

## A novel CYBB mutation with the first genetically confirmed case of chronic granulomatous disease in South Africa

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A case of a child with chronic granulomatous disease (CGD) presenting with recurrent mycobacterial infections and invasive *Aspergillus fumigatus* disease is described. Genetic analysis confirmed X-linked CGD with a novel mutation in exon 10 of the

CYBB gene – the first South African report of genetically confirmed CGD.

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Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease of phagocytic cells, resulting from impaired function of one of five essential subunits of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex within neutrophils, eosinophils, monocytes and macrophages. This enzyme complex mediates intracellular killing of phagocytosed micro-organisms. The NADPH oxidase complex is composed of membrane-bound glycoproteins gp91<sup>phox</sup> and p22<sup>phox</sup>, and cytosolic subunits p47<sup>phox</sup>, p67<sup>phox</sup>, p40<sup>phox</sup> and *rac*. Although mutations in genes encoding any one of these protein subunits can result in the disease, 70% of cases are due to mutations in the CYBB gene causing gp91<sup>phox</sup> deficiency, which is responsible for X-linked CGD.<sup>1,2</sup> Autosomal recessive forms of CGD due to mutations in genes encoding p47<sup>phox</sup>, p22<sup>phox</sup>, p67<sup>phox</sup> and p40<sup>phox</sup> have been described.<sup>2</sup>

Failure to mediate oxidative killing results in susceptibility to recurrent life-threatening bacterial and fungal infections and increased risk of mycobacterial infections.<sup>1</sup> In the past, mortality from CGD was exceptionally high, but antibiotic and antifungal prophylaxis and therapy, and haematopoietic stem cell transplantation has improved outcomes significantly and reduced mortality to 5%.<sup>1</sup> The worldwide prevalence of CGD is estimated to be 1 in 200 000 - 250 000 live births.<sup>1</sup> Since 1983, 6 cases in South Africa have been reported in the literature, all diagnosed by immunological testing.<sup>3</sup> We describe the first genetically confirmed case of CGD in this country.

### Case report

In June 2010, a 5-year-old boy with a past history of recurrent tuberculosis (TB) presented to his paediatrician with left lower lobe consolidation, generalised lymphadenopathy and marked failure to

thrive. Bacterial and mycobacterial cultures were negative and, in view of his past history, he was suspected of having drug-resistant TB. He had been well up until 15 months of age, when pulmonary TB was diagnosed, and he received 6 months of anti-TB medication. However, he presented a year later with abdominal TB. He responded to a second course of therapy, but at the age of 4 years presented with a discharging sinus over the right arm and right axillary scrofuloderma. Histological analysis of the skin biopsy confirmed the presence of intracellular acid-fast bacilli, although mycobacterial cultures were negative. He responded to a third course of anti-TB medication. There was no preceding history of severe acute bacterial infections.

Upon transfer to Red Cross War Memorial Children's Hospital, clinical evaluation revealed wasting, generalised lymphadenopathy, and left lower lobe consolidation on respiratory examination. There was tenderness over the thoracic spine but no gibbus formation. Although globally weak, no neurological abnormalities were present. Chest computed tomography (CT) showed opacification in the left lower lobe, multiple bilateral upper lobe parenchymal nodules, a contiguous left paravertebral density along thoracic vertebrae 7 - 11, erosion of ribs 7 and 8, and partial height loss of corresponding thoracic vertebral bodies. A lung biopsy showed granulomatous inflammation and septated hyphae with dichotomous branching resembling *Aspergillus* infection. Fungal cultures, however, were negative. He was initially commenced on amphotericin B but changed to intravenous voriconazole a week later.

The clinical course improved and he was converted to oral voriconazole after 3 weeks. However, a month into therapy, he developed collapse of thoracic vertebrae 6 - 8 and required spinal cord decompression. Culture from the spinal mass confirmed *Aspergillus fumigatus* infection. The myelopathy improved gradually. However, 6 months later, mild bilateral lower limb hypertonia persisted. Follow-up chest CT showed calcification of the pulmonary nodules but persistence of the paravertebral mass and left lower lobe consolidation. The patient remains on long-term therapeutic oral voriconazole and cotrimoxazole prophylaxis.

Immunological work-up demonstrated evidence of reduced neutrophil killing capacity, i.e. a monocyte oxidative reaction of 14.26% (normal 70 - 100%) and neutrophil oxidative reaction of 17.44% (normal 95 - 100%), which suggested a diagnosis of CGD. This was confirmed on repeat neutrophil burst testing. Immunoglobulin concentrations, complement screen and lymphocyte subset analysis were relatively normal.

