CASE REPORT
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Phenotypic Diversity of Chronic Granulomatous Disease within a Family Carrying the Same *NCF1* Gene Mutation

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ABSTRACT

Chronic Granulomatous Disease (CGD) is a defect or abnormality in the immune system that produces severe and persistent signs and symptoms in affected individuals.

Phenotypic diversity and genetic heterogeneity exist among patients with inborn errors of immunity (IEI). Symptoms may vary even when the mutations are identical; conversely, patients with different mutations may have similar clinical features. The expression of phenotype may be determined by the gene sequence, epigenetic changes, and sometimes environmental factors. Some of these outcomes are influenced by the individual's past immunological exposure.

This study discusses two CGD cases, a father and son; after the diagnosis of CGD in the child and confirmation of the genetic mutation, the same mutation was also identified in the father.

Therefore, physicians should have more awareness that a single genetic mutation can have different clinical manifestations.

Keywords: Chronic granulomatous disease; Consanguinity; Mutation; Neutrophil cytosolic factor 1; Phenotype

INTRODUCTION

Chronic Granulomatous Disease (CGD) is considered a rare hereditary immune disease characterized by a reduced oxidative stress response by neutrophils, leading them to become incapable of killing bacteria or fungi.[1] This genetic defect prevents the generation of superoxide, thereby impairing

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phagocytic capacity of various host cells; as a result, they are left extremely susceptible to severe bacterial and fungal infections from a very early age. These include species of Staphylococcus and Aspergillus, which cause pneumonia, lung abscess, skin and soft tissue infections, lymphadenitis, osteomyelitis, and hepatic abscesses. These manifestations include recurrent pyogenic abscesses in regional lymph nodes, lungs, liver, and granulomatous inflammatory reactions.[2]

There are two primary forms of CGD: autosomal recessive CGD and X-linked. Autosomal-recessive CGD is caused by biallelic mutations in any of the genes encoding components of the phagocyte NADPH oxidase complex: Cytochrome b-245, alpha polypeptide (*CYBA),* Cytochrome b-245, beta polypeptide *(CYBB),* Neutrophil Cytosolic Factor *NCF1, NCF2, and NCF4.* X-linked CGD is most commonly caused by mutations in *CYBB*, which encodes gp91phox, the catalytic subunit of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase complex. Mutations in the *NCF1* gene, which encodes the p47-phox protein, have been reported to cause approximately 23% of CGD cases.[3] Ninety-three percent of mutations are homozygous dinucleotide deletion (ΔGT) at the GTGT repeat in exon 2, resulting in a frameshift and an early termination of translation at amino acid position 51.[4] The *NCF1* locus is bordered by two nearly identical pseudogene sequences that may contribute to sporadic patterns seen in some NCF1-related CGD cases.[5] This study describes two CGD cases (father and son) with different clinical symptoms, despite an identical homozygous NCF1 mutation in exon 2, c.75-76del.

Patients develop granulomas from both infectious causes, such as Staphylococcus and Nocardia, and non-infectious inflammation, such as chronic colitis.[6] CGD is commonly linked to disseminated Bacillus Calmette-Guérin (BCG) disease, showing how important healthy immune cells are for controlling bacterial infections.[7] The condition typically follows autosomal recessive inheritance and is more common in families with consanguinity.[8,9] Interestingly, people with disease-causing mutations do not always show symptoms or may have mild disease.[10]

