code availability:

Not applicable

Authors' contributions:

MM contributed to perform the experiments, analysis, and interpretation of data and also revised the manuscript. She led the project, supervised the study, contributed to the design of the study. RKH drafted the manuscript. NK involved in the clinical diagnosis of the patient. All authors discussed the results and approved the final manuscript.

Author details:

¹ Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences (IUMS), ShahidHemmat Highway, Tehran, Iran.

² Department of medical genetics, Ali-Asghar Children's Hospital, Zafar St., ShahidModarres Highway, Tehran, Iran.

³ Student Research Committee, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.

References

1. Bishara J, Keens TG, Perez IA. The genetics of congenital central hypoventilation syndrome: clinical implications. *Appl Clin Genet* (2018) 11:135-44. Epub 2018/12/12. doi: 10.2147/tacg.S140629. PubMed PMID: 30532577; PubMed Central PMCID: PMC6241683.
2. Hammel M, Klein M, Trips T, Priessmann H, Ankermann T, Holzinger A. Congenital Central Hypoventilation Syndrome due to PHOX2b gene defects: inheritance from asymptomatic parents. *Klin Padiatr* (2009) 221(5):286-9. Epub 2009/08/27. doi: 10.1055/s-0029-1220941. PubMed PMID: 19707990.
3. Weese-Mayer DE, Marazita ML, Rand CM, Berry-Kravis EM, Classic C. Congenital central hypoventilation syndrome. *Sleep in childhood neurological disorders* (2011):295-312.
4. Amiel J, Laudier B, Attié-Bitach T, Trang H, de Pontual L, Gener B, et al. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nature genetics* (2003) 33(4):459-61.
5. Repetto GM, Corrales RJ, Abara SG, Zhou L, Berry-Kravis EM, Rand CM, et al. Later-onset congenital central hypoventilation syndrome due to a heterozygous 24-polyalanine repeat expansion mutation in the PHOX2B gene. *Acta paediatrica* (2009) 98(1):192-5.
6. Bachetti T, Matera I, Borghini S, Duca MD, Ravazzolo R, Ceccherini I. Distinct pathogenetic mechanisms for PHOX2B associated polyalanine expansions and frameshift mutations in congenital central hypoventilation syndrome. *Human molecular genetics* (2005) 14(13):1815-24.
7. Trochet D, O'Brien LM, Gozal D, Trang H, Nordenskjöld A, Laudier B, et al. PHOX2B genotype allows for prediction of tumor risk in congenital central hypoventilation syndrome. *The American Journal of Human Genetics* (2005) 76(3):421-6.

8. Trochet D, de Pontual L, Estêvão MH, Mathieu Y, Munnich A, Feingold J, et al. Homozygous mutation of the PHOX2B gene in congenital central hypoventilation syndrome (Ondine's Curse). *Human mutation* (2008) 29(5):770-.
9. Berry-Kravis EM, Zhou L, Rand CM, Weese-Mayer DE. Congenital central hypoventilation syndrome: PHOX2B mutations and phenotype. *American journal of respiratory and critical care medicine* (2006) 174(10):1139-44.
10. Miller S, DD D. Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* (1988) 16(3):1215.
11. Mojbafan M, Bahmani R, Bagheri SD, Sharifi Z, Zeinali S. Mutational spectrum of autosomal recessive limb-girdle muscular dystrophies in a cohort of 112 Iranian patients and reporting of a possible founder effect. *Orphanet Journal of Rare Diseases* (2020) 15(1):14.
12. Serensits T, Xuanhe Z, Shouyong N, Liu J. SoftGenetics Application Note. (2007).
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine*(2015). p. 405-23.
14. Weese-Mayer DE, Berry-Kravis EM, Ceccherini I, Keens TG, Loghmanee DA, Trang H. An official ATS clinical policy statement: congenital central hypoventilation syndrome: genetic basis, diagnosis, and management. *American journal of respiratory and critical care medicine* (2010) 181(6):626-44.
15. Trang H, Dehan M, Beaufils F, Zaccaria I, Amiel J, Gaultier C, et al. The French congenital central hypoventilation syndrome registry. *Chest* (2005) 127(1):72-9.
16. Shimokaze T, Sasaki A, Meguro T, Hasegawa H, Hiraku Y, Yoshikawa T, et al. Genotype–phenotype relationship in Japanese patients with congenital central hypoventilation syndrome. *Journal of human genetics* (2015) 60(9):473.
17. Weese-Mayer DE, Berry-Kravis EM, Marazita ML. In pursuit (and discovery) of a genetic basis for congenital central hypoventilation syndrome. *Respiratory physiology & neurobiology* (2005) 149(1-3):73-82.
18. Matera I, Bachetti T, Puppo F, Di Duca M, Morandi F, Casiraghi G, et al. PHOX2B mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset Central Hypoventilation syndrome. *Journal of medical genetics* (2004) 41(5):373-80.
19. Di Lascio S, Benfante R, Di Zanni E, Cardani S, Adamo A, Fornasari D, et al. Structural and functional differences in PHOX2B frameshift mutations underlie isolated or syndromic congenital central hypoventilation syndrome. *Human mutation* (2018) 39(2):219-36.
20. Weese-Mayer DE, Berry-Kravis EM, Zhou L, Maher BS, Silvestri JM, Curran ME, et al. Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. *American journal of medical genetics Part A* (2003) 123(3):267-78.

21. Adimi Naghan P, Gheraati M, Ghaffaripour HA, Vasheghani M, Mirenayat MS. Three Cases of Hypoventilation in the First Decade with an Unusual Presentation and Rare Etiology: A Case Report. Journal of Comprehensive Pediatrics 10(3).

Figures

Figure 1

pedigree of the proband. The proband is shown by arrow in the figure.

Figure 2

Sanger sequencing results of the proband and his parents: A) chromatogram of the patient, B) chromatogram of the patient's father C) chromatogram of the patient's mother