

## A Complete Collection of Genetic Variations That Cause Chronic Granulomatous Disease

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### Abstract

Chronic granulomatous disease is an immunodeficiency that manifests in young children as susceptibility for recurrent bacterial and fungal infections. The disease is caused by mutations in the genes that encode the components of the leukocyte NADPH oxidase enzyme. This enzyme generates reactive oxygen compounds needed for killing pathogenic microorganisms and comprises five protein components. One of these components is gp91phox, encoded by CYBB, a gene on the X chromosome. Mutations in this gene cause the X-linked, most prevalent form of chronic granulomatous disease, affecting about 65% of the patients. The other four components are encoded by autosomal genes. In two recent articles, all variations in these five genes, published and unpublished, have been collected from almost 5000 patients, thus substantially aiding in the correct interpretation of genetic diagnosis.

**Keywords:** Chronic granulomatous disease; Mutations; NADPH oxidase; CYBB; CYBA; NCF1; NCF2; X chromosome

**Abbreviations:** CGD: Chronic Granulomatous Disease; CYBA: Cytochrome-B Alpha subunit; CYBB: Cytochrome-B Beta subunit; CYBC1: Cytochrome b558 Chaperone-1; FACS: Fluorescence Activated Cell Sorting; G6PD: Glucose-6-Phosphate Dehydrogenase; GP: Glycoprotein; H<sub>2</sub>O<sub>2</sub>: Hydrogen Peroxide; HSPC: Hematopoietic Stem/Progenitor Cell; Indel: Insertion-deletion; kDa: Kilodalton; NADPH: Nicotinamide-Adenine-Dinucleotide Phosphate reduced; NCF: Neutrophil Cytosolic Factor; Nox: NADPH oxidase; O<sub>2</sub><sup>-</sup>: Superoxide; P: Protein; Phox: Phagocyte Oxidase; RAC: Ras-related C3 botulinum toxin substrate; ROS: Reactive oxygen species; X-CGD: X-linked CGD

### Introduction

Chronic Granulomatous Disease (CGD) is a rare (1:250,00 newborns) immunodeficiency caused by mutations in the genes that encode the components of the leukocyte NADPH oxidase [1]. The disease manifests usually in young children as a susceptibility to recurrent, life-threatening infections with bacteria, fungi and yeasts. In healthy individuals, these microorganisms are ingested by phagocytic leukocytes (neutrophils, eosinophils, macrophages) and killed intracellularly. An important component in the killing process is the formation of Reactive Oxygen Compounds (ROS) by these leukocytes. The responsible enzyme, called NADPH oxidase, releases superoxide (O<sub>2</sub><sup>-</sup>), which is subsequently converted to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), into the phagocytic vacuole that contains the ingested microorganisms. Together with proteolytic enzymes, also released into this vacuole, this creates a very toxic environment that kills the pathogens. In CGD patients, the NADPH oxidase is non-functional, leading to a defect in this killing process. Infection of the skin, lungs, gastrointestinal tract and lymph nodes draining these organs are common, as well as abscesses in liver, bones, kidney or

brain. Infecting organisms include a variety of bacteria and fungi, such as *Staphylococcus aureus*, *Aspergillus* species, *Burkholderia cepacia*, *Serratia marcescens*, *Klebsiella* species and *Salmonella* species [1,2]. Treatment of CGD patients consists of aggressive antibiotic and antifungal therapy, and surgical drainage of abscesses. This is usually followed by life-long prophylactic antibiotic and antifungal therapy. Cure can only be obtained by bone-marrow transplantation [2,3]. Gene therapy is being developed but is still hampered by loss of gene-corrected Hematopoietic Stem/Progenitor Cells (HSPCs) during survival and outgrowth *in vivo* [4,5].

### Literature Review

Diagnosis of CGD patients requires clinical, cellular and genetic observations. The hallmark of CGD is the lack of ROS formation by phagocytic leukocytes. Several methods are available for this analysis [6]. A commonly used test is a whole-blood DHR assay, in which neutrophils are activated with phorbol ester, and the H<sub>2</sub>O<sub>2</sub> released by these cells oxidizes Dihydrorhodamine-1,2,3 (DHR) to Rhodamine-1,2,3; this product can be measured by FACS. However, for recognition of heterozygous family members, for prenatal detection of patients and for choosing a suitable bone-marrow donor, genetic analysis is mandatory. For this purpose, the genes encoding the various NADPH oxidase components must be investigated.

