

CASE REPORT

Iran J Allergy Asthma Immunol
October 2016; 15(5): 426-429.

A Novel *CYBB* Mutation in Chronic Granulomatous Disease in Iran

Shaghayegh Tajik¹, Mohsen Badalzadeh¹, Mohammad Reza Fazlollahi¹, Massoud Houshmand²,
Fariborz Zandieh³, Shamim Khandan¹, and Zahra Pourpak¹

¹ Immunology, Asthma and Allergy Research Institute, Tehran University of
Medical Sciences, Tehran, Iran

² Department of Medical Genetics, National Institute of Genetic Engineering and
Biotechnology (NIGEB), Tehran, Iran

³ Department of Asthma, Allergy and Immunology, Bahrami Children Hospital,
Tehran University of Medical Sciences, Tehran, Iran

Received: 14 February 2016; Received in revised form: 29 April 2016; Accepted: 6 May 2016

ABSTRACT

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disorder due to a genetic defect in one of the components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. This complex is composed of membrane-bound gp91-*phox* and p22-*phox* subunits, and cytosolic subunits consisting of p47-*phox*, p67-*phox*, and p40-*phox*. A mutation in *CYBB* gene encoding gp91-*phox* located on chromosome Xp21.1, leads to X-linked CGD. Herein, we report a 4-year-old Iranian boy presented with episodes of recurrent fever, cervical lymphadenopathy, and abdominal abscesses. Mutation analysis of the *CYBB* gene in the patient indicated a one-nucleotide deletion, c.316delT, resulting in p.W106GfsX.

Keywords: Chronic granulomatous disease (CGD); gp91-*phox*; *CYBB*; Iran; Novel; Mutation

INTRODUCTION

Chronic granulomatous disease (CGD) is a rare genetically heterogeneous primary immunodeficiency resulting from a defect in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex.^{1,2} This enzyme mediates intracellular killing of microbes by phagocytes.³

The NADPH oxidase is composed of membrane-

bound subunits including gp91-*phox* and p22-*phox*, and cytosolic subunits that consist of p47-*phox*, p67-*phox* and p40-*phox*.⁴⁻⁶ Mutations in *CYBB* gene encoding the gp91-*phox* subunit of the NADPH oxidase of phagocytes lead to an X-linked (XL) predominant type of CGD in the United States as well as in European countries.^{2,7} However, XL-CGD is known as the rarest form of CGD in the Middle Eastern countries including Iran.⁸⁻¹¹ At the present study, we identified a novel mutation in a boy suffering from XL-CGD and his mother as a carrier case.

Corresponding Author: Zahra Pourpak, MD, PhD;
Immunology, Asthma and Allergy Research Institute, Children's
Medical Center, Tehran University of Medical Sciences, Tehran,
Iran. Tel: (+98 21) 6693 5855, Fax: (+98 21) 6642 8995, E-mail:
pourpakz@tums.ac.ir

CASE REPORT

A 4-year-old boy was referred to Immunology,

A Novel *CYBB* Mutation in CGD

Asthma and Allergy Research Institute (IAARI) with fever, abdominal pain, a history of axillary lymphadenopathy (LAP) with purulent discharge, and recurrent lymphadenitis when he was 18 months old. Parental consanguinity was positive with no familial history of autoimmune or immunodeficiency disorders. Physical examination at the time of admission revealed splenomegaly and hepatomegaly which was confirmed by heterogenic view and mild increase in normal spleen dimensions (27×87×71 mm) in sonographic evaluation. Additionally, spiral abdominopelvic CT scan showed a small collection (24×12 mm) at omental area anterior to hepatic flexure of ascending colon with peripheral fat stranding, and cecum wall thickening. Sonography of the neck area proved the presence of several adenopathies at right mandibular angle (Maximum: 18×15 mm), left supraclavicular (10×21 mm), and bilateral cervical region. Radiological assessment showed an anterior mediastinal mass that supposed the probability of an underlying mediastinal adenopathy. Immunological screening and relative laboratory tests were done, results were as follows: WBC=22.3 ($10^3/\mu\text{L}$) with 84.1% neutrophils and 10.9% lymphocytes, IgA=199 (mg/dl), IgM=346 (mg/dl),