Although there was no reported family history of primary immunodeficiencies, 2 maternal uncles and 1 maternal male cousin died in infancy of unknown causes that suggested an X-linked inheritance pattern (Fig. 1). Sequencing of the CYBB gene on peripheral blood samples was completed by the University of

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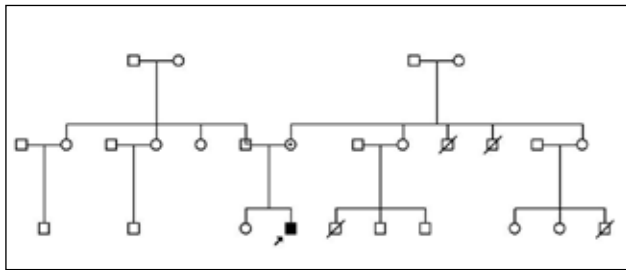


Fig. 1. Pedigree of the family showing the affected patient (■) and carrier female (mother), genetically normal male (father) and male deaths in the maternal family. (Cyrillic Pedigree Editor Version 2.0.1.0)

Hong Kong;<sup>4</sup> 13 exon fragments were amplified using HotStarTaq Plus PCR system (Qiagen GmbH, Germany). Homology analysis of the sequenced data with CYBB genomic DNA was performed through the NCBI database (<http://www.ncbi.nlm.nih.gov/BLAST>) and the Ensembl SNPs database (<http://www.ensembl.org>). Analysis of the CYBB gene of the patient revealed a novel insertion-deletion mutation in exon 10, while his mother was found to be a heterozygous carrier of the mutation (Fig. 2).

## Discussion

We describe a patient with CGD and a novel mutation in the CYBB gene. If the South African incidence is similar to international

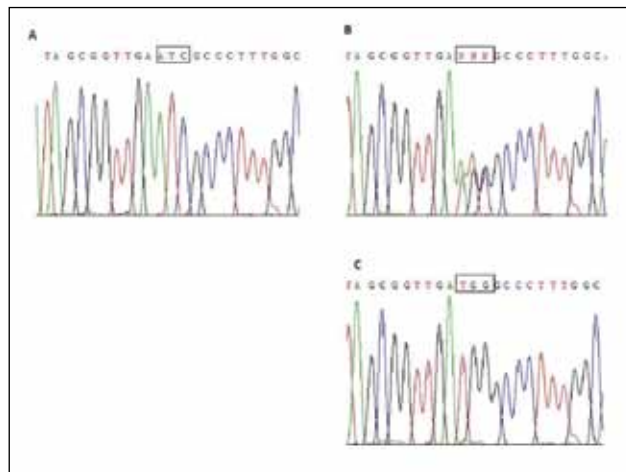


Fig. 2. X-CGD molecular diagnosis by PCR-direct sequencing of CYBB gene (naming according to Ref. sequence of CYBBbase, <http://bioinf.uta.fi/CYBB-base/>). (A) Index patient: X-linked indel mutation 1178-1180 - "TGG" deletion and "ATC" insertion at nucleotide position 1178-1180, Asp-Gly (codon GAT-GGG) change to Glu-Ser (codon GAA-TCG) at amino acid position 388-389. c.1178\_1180delinsATC, p.DG388\_389ES, (B) Mother: Heterozygous mutation, carrier, and (C) Father: genetically normal.

prevalence, 4 - 5 new cases of CGD should be identified in the country per year, which suggests that many children with CGD are not being diagnosed, most probably owing to failure to recognise the clinical manifestations.

Three mycobacterial infections and invasive aspergillosis in our patient is consistent with CGD. Acute bacterial infections with *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens* and *Nocardia sp.* predominate, while *Aspergillus* – the most frequently associated fungal infection – is the leading cause of morbidity and mortality.<sup>1,2</sup> Several CGD patients with Bacille-Calmette-Guérin (BCG) disease and TB have been described in regions endemic for tuberculosis.<sup>4</sup> Other CYBB mutations not related to CGD have also been associated with X-linked recessive mendelian susceptibility to mycobacteria.<sup>5</sup> Other primary immunodeficiency diseases (PIDs) that cause recurrent mycobacterial infections include X-linked hyper-IgM syndrome, severe combined immunodeficiency syndrome and deficiencies of the IL-12/23-IFN- $\gamma$  axis.

Sequencing of the CYBB gene documented a novel insertion-deletion mutation in exon 10 which resulted in amino acid alterations at positions 388 and 389. Over 400 different mutations of the CYBB gene have been described.<sup>6</sup> While mutational analysis is important for definitive diagnosis, it is not routinely available, however, and immunological evaluation with neutrophil burst testing is sufficient to make a diagnosis and initiate treatment. Prophylactic antibiotic therapy with cotrimoxazole has been effective in reducing serious bacterial infections, while prophylactic itraconazole has substantially decreased the number of *Aspergillus* infections.<sup>1,2</sup> Interferon-gamma has also been shown to be a highly effective prophylactic therapy, but the cost remains prohibitive in resource-limited settings.<sup>1</sup>

Improved recognition of CGD and early diagnosis is the cornerstone in reducing morbidity and mortality, so that prophylaxis may be initiated early, infections may be treated promptly and possibly curative haematopoietic stem cell transplantation considered.

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