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ORIGINAL ARTICLE

Novel *CYBA* mutation in a family with BCGitis

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ABSTRACT

Chronic granulomatous disease is a non-prevalent genetic disorder due to different structural gene mutations encoding components of nicotinamide adenine dinucleotide phosphate oxidase complex. Nicotinamide adenine dinucleotide phosphate oxidase is a complex made by a group of five proteins (subunit) and plays an important role in the innate immune system. Five structural genes are responsible for encoding each subunit, in which cytochrome b-245 alpha chain (also known as p22-phox) is encoded by *CYBA* gene. *CYBA* gene mutation leads to a group of autosomal dominant chronic granulomatous disease. Decreased level or lack of nicotinamide adenine dinucleotide phosphate oxidase leaves affected individuals vulnerable to many types of infections and excessive inflammation. In this study, a family affected by BCGitis caused by a novel intronic autosomal recessive *CYBA* mutation (88,713,158 C > T) has been described. The proband is a 5-year-old girl with chronic granulomatous disease who was referred to the clinic due to BCGitis. The culprit mutation was detected following whole genome sequencing and was confirmed among the family members by Sanger sequencing. Being symptom-free at the time of diagnosis, despite the proband's mother homozygosity, was a characteristic feature of this report. Remarkably, none of the *CYBA*-mutated members, as a known chronic granulomatous disease causing gene, has expressed symptoms other than regional lymph node enlargements. This might explain the gene mutation site importance in demonstrating different manifestations.

KEYWORDS

chronic granulomatous disease, *CYBA* gene, BCGitis

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INTRODUCTION

Chronic granulomatous disease (CGD) is an inherited immunodeficiency disorder, which exposes the body to recurrent bacterial and fungal infectious diseases, followed by granuloma formations due to dysregulated inflammatory mechanisms [1]. Reactive oxygen species are produced by phagocytes through activation of a multicomponent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (PHOX), which plays a pivotal role in phagocyte respiratory burst (Figure 1). Defects in each subunit of PHOX can lead to CGD [2, 3].

Mutations in five genes can result in CGD different phenotypes [4]. *CYBA* gene, which is located on chromosome 16q24, encodes P22*phox* protein. *CYBA* mutation has been determined in approximately 5% of CGD patients and is responsible for one of the four forms of autosomal recessive CGD (AR-CGD) [5, 6]. CGD is usually diagnosed before the age of 5 years following wide range of bacterial and fungal infections, as the first manifestation of the disease. Diagnosis may occur in older ages depending on the level of NADPH enzyme remains working in the phagocytes [7]. Infections of the lung, skin, lymph nodes, liver, and gastrointestinal tract are the most frequent symptoms in CGD patients [8]. Localized mycobacterial infections are more frequent in CGD patients in comparison to normal population and it is a significant problem in regions highly endemic for tuberculosis [2, 7]. Diagnostic assays should be considered for individuals clinically susceptible to CGD. Tests are based on measuring NADPH oxidase products. Nitroblue tetrazolium (NBT) reduction is the oldest and the most recognized test for CGD. At present, the flow cytometry-based dihydrorhodamine (DHR) oxidation method is preferred, as a result of its high sensitivity. Diagnosis was confirmed by genetic analysis [9, 10].

REPORTS

Clinical manifestations

The family pedigree is shown in Figure 2. The affected members were assessed by a group of immunogenetics experts.

Family member V:4. The proband was a 5-year-old Iranian girl referred to the Immunogenetics Clinic of "Children's Medical Center" with multiple enlarged axillary and cervical lymph nodes. Soon after, BCGitis was diagnosed by lymph node biopsy and proper treatment with isoniazid and rifampin was started.

Her parents and both grandparents were all first cousins and a positive family history of swollen lymph nodes was presented in her mother, aunt, and uncle. One of her uncles died because of lymphoma in early childhood. Her younger brother had no any positive history of diseases. Following a normal complete blood cell (CBC) count, NBT test was performed. The results showed an enormous decrease of 65% in NBT. DHR flow cytometry showed 39.61% of positive phagocytes after phorbol myrisate acetate stimulation with neutrophil oxidative index of 15.5 (Table I).

Family member IV:8. The proband's mother was 33 years old with no positive symptoms at the time of reference. During a detailed history taking, she reported a brief period of anti-tuberculosis drugs consumption due to swollen lymph nodes in early childhood. Nevertheless, she was symptom-free for the rest of her life and genetic analysis declared the same homozygous mutation as her daughter. CBC components were in the normal range, while DHR flow cytometry showed positive results for CGD (Table I).

Family member IV:6. Six months later, a 7-year-old boy (family member IV:6) was referred to the pediatrics' immunogenetics clinic for further evaluation of swollen

Figure 1. The assembled NADPH oxidase complex activity. Defective activity of each component will result in CGD. *CYBA- 16q24-* mutation is responsible for <5% of CGD cases via affecting the P22^{phox} subunit. *CYBB- Xp21.1, NCF1- 7q11.23, NCF2- 1q25*, and *NCF4- 22q13.1* mutations affect NOX2, P47^{phox}, P67^{phox}, and P40^{phox}, respectively

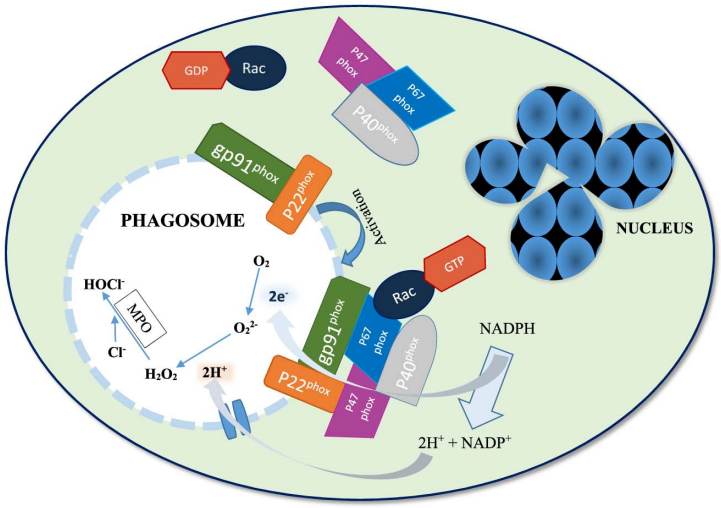


Figure 2. The consanguinity pattern and involvement of kindreds with (88,713,158 C > T) *CYBA* mutation

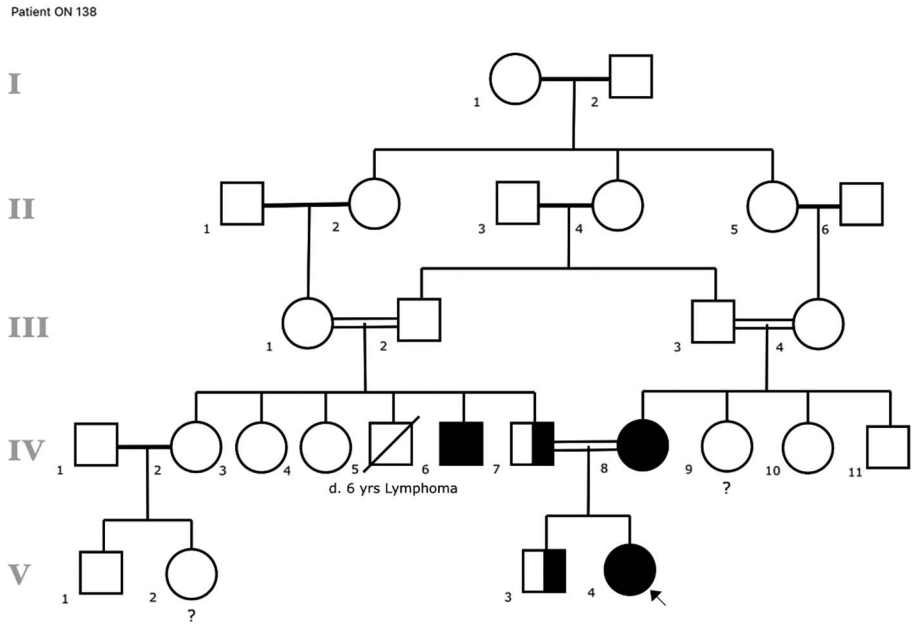


Table I. Dihydrorhodamine flow cytometry

		V:4	IV:8	IV:6	Reference range
WBC (cell/μl)		4,960	5,360	9,400	4,000–11,000
% Neutrophil		45	52.6	60.4	50–70
Absolute neutrophil count (cell/μl)		2,232	2,819	5677.6	1,700–7,000
% PMA ox-DHR+ (%)		39.61	3.34	96.74	>95
Neutrophil oxidative index (NOI)		15.5	16.4	36.2	>100
MFI	Resting	0.2	0.2	0.1	
	Cells + DHR	1.48	1.03	0.41	
	Cells + DHR + PMA	22.90	17.36	14.83	

Note: WBC: white blood cell; DHR: dihydrorhodamine; PMA: phorbol myrisate acetate; MFI: mean fluorescence intensity.

lymph nodes. The primary workups were suggestive for CGD by the normal CBCs and decreased level of DHR flow cytometric test (Table I). Treatment with anti-tuberculosis drugs was started immediately after the diagnosis had been performed.

Other family members. Family member IV:9 had similar symptoms in a similar age to those of family member IV:8, and the disease was limited by administrating undetermined medication years ago. Family member V:2 was a 2-year-old girl with a suspected history of BCGitis in which the swollen axillary lymph nodes were regressed without any proper treatments. Further history did not show any positive symptoms in other family members; moreover, the family members other than V:3,4 and IV:7,8 were not available or declined to participate in genetic testing.

MATERIAL AND METHODS

To confirm the diagnosis, whole exome sequencing was performed for the proband member. We used Agilent V5 + UTR library preparation and an Illumina HiSeq 4000 sequencing platform Ahvaz, Iran. The paired-end sequencing was done with the reading length of 101 base pairs and coverage of 100×. The bioinformatics analysis used frequency filters with public and in house databases (e.g., ExAc, GnomAD, and GME). Subsequently, we used

Sanger sequencing to validate the variation in the patients and other family members.

RESULTS

Gene mutation analysis showed a novel intronic homozygous CYBA (88,713,158 C > T) DNA change (Table II; Figure 3) and could be likely deleterious based on most predictors. Sanger sequencing confirmed that the patient and her (affected) mother were homozygous but her father and healthy brother were carriers heterozygous for the current mutation. Surprisingly, her mother was affected by the same homozygous mutation but did not suffer from the related symptoms such as swollen lymph nodes at the time (Figure 2).

Other family members including both her father and younger brother were carriers and completely disease-free. Significantly, all affected members, even with confirming laboratory data for CGD, have never experienced various symptoms of the disease except regional lymph node enlargements.

DISCUSSION

CGD is a rare primary immunodeficiency caused by a defect in NADPH oxidase molecular structure. A defect in the enzyme activity leads to the impaired intracellular killing of phagocytic cells [10]. CGD patients are diagnosed mostly in the age of infancy or childhood due to specific symptoms of recurrent infections, although the late onset of disease presentations in adulthood is also likely [11]. In this report, a case of AR-CGD caused by a novel intronic *CYBA* mutation has been explained. The clinical records and *CYBA* mutations were reviewed for analysis of symptoms in the proband's family members. The patient had met the criteria of the disease including positive clinical symptoms and confirming laboratory findings. Meanwhile, her mother had the same homozygous mutation without experiencing any complications, at the time of diagnosis.