IgG=1593 (mg/dl), AST=103 (IU/L), ALT=102 (IU/L), CRP=63 (mg/L) and ESR-1 hour=94 (mm/hr). Peripheral blood smear analysis revealed leukocytosis, with lymphocyte dominancy and an approximately 5% pleomorphic atypical lymphocytes.

Along with immunological screening tests, the Nitroblue tetrazolium (NBT) slide test (neutrophil oxidative reaction) was 0% in the patient, 50% in his mother and 100% in his father (Figure 1). The procedure was performed as explained in a previous study.⁸

Also, the neutrophil dihydrorhodamine (DHR) test manifested nearly the absence of fluorescence on granulocyte stimulation. The stimulation index (SI) was 1.1, which was compatible with XL-CGD.

After obtaining written informed consent from the patients' parents, 1 milliliter of blood samples from the patient and his parents were dissolved in ethylene diamine tetra acetic acid (EDTA) to be used for genetic diagnostic assays.

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures¹² PCR was performed in a final volume of 30 μl containing 100 μM dNTP, 1X Taq DNA polymerase buffer,

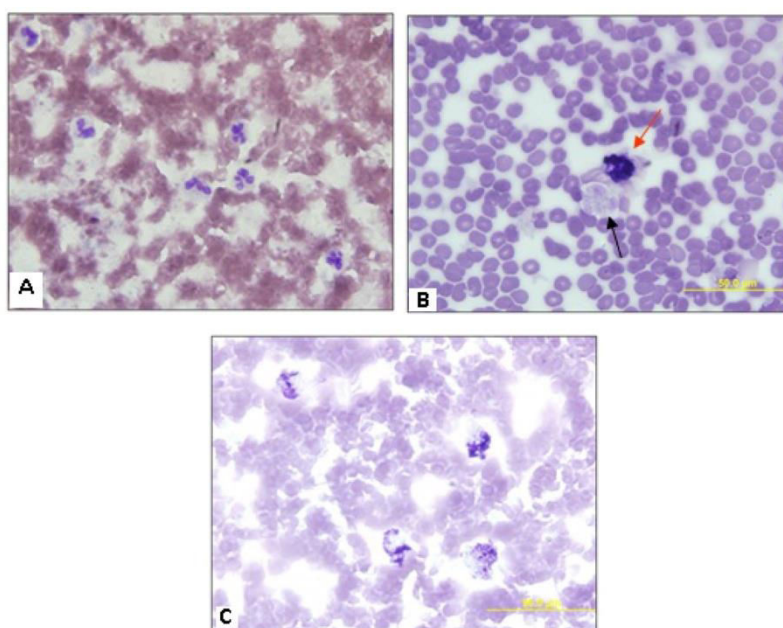


Figure 1. Neutrophil functional assay. Nitroblue tetrazolium (NBT) slide tests showed percentage of neutrophils with activation of respiratory burst: 0% in patient with X-linked chronic granulomatous disease (XL-CGD) (A), 50% in patient's mother as a carrier for XL-CGD (B) and 100% in patient's father (C).

Red arrow: cell containing normal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme.

Black arrow: cell containing defective NADPH oxidase enzyme.