The leukocyte NADPH oxidase consists of five protein components, i.e. gp91phox and p22phox in the cell membrane and p40phox, p47phox and p67phox in the cytosol (gp=glycoprotein, p=protein, the numbers refer to the molecular weight in kDa, phox stands for phagocyte oxidase) [1-3]. Gp91phox (also called Nox2) is the enzymatic subunit; its expression in the membrane is stabilized by p22phox. During cell activation by phagocytosis of microorganisms, the heterotrimer of the three cytoplasmic subunits forms a complex with the plasma membrane subunits. In the assembled oxidase complex, gp91phox is able to accept electrons from NADPH in the cytosol and

to transport these to the apical side of the plasma membrane, into the phagocytic vacuole. Here, these electrons can react with molecular oxygen to superoxide and subsequently to H<sub>2</sub>O<sub>2</sub> [1-3].

Mutations in any one of the genes encoding these five NADPH oxidase components can cause CGD. Most CGD patients (about 65%) have mutations in CYBB, the gene encoding gp91phox. CYBB is located on the X chromosome, so most of the X-CGD patients are boys. However, females with only few leukocytes with an active, non-mutated X chromosome can also present as CGD patients [7]. The other four NADPH oxidase components are encoded by autosomal genes. Mutations in these genes are found in about 35% of all CGD patients; most of these mutations (~25%) are present in NCF1, the gene encoding p47phox. One NCF1 mutation in particular is present very frequently. That is a GT deletion at the start of exon 2 in NCF1, originating from exchange of genetic material between NCF1 and one of the two pseudogenes surrounding NCF1. Mutations in CYBA (p22phox) and NCF2 (p67phox) are found in about 5% of all CGD patients; mutations in NCF4 (p40phox) are very rare [8,9].

Mutational diagnosis can be hampered by unknown consequences of the genetic variations found. In case of large deletions, frameshift mutations or nonsense mutations leading to premature stop codons, one can safely assume these to cause the disease. However, missense mutations (leading to amino-acid substitutions), splice site mutations or small insertion-deletions (indels) can either be disease causative or harmless polymorphisms. Thus, a complete list of all variations ever found in proven CGD patients can be very helpful for correct interpretation of genetic analysis. A new update of these mutations in X-CGD and in autosomal CGD has recently been published [8,9]. This is the result of a world-wide collaboration of more than 20 clinical and research centers, screening of the literature for published mutations and contributing unpublished mutations from their own efforts.

These publications contain not only the actual mutations, but also the type of mutation (nonsense, missense, deletion, insertion, indel, splice site or transcription site mutation), the number of patients with these mutations, the occurrence of female X-CGD patients, and the effects of the mutations on the expression of the respective proteins (complete lack, diminished expression and activity, or normal expression but lack of activity). In total, the mutations of about 3000 X-CGD patients and about 1800 autosomal CGD patients are included. In addition, proven polymorphisms in the CGD genes are also listed, again helpful for correct interpretation of genetic analysis.

## Discussion

Finally, mutations are included in three genes that have recently been identified to induce CGD-like symptoms. One is G6PD, the gene that encodes glucose-6-phosphate dehydrogenase. In case of a mutation that induces a very low activity of this protein in phagocytic leukocytes, this causes a shortage of NADPH in these cells, and thus insufficient substrate for the NADPH oxidase [10]. A second gene is

CYBC1, encoding cytochrome-b558 chaperone-1, a protein necessary for correct maturation and expression of gp91phox. Mutations in this gene can cause lack of gp91phox expression, and thus X-CGD [11]. Finally, RAC is a GTPase protein in leukocytes involved in the activation process of the NADPH oxidase (RAC1 in macrophages, RAC2 in neutrophils). One particular RAC2 mutation has been identified that disturbs this activation process [12]. This is now included in the recent autosomal CGD mutation update [9].

## Conclusion

In these two publications contain important data for clinicians, researchers and students of CGD and of the leukocyte NADPH oxidase.

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