Novel mutations in the *CYBB* gene causing X-linked chronic granulomatous disease: a case report of 2 patients

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ABSTRACT

Introduction: Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency caused by mutations in the NADPH complex characterized by recurrent infections, inflammation and autoimmunity. While autosomal recessive forms exist, X-linked CGD makes up the majority of cases, which is caused by mutations in the *CYBB* gene. Patients are at high risk for infections with catalase positive bacteria and fungi. The prognosis has improved significantly with improvements in disease detection and management, including prophylactic antibiotic and antifungal therapy. Hematopoietic stem cell transplantation (HSCT) is a curative option for patients with a suitable donor.

Aim: To report the clinical presentation, immune features and genetic mutations in 2 patients with novel mutations in the *CYBB* gene causing X-linked CGD who underwent HSCT.

Results: *Case 1:* Patient 1 is a 14-year-old patient who initially presented with disseminated aspergillosis at the age of 3. He was noted to have an abnormal neutrophil oxidative burst index (NOBI) and genetic testing revealed a mutation in the *CYBB* gene (c.883_87dupGTGGT) consistent with CGD. He successfully underwent HSCT at age 4. At age 10 he developed a primary intracranial rhabdomyosarcoma in the posterior cranial fossa. *Case 2:* Patient 2 is a 4-year-old male who was worked up for CGD after developing a perianal abscess at 1 month of age followed by Moraxella bacteremia at 2 months of age. He had 2 abnormal NOBIs and genetic testing identified a novel mutation in the *CYBB* gene that was thought to explain his phenotype (c.941delA). He underwent an HSCT (10/10 HLA matched unrelated donor). Both patients have had normalization of their NOBI post-transplant and remain free of significant infections.

Discussion: We report the clinical presentation, immune features and genetic mutations in 2 patients with novel mutations in the *CYBB* gene causing X-linked CGD. Identifying pathogenic mutations causing CGD is important for a better understanding of genotype–phenotype associations and disease course in this patient population.

Statement of novelty: We describe 2 pediatric patients diagnosed with X-linked chronic granulomatous disease due to novel mutations in the *CYBB* gene.

Introduction

Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency leading to recurrent infections, autoimmunity and chronic inflammation. It is caused by a mutation in any of the 5 structural subunits of the NADPH complex in neutrophilic granulocytes and monocytes, leading to defective reactive oxygen species production necessary for killing bacteria and fungi (Segal et al. 2000). The incidence of CGD varies around the world with estimates from 1:200 000 in the United States, 1:250 000 in Europe, 1:1 000 000 in Italy

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and 1:70 000 in the Israeli Arab population (Winkelstein et al. 2000; Martire et al. 2008; van den Berg et al. 2009; Wolach et al. 2017). While the vast majority of cases are X-linked in nature, rates of autosomal recessive CGD tend to predominate in countries with a high prevalence of consanguinity (van den Berg et al. 2009; Fattahi et al. 2011; Köker et al. 2013; Wolach et al. 2017).

The NADPH oxidase complex is made up of membrane-bound and cytosolic proteins that work together upon phagocyte activation. The 2 cell membrane proteins are catalytic and include glycoprotein gp91^{phox} and non-glycosylated protein p22^{phox}. Together they form a heterodimer known as cytochrome b_{558}. The 3 cytosolic proteins include p47^{phox}, p67^{phox} and p40^{phox}. When phagocytes are activated the cytosolic proteins translocate to cytochrome b_{558} and recruit Rac 1/2 causing a conformational change in gp91^{phox}. This allows NADPH in the cytosol to donate an electron and form superoxide ions which generate further reactive oxygen species and are detrimental to phagocytosed organisms.

The most commonly affected NAPDH subunit in CGD is the transmembrane protein glycoprotein gp91^{phox}, encoded by *CYBB* on the X chromosome, accounting for about two-thirds of CGD cases. Mutations in *NCF1* (p47^{phox}), *CYBA* (p22^{phox}) and *NCF2* (p67^{phox}) are autosomal recessive and account for approximately 20%, 5%, and 5% of cases, respectively (Winkelstein et al. 2000; Jones et al. 2008; Martire et al. 2008; van den Berg et al. 2009; Kuhns et al. 2010). A single case report of an *NCF4* (p40^{phox}) mutation causing CGD has been reported (Matute et al. 2009).

Infections tend to occur in the skin, lymph nodes, lungs and liver and are generally caused by catalase-positive organisms (e.g., *Aspergillus*, *Nocardia*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens* and *Salmonella*) (Segal et al. 2000; Winkelstein et al. 2000; Jones et al. 2008; Martire et al. 2008; van den Berg et al. 2009; Marciano et al. 2015). The rate of invasive fungal infections is higher in CGD than any other primary immunodeficiency with up to 20%–40% of patients affected, primarily occurring in the chest wall and the lungs. The most commonly isolated fungi include *Aspergillus fumigatus* and *A. nidulans* (Beauté et al. 2011; Blumental et al. 2011; Falcone and Holland 2012; Marciano et al. 2015). Patients with CGD can also suffer from immune dysregulation with the gastrointestinal tract (diarrhea, oral ulcers, anal fistulae, vomiting, anorexia, abdominal pain), lungs (dyspnea, granulomata, micronodules, pleural thickening, lymphadenopathy, interstitial lung disease, fibrosis), urogenital tract (hydronephrosis, cystitis, granulomatous orchitis) and eye involvement (chorioretinitis, uveitis, ocular granuloma. Patients are also at risk for autoimmune conditions such as lupus, vasculitis and dermatomyositis (Magnani et al. 2014).

Most patients with CGD are diagnosed before the age of 5, however, it can present at any age from infancy to late adulthood (Jones et al. 2008; Martire et al. 2008; van den Berg et al. 2009). X-linked CGD tends to lead to both an earlier presentation and mortality (Winkelstein et al. 2000; van den Berg et al. 2009). With better disease recognition and management there has been a significant improvement in life expectancy with recent studies reporting a survival rate of as high as 90% at 10 years of age (Kuhns et al. 2010). Previous mortality rates