CASE REPORT

CYBB Gene Mutation Detection in an Iranian Patient with Chronic Granulomatous Disease

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ABSTRACT

In this study, we report a mutation in CYBB gene in a patient with X-CGD (diagnosed on the base of family history, NDT test, DHR 123 assay). Mutation in CYBB gene was detected using SSCP analysis (single-strand conformation polymorphism) followed by sequencing. During screening for mutations in the CYBB gene we observed 880 C → T in exon 8. This mutation resulted in 290 Arg → Stop. We also observed a change (-270 C → A) in the promoter region which needs further investigation.

We would like to pursue this study by analyzing more X-CGD patients to find out the CYBB mutation spectrum in Iranian patients.

Keywords: Chronic Granulomatous Disease; CYBB Protein, Human; Gene; Mutation

INTRODUCTION

Chronic Granulomatous Disease (CGD, OMIM No. 306400) is an inherited phagocytic disorder resulting to reduced nicotinamide dinucleotide phosphate (NADPH) oxidase complex activity, which results in defective superoxide generation and intracellular killing.1 CGD patients present with recurrent life-threatening bacterial and fungal infections, chronic granulomas and poor wound healing.2 CGD may be inherited as an X-linked (X) or autosomal recessive (AR) manner. However, X-CGD is far more common, accounting for about 70% of all CGD cases.3 X-CGD is because of mutation in CYBB gene (GenBank Accession Nos.AF469757-AF469769). This gene is located on chromosome Xp21.1, spans 30 kb and contains 13 exons. CYBB encodes gp91phox (also known as cytochrome b22R), a key transmembrane protein in the phagocyte NADPH oxidase system.

This protein and p22phox are the two membrane subunits of flavocytochrome b558 and are essential components of the phagocyte NADPH oxidase system.1,3

Diagnosis of X-CGD is made by demonstrating absent or markedly reduced oxidase activity in stimulated neutrophils. Screening for CGD is accomplished by using the nitroblue tetrazolium test or a more sensitive dihydrorhodamine (DHR) 123 assay. Quantitative methods for evaluating oxidase activity include the measurement of superoxidase production by ferricytochrome c assay, chemiluminescence or protein expression characterization by Western blot analysis.4 Most X-CGD patients demonstrate little or no activity on DHR assay, as reflected by a very low granulocyte stimulation index (range of 0.9-2.2) as well as the absence of the gp91phox protein and superoxide production. However, a subset
of X-CGD patients has atypical phenotypes reflected by variable levels of the gp91phox protein and superoxide production, requiring CYBB mutation analysis to establish the definitive genotype.5,8

Over 300 CYBB mutations have been registered in an internationally maintained X-CGD database (X-CGD base). Most mutations are distributed throughout the 13 exons or at exon/intron boundaries and almost 200 of these mutations are unique. Although less than 1% of the mutations reported in the X-CGD base has been found in the promoter/enhancer region, nearly 10% are splice mutations, which remove a donor or acceptor splice site.6,7

To date, most of the published CYBB mutation analysis methods typically start with radioactive screening methods, such as single-stranded conformation polymorphism (SSCP) analysis of DNA. The limitations of SSCP are well recognized, and it may miss 5-30% of all sequence changes, depending on the size of DNA fragment and the position of the basis substitution within the fragment of the gene under study.2

In this study, we report a mutation in CYBB gene in a patient with X-CGD (diagnosed on the basis of family history, NDT test, DHR 123 assay). Mutation in CYBB gene was detected using SSCP analysis (single-strand conformation polymorphism) followed by sequencing.

CASE REPORT

Patient

Patient is male, 4 years old. He had recurrent episodes of bacterial infection and was diagnosed as having X-Linked CGD by functional analysis in the family. Blood samples were obtained after his parents' written consent.

Functional and Phenotypic Analysis of Polymorphonuclear Neutrophils (PMN)

Biological diagnosis of CGD was based on PMN oxidative burst measurements. Neutrophils were obtained by centrifugation of heparinized blood over a polymorphoprep density gradient. The capacity of PMN to produce ROS was assessed by the NBT (nitroblue tetrazolium) slide test, quantitative NBT and flow cytometric dihydrorhodamine assay (DHR test).

DNA Extraction and SSCP Analysis

Genomic DNA was extracted from white blood cells by proteinase K digestion and salting out precipitation of DNA. Promoter region and 13 exons of the CYBB gene were amplified by independent PCR runs. PCR was performed in a final volume of 50 µl containing 200 µM dNTP, 1x Tag DNA polymerase buffer, various concentration of MgCl2, 2U of Tag DNA polymerase (Cinagen, Iran), 200 ng Genomic DNA and 10 pmol of each primer. After initial denaturation for 10 min at 95°C, 35 cycles of amplification were done as follows: 30 sec at 95°C, 20 sec at 51 or 55°C and 20 sec at 72°C. PCR products evaluated on 2% agarose gel. SSCP analysis was performed on 6% polyacrylamid gel (with or without glycerol) followed by silver staining.

DNA Sequencing

Sequence analysis of PCR products with abnormal SSCP patterns were done after purification of PCR products (PCR product purification kit, Roche). Both strands were sequenced using Big Dye Terminator System. Sequencing results were analyzed by bioinformatics tools (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

The patient with clinically diagnosed CGD was further studied by PMN functional oxidative burst assays. Significant ROS (Reactive Oxygen Species) production was absent in the patient.

PMN oxidative burst assay was also done for his mother. Flow cytometric analysis of H2O2 production identified two functional PMN subpopulations, confirming the carrier status of mother and excluding de novo mutations (which accounts for about 10% of cases of CGD). PMN oxidative burst was also done for his father and who was normal (Table 1).

Table 1. Results of NBT and DHR tests.

<table>
<thead>
<tr>
<th></th>
<th>NBT Slide Test</th>
<th>Quantitative NBT</th>
<th>DHR Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0%</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Mother</td>
<td>78%</td>
<td>0.36</td>
<td>1, 22</td>
</tr>
<tr>
<td>Father</td>
<td>100%</td>
<td>0.51</td>
<td>23</td>
</tr>
<tr>
<td>Control</td>
<td>100%</td>
<td>0.42</td>
<td>21</td>
</tr>
<tr>
<td>Normal Values</td>
<td>90-100%</td>
<td>≥ 0.2</td>
<td>≥ 20</td>
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All 13 exons and the promoter region of CYBB gene were examined by SSCP analysis. During screening for mutations in the CYBB gene with SSCP, abnormal band pattern was observed in exon 8 and promoter region (Figure 1). Sequencing of exon 8 and promoter region showed a nonsense mutation in exon 8 and a polymorphism in the promoter region. The mutation was found to be 880C→T in exon 8.

This mutation resulted in 290 Arg→Stop. We also observed a change (-270 C→A) in the promoter region which needs further investigation.

DISCUSSION

880C→T mutation has previously been reported in the Human Gene Mutation Database (http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/120513.html). Rae et al, have shown that mutations from a coding to a nonsense codon results in X91ο phenotype in all patients. Nonsense mutations were found in 23% of all cases. Nonsense mutations, including stop codones, always manifest X91ο phenotype of CGD.9

Analyzing missense and nonsense mutations in X-Linked CGD (excluding the polymorphism), showed that 34% mutations were in CpG sequences and remarkably not found in other than G→A and C→T mutations. Always these CG→TG nonsense mutations concerned the CGA codon for arginine mutated to the stop codon TGA.3

REFERENCES

CYBB Gene Mutation in CGD