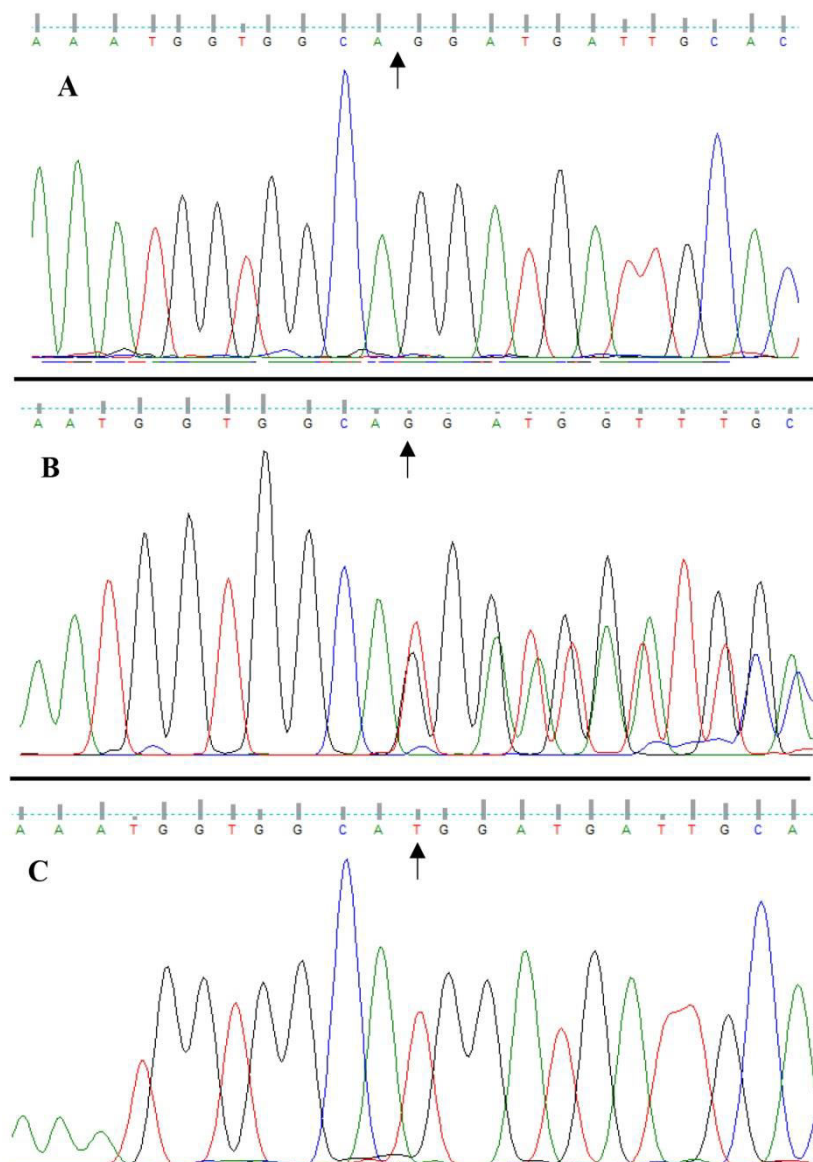


Figure 2. X-linked chronic granulomatous disease (XL-CGD) molecular diagnosis by PCR sequencing of *CYBB* gene. A: Index patient, X-linked mutation in exon 4 “T” deletion at nucleotide position 316, Trp change to Gly at amino acid position 106. c.316delT, p.W106GfsX. B: Patient’s mother: heterozygous mutation, carrier, and C: Patient’s father: genetically normal.

various concentration of $MgCl_2$, 2U Taq DNA polymerase (Cinagene, Tehran, Iran), 200 ng genomic DNA, and 10 pmol of each primer. PCR product was evaluated on 1.5% agarose gel and then was sequenced by ABI 3730XL genetic analyzer (Applied Biosystem, USA).

Homology of *CYBB* genomic DNA and the sequenced data were analyzed by the NCBI database (<http://www.ncbi.nlm.nih.gov/BLAST>) and the Ensembl SNPs database (<http://www.ensembl.org>).

Analysis of the *CYBB* gene in the patient revealed a novel deletion in exon 4, c.316delT, which leads to a frameshift in open reading frame in the mRNA, p.W106GfsX (Figure 2A). The patient’s mother showed the mutation as heterozygous (Figure 2B) but his father showed no mutation (Figure 2C).