Case Presentation

Two cases of CGD were observed in a father and son, both carrying the same mutation, yet presenting with varying degrees of severity. The patient was a 26-month-old child from consanguineous parents (Figure 1), admitted to Mofid Children's Hospital for a combination of problems, such as anorexia, prolonged fever, and swelling of some lymph nodes of the neck, submandibular, and lateral regions. The patient had been hospitalized at another facility for 10 days prior to this admission without any improvement. Some details from past medical or family history were deemed not relevant to the current presentation. He had received the complete vaccination schedule, including BCG. The patient's vital signs were within normal limits. On examination, aside from left submandibular lymphadenitis, there were no significant findings. Laboratory findings indicated a white blood cell count of 10, 800 cells/μL with differential: 58% polymorphonuclear cells, 35% lymphocytes, and 7% mixed cells (monocytes, eosinophils, and basophils) in differentiation. The hemoglobin concentration was 9.1 g/dL, and the platelet count was 396 000/mm^3. The qualitative result of C-reactive protein (CRP) concentration was 1+ positive. The erythrocyte sedimentation rate (ESR) was found to be 72 mm/h; subsequently, no underlying infections were found in the blood cultures. The father's medical history was of recurrent episodes of aphthous lesions and dental caries, with no other significant medical conditions being reported. Broad-spectrum antibiotics (clindamycin and ceftriaxone) were initially administered as medical treatments. Gastric washing and lymph node aspiration were performed, and the material was sent for Gram staining, PCR, and culture to the hospital. A lymph node biopsy was performed, and PCR assays, as well as Gram staining procedures and microbial cultures for Mycobacterium tuberculosis, non-tuberculous mycobacteria, fungi, and bacteria, were performed on the lymph node specimens. Serratia marcescens was cultured from the lymph node biopsy, and the antibiotic therapy was changed to meropenem and cotrimoxazole accordingly. An acid-fast bacilli smear of the sputum specimen and purified protein derivative (PPD) skin test for tuberculosis were negative. An immunological workup was performed because of the long course of lymphadenitis that did not respond to antibiotics and due to the presence of Serratia marcescens. Histopathological examination of the lymph nodes showed necrotizing granulomatous lymphadenitis. Following an immunological workup nitroblue tetrazolium (NBT) test of the patient was zero, which confirmed the diagnosis of CGD. Dihydrorhodamine (DHR) flow cytometry assay showed a mean fluorescence intensity (MFI) of 3.6 in the

patient. Flow cytometry analysis for lymphocyte subsets with markers Cluster of Differentiation 3 (CD3), CD4, CD8, CD16, CD56, CD19, and CD20 were within normal ranges. The patient's condition at discharge was stable.

At follow-up, whole-exome sequencing was performed for the purpose of prenatal diagnostic assessment. The test showed a known common pathogenic homozygous deletion mutation (ΔGT) in exon 2 of the *NCF1* gene (NM-000265.6: c.75-76del). Sanger sequencing, fragment analysis, and allele-specific PCR confirmed homozygosity for the detected mutation in the patient. Surprisingly, segregation analysis showed that the proband's father was also homozygous for the same variant, while the mother and sister were heterozygous for the referred variant (Figure 2). The NBT test result of the father was zero, which confirmed the diagnosis of CGD. Although the father had no remarkable history, except for periods of aphthous lesions and tooth decay, and there was an episode of febrile infection in adolescence that was quickly treated, the diagnosis of CGD is also confirmed for the father.

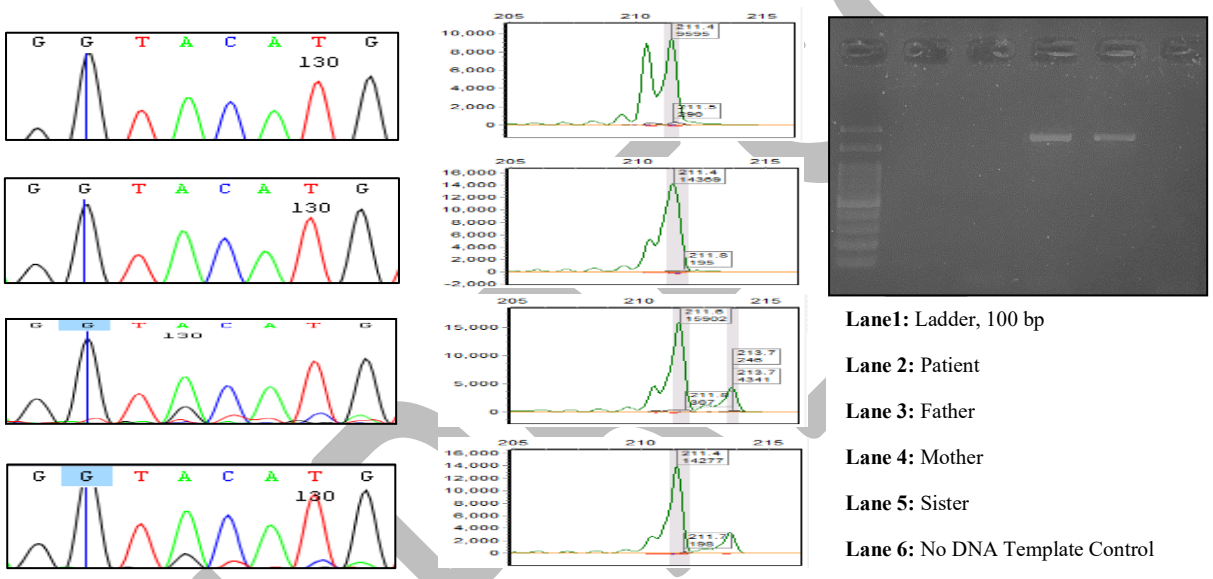
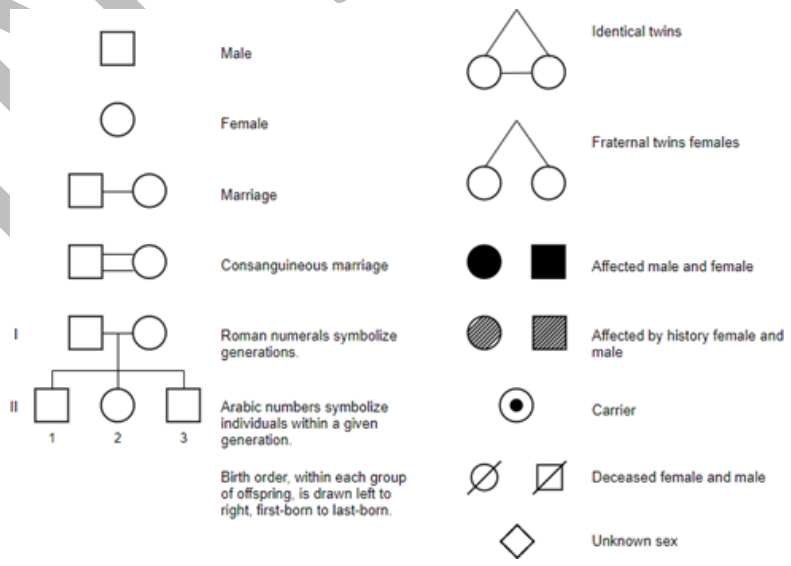


Figure 1. Sequencing chromatograms, separated fluorescently labelled amplified Neutrophil Cytosolic Factor 1 (*NCF1*) fragments by capillary electrophoresis and Allele-specific PCR (patient, affected father, asymptomatic mother and sister in order from top to bottom).



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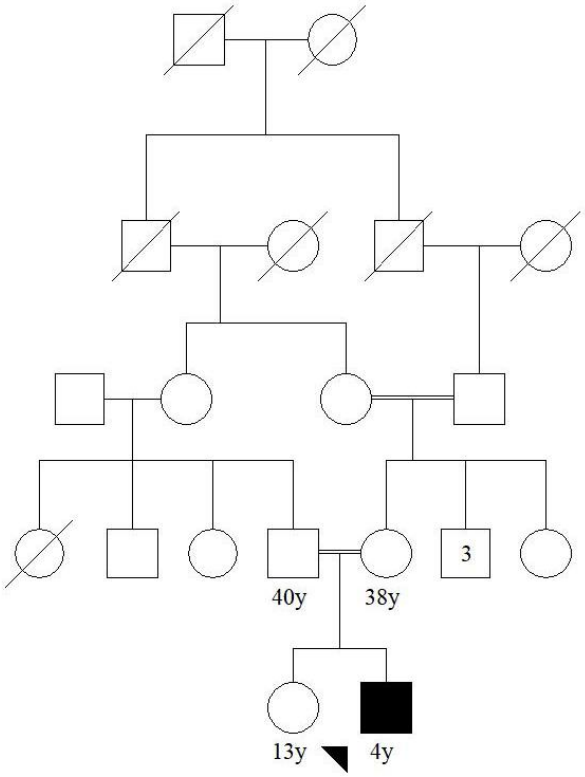

Figure 2. Pedigree of the family showing the single affected case of a first cousin parent. Affected individual is indicated by filled symbols. The proband is marked with an arrow.

DISCUSSION

Inborn errors of immunity diseases are suspected when an individual experiences severe, recurrent infections or infections caused by atypical, opportunistic pathogens, or when there are atypical autoimmune, allergic, autoinflammatory, or neoplastic manifestations.

Similar to the study by Serdar Nepesova et al our patient experienced lymphadenitis caused by Serratia marcescens, a rare pathogen that strongly suggests CGD and should raise early suspicion for immunodeficiency.[11]

Our patient exhibited the initial clinical presentation of the disease characterized by cervical lymphadenitis. The lymphatic involvement aligns with the studies conducted by Fattahi et al and Esmaeil Mortaz et al, which reported lymphatic system complications as the most common feature in CGD patients, affecting up to 65.6% of cases.[12,13] However, in the study by Serdar Nepesova et al, pulmonary involvement was the most frequently reported site of involvement in this group of patients.[11]

While Fattahi et al found Aspergillus species to be the most frequent pathogen,[12] in our study, we observed Serratia marcescens in only one patient, despite it being reported as a common organism in infections affecting these patients in other studies.[13]

The patient had autosomal recessive CGD (AR-CGD), which is consistent with Fattahi et al's findings of high AR-CGD rates (87%) linked to consanguinity, which was also observed in our case.[12] This pattern is particularly prevalent in Iran due to high rates of consanguineous marriages, contrasting with Western populations where X-linked forms predominate.[12,13]

As mentioned above, our patient had a pathogenic homozygous deletion (ΔGT) in exon 2 of the NCF1 gene.

