

## Mutations in *CYBB* and Chronic Granulomatous Disease – an Overview

### Introduction

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency disorder with an estimated incidence of between 1 in 200,000 and 1 in 250,000 births (1, 2). CGD is a heterogeneous disorder, characterized by recurrent, severe bacterial and fungal infections and chronic inflammatory granulomas (for comprehensive reviews, refer to references 3-5). While infections associated with CGD can be life-threatening, they can be successfully controlled with the use of prophylactic antibiotics and interferon- $\gamma$  therapy, making early diagnosis important (3). To date, loss-of-function mutations in any one of six different genes have been implicated in CGD (4). In about 70-75% of cases, CGD is due to X-linked loss-of-function mutations in the gene *CYBB* (5, 6).

### Molecular Pathophysiology

The *CYBB* gene product, gp91<sup>phox</sup> (91 kDa subunit of phagocyte oxidase), is a subunit of the phagocyte NADPH oxidase, which generates hydrogen peroxide and superoxide radicals necessary for the microbicidal response of phagocytes. The NADPH oxidase is comprised of at least six subunits which are dispersed throughout the cell in resting phagocytes (reviewed in 7). The enzymatic component of NADPH oxidase, flavocytochrome b<sub>558</sub>, consists of two integral membrane proteins, gp91<sup>phox</sup> and p22<sup>phox</sup>. gp91<sup>phox</sup> is the redox center of the enzyme (8), while p22<sup>phox</sup> is thought to be important for membrane anchoring and complex stabilization (9). The remaining subunits, which include p67<sup>phox</sup>, p47<sup>phox</sup>, p40<sup>phox</sup>, and Rac2, serve to activate the NADPH oxidase function of the membrane components. These subunits reside in the cytosol in unstimulated cells and translocate to the membrane upon phagocyte stimulation. Activated NADPH oxidase accepts electrons from NADPH and donates them to molecular

oxygen, producing superoxide (O<sub>2</sub><sup>-</sup>). Superoxide is subsequently converted to microbicidal compounds such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hypochlorous acid (HOCl) (reviewed in 9).

The leukocytes of patients with mutations in *CYBB* are unable to produce the reactive oxygen species necessary for intracellular killing of phagocytized pathogens (10). Patients with CGD are particularly at risk for infection by organisms that contain catalase, which neutralizes hydrogen peroxide, making catalase-positive organisms particularly resistant to intracellular killing. Since mutations in *CYBB* do not affect leukocyte mobility, defective monocytes are able to reach the sites of infection, where they can accumulate and give rise to dysregulated inflammatory reactions, resulting in granuloma formation. Inefficient degradation of debris may further contribute to granuloma development (11, 12).

### Clinical Presentation

CGD is characterized by recurrent, severe bacterial and fungal infections and chronic inflammatory granulomas in the skin, gastrointestinal tract, and genitourinary tract. Patients with CGD are particularly susceptible to *Staphylococcus aureus* and *Aspergillus* species, as well as a variety of gram-negative enteric bacteria, including *Serratia marcescens*, *Burkholderia cepacia*, and *Salmonella* species (3). Pneumonia or sepsis due to *Aspergillus* or *B. cepacia* infection are the most common causes of death in CGD patients (4). Presence of inflammatory granulomas may result in colitis, enteritis, or granulomatous obstructions of the urinary tract or the gastric outlet (3, 5). Additional symptoms include fibrosis, fever, and other signs of systemic infection in the absence of obvious infection (13) and poor wound healing (14). Hypergammaglobulinemia is often observed due to persistent antigenic stimulation in response to chronic infection.

Mutations in *CYBB* that result in a complete lack of flavocytochrome  $b_{558}$  account for the majority of defects in *CYBB*, and result in more severe symptoms than mutations that allow residual enzyme synthesis (6). Very large deletions in the *CYBB* gene affecting neighboring genes have also been observed, giving rise to additional symptoms such as Duchenne muscular dystrophy, retinitis pigmentosa, and McLeod's syndrome (5).

Female carriers of mutations in *CYBB* are typically not affected by CGD, but nearly half of all X-CGD carriers suffer from recurrent stomatitis or gingivitis; photosensitivity and discoid lupus erythematosus have also been observed. In rare cases, skewed X-inactivation will result in symptoms similar to those observed for classic X-CGD (reviewed in 3).

## Diagnosis

X-CGD is suspected in male infants and children presenting with recurrent, severe bacterial and fungal infections in conjunction with infectious dermatitis or abscesses in the skin or organs due to inflammatory granulomas. Although patients with X-CGD are usually diagnosed during the first two years of life, delayed onset of symptoms has been observed (5). CGD is typically diagnosed using chemical assays to detect reduced or absent oxidase activity in activated neutrophils. The nitroblue tetrazolium (NBT) test is commonly used, although other methods are available (14). However, these assays do not allow reliable detection of carrier status, since

skewing of X-inactivation toward the X chromosome carrying the normal *CYBB* copy may obscure the effects of heterozygous mutations in *CYBB*. In addition, a significant percentage of mutations arise *de novo* (5). Detection of carrier status is important, since female carriers of mutations in *CYBB* place their sons at a 50% risk of being affected with the disease. Since published studies have established a clear relationship between mutations in *CYBB* and X-CGD, a diagnosis of *CYBB*-related X-CGD can be confirmed or established through genetic testing. Genetic testing also allows differential diagnosis of X-linked and autosomal recessive forms of CGD, as well as detection of carriers, enabling improved genetic counseling and early diagnosis and timely treatment of affected descendants.

## Treatment

The standard of treatment for X-CGD includes use of prophylactic antibiotics and interferon- $\gamma$ , coupled with aggressive treatment of infections. Corticosteroids can be used to treat complications arising from chronic granulomas (reviewed in 3). Although considered risky, stem-cell transplantation offers the prospect of a cure for CGD and has been used successfully in a number of cases (reviewed in 15). Research into the development of gene therapy-based treatment of CGD is ongoing, but has not yet yielded a viable treatment option (reviewed in 16, 17).

## References

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