The patient was hospitalized and after receiving broad-spectrum antibiotics, fever and clinical symptoms relieved and the patient was discharged with a prophylactic treatment of cotrimoxazole and

A Novel *CYBB* Mutation in CGD

gamma interferon. The patient was also candidate for hematopoietic stem cell transplantation (HSCT).

DISCUSSION

In this study, we reported clinical symptoms and a novel mutation in *CYBB* gene in an Iranian patient with XL-CGD. So far, over 700 different mutations in *CYBB* gene have been reported in Human Gene Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk/ac/all.php>). Mutation found in the present study, c.316delT, changes Tryptophan 106 to Glycine and generates a stop codon immediately afterwards. In the previous report on 11 Iranian patients with XL-CGD, nine different mutations have been reported.¹²

Considering that the XL-CGD is known as the rarest form of CGD in Iran,⁸ reporting this novel mutation will help to complete the genetic panel of CGD, which could be helpful in early genetic diagnosis, prenatal diagnosis and timely bone marrow transplantation for the patient treatment.

ACKNOWLEDGEMENT

The study was supported by Immunology, Asthma & Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran.

REFERENCES

1. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM. Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine* 2000; 79(3):170-200.
2. Winkelstein JA, Marino MC, Johnston Jr RB, Boyle J, Curnutte J, Gallin JI, et al. Chronic granulomatous disease: report on a national registry of 368 patients. *Medicine* 2000; 79(3):155-69.
3. Yu L, Zhen L, Dinauer MC. Biosynthesis of the phagocyte nadph oxidase cytochrome b 558 role of heme incorporation and heterodimer formation in maturation and stability of gp91 phox and p22 phox subunits. *J Biol Chem* 1997; 272(43):27288-94.
4. de Oliveira-Junior E, Bustamante J, Newburger P, Condino-Neto A. The human NADPH oxidase: primary and secondary defects impairing the respiratory burst function and the microbicidal ability of phagocytes. *Scand J Immunol* 2011; 73(5):420-7.
5. Holland SM. Chronic granulomatous disease. *Clin Rev Allergy Immunol* 2010; 38(1):3-10.
6. Roos D, De Boer M, Kuribayashi F, Meischl C, Weening R, Segal A, et al. Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. *Blood* 1996; 87(5):1663-81.
7. Van Den Berg JM, Van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. *PLoS one* 2009; 4(4):e5234.
8. Fattahi F, Badalzadeh M, Sedighipour L, Movahedi M, Fazlollahi MR, Mansouri SD, et al. Inheritance pattern and clinical aspects of 93 Iranian patients with chronic granulomatous disease. *J Clin Immunol* 2011; 31(5):792-801.
9. Bakri FG, Martel C, Khuri-Bulos N, Mahafzah A, El-Khateeb MS, Al-Wahadneh AM, et al. First report of clinical, functional, and molecular investigation of chronic granulomatous disease in nine Jordanian families. *J Clin Immunol* 2009; 29(2):215-30.
10. Köker M, Sanal Ö, De Boer M, Tezcan I, Metin A, Ersoy F, et al. Mutations of chronic granulomatous disease in Turkish families. *Eur J Clin Invest* 2007; 37(7):589-95.
11. Wolach B, Gavrieli R, de Boer M, Gottesman G, Ben-Ari J, Rottem M, et al. Chronic granulomatous disease in Israel: clinical, functional and molecular studies of 38 patients. *Expert Rev Clin Immunol* 2008; 129(1):103-14.
12. Teimourian S, Rezvani Z, Badalzadeh M, Kannengiesser C, Mansouri D, Movahedi M, et al. Molecular diagnosis of X-linked chronic granulomatous disease in Iran. *Int J Hematol* 2008; 87(4):398-404.