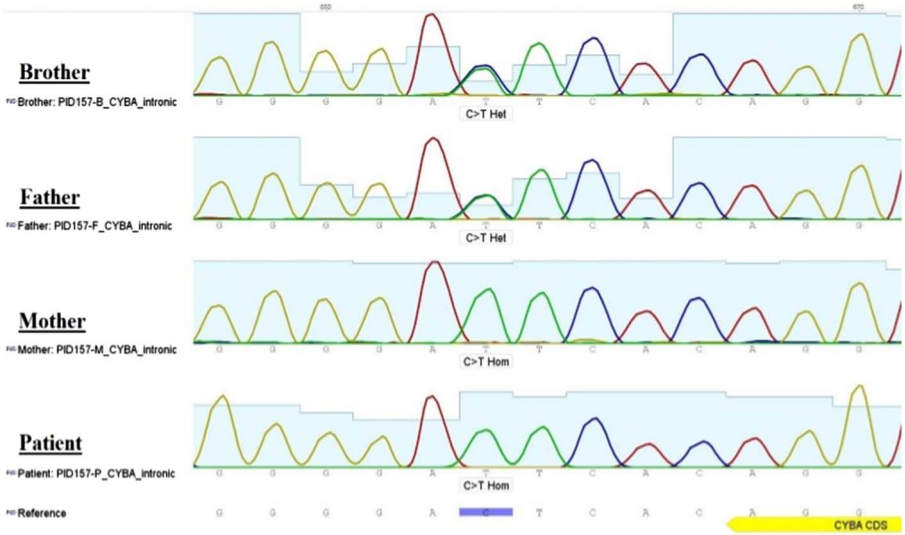
Other family members were genetically heterozygous and disease-free. In this case, DHR flow cytometric assay helped the diagnosis of CGD, and *CYBA* mutation analysis clarified the distribution of the mutated gene among the family members. This mutation was in accordance with the clinical

Table II. *CYBA* gene mutation analysis

Gene	DNA change	Protein change	dbSNP rsID	Associated disease	Inheritance	Zygosity
CYBA	1688713158 88713158 C→T	Intronic	–	CGD, autosomal, due to deficiency of *CYBA*	Autosomal recessive	Patient:Hom
						Mother:Hom
						Father:Het
						Brother:Het

Note: CGD: chronic granulomatous disease.

Figure 3. Family Sanger sequencing. The homozygous 88,713,158 C > T *CYBA* mutation was confirmed in the proband and her mother. both father and brother of patient were healthy carriers



presentations and was confirmed in the patient, her healthy brother and her parents by Sanger sequencing. Although being asymptomatic in defiance of the homozygous CYBA mutation of the patient's mother at the time of diagnosis still remains interrogative, more genetic evaluations are recommended. This situation might suggest some unknown mechanisms involved in the disease pathophysiology. Moreover, lymph node enlargement is the only symptom among the whole family members and none of the affected individuals has experienced other common symptoms of CGD. Some reports indicate specific mutations of CGD-causing genes that result in macrophage defects alone and have no disease-causing effect on monocytes and granulocytes, in which a variety of tuberculosis infections are the exclusive symptoms of the disease [12]. Although the discovered *CYBA* mutation in the presenting family has expressed BCGitis exclusively, complementary evaluations may declare undetermined downstream pathways and clarify the role of different *CYBA* gene variants in the disease manifestations.

ABBREVIATIONS

CGD : chronic granulomatous disease
NADPH : nicotinamide adenine dinucleotide phosphate
PID : primary immunodeficiency disease
CBC : complete blood cell count
DHR : dihydrorhodamine
PMA : phorbol myrisate acetate
NOI : neutrophil oxidative index
ROS : reactive oxygen species
NBT : nitroblue tetrazolium

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ER drafted the manuscript, contributed in data collection, and was in charge of project management. GP made the initial diagnosis and referred the patient to the PID center. NP was involved in planning and patients' follow-up. MS performed genetic analysis. ZA contributed to the patients' follow-up and data collection. NR made the final diagnosis and supervised the whole project. All authors read, critically revised, and approved the final version of the manuscript.

Conflict of Interest: The authors declare no competing interests.

Ethics: The study was approved by the research ethics committee of Tehran University of Medical Sciences and written informed consent was signed by the participants' parents. A written consent form for data publication was obtained from the participants' parents.

Availability of Data and Materials: The data sets used during this study are available from the corresponding author.

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