In the investigation of genotype-phenotype correlations in CGD patients, various phenotypes have been observed in these individuals. Studies have shown that patients with X-linked CGD show more severe clinical manifestations and experience an earlier age of onset.[14]

A commonly known defect in *NCF1* is a nucleotide deletion, specifically c.75_76delGT, at the beginning of

exon 2. The *NCF1* defect is the most common mutation identified in patients with autosomal recessive CGD. According to Rawat et al, out of 141 patients studied, in 45, molecular findings suggested pathogenic variants within the *NCF1* gene. Most patients with autosomal recessive CGD due to mutations within the *NCF1* gene are generated from the deletion of the two nucleotides located in exon 2 of the gene.[15] As shown in the study by Tajik et al, the prevalence of *NCF1* deficiency in Iran is higher than in India and Turkey.[16]

The most important and interesting point of this article is that we have reported two cases of CGD where the father was homozygous for *NCF1* instead of being a carrier but had very mild symptoms.

Despite sharing the same genotype, only the child exhibited severe CGD symptoms. The father, although homozygous for the same mutation, showed very mild symptoms.

There is a similar report in a study conducted by Wolach et al on 17 unrelated Kavkazi CGD patients belonging to 14 unrelated families; patients showed different manifestations despite the same *NCF1* gene mutation (c.579G>A). [17]

The relationship between genotypes and clinical phenotypes is often not straightforward, as individuals with disease-associated mutations may not express all disease features or may be asymptomatic.[10]

The reasons behind these variations involve multiple factors working together.

Penetrance and expressivity are concepts that explain how certain traits manifest in individual people. In simple terms, penetrance can be defined as the proportion of affected individuals in a population with the same mutation showing clinical signs of the relevant disorder. On the contrary, expressivity indicates the range of ways and the severity of these characteristics among individuals who share the gene mutation.[18]

Furthermore, the phenotypic variability observed in our cases-where both the father and the son share a homozygous pathogenic *NCF1* mutation but display markedly different clinical manifestations-can be attributed to a combination of genetic and environmental factors. Modifier genes are increasingly recognized as key contributors to the heterogeneity seen in monogenic disorders like CGD. Variations in genes involved in immune regulation, such as Toll-like receptors (TLR2, TLR4) and cytokines like interleukin-10 (IL-10) and tumor necrosis factor (TNF), can modulate the innate immune response and inflammatory pathways, thereby influencing susceptibility to infections and disease severity.[19] Additionally, the role of epigenetic modifications and regulatory elements in non-coding genomic regions may affect *NCF1* expression and downstream immune responses.[18] Environmental exposures also play a significant role in determining clinical outcomes. Differences in microbial exposure, vaccination history (particularly BCG), antibiotic use, and hygiene can shape immune system maturation and infection risk.[20–23] For instance, early-life exposure to specific pathogens may trigger symptomatic disease in one individual, while another with the same genetic mutation remains asymptomatic due to limited exposure. Moreover, recent studies suggest that gut microbiota composition may influence systemic inflammation in CGD, adding another layer of environmental modulation.[24]

These findings underscore the complex interplay between genotype, immune regulation, and environmental context, and explain why even individuals within the same family carrying identical mutations may exhibit different phenotypes.

We described a mutation in the *NCF1* gene that was found in both a father and his son, causing CGD. Despite sharing the same mutation, their clinical phenotypes differed markedly. Genetic mutations often have variable clinical expressivity or can be phenotypically normal. Thus, the detection of these mutations is particularly important for treatment decisions and the timing of therapeutics. Genetic mutations may result in a wide range of disease severity and age of onset. Studying patients with the same mutation shows us which factors modify how the gene behaves. Environmental influences then help explain why the disease looks different in different settings, which is essential for designing better targeted treatments.

STATEMENT OF ETHICS

Ethical approval for this study was obtained from Shahid Beheshti University ethics committee (approval number: IR SBMU.MSP.REC.1400.030).

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, (Dr. Shahrzad Fallah) upon reasonable request.

AI ASSISTANCE DISCLOSURE

The authors declare that generative artificial intelligence (Chat GPT) was used to assist in the writing of this manuscript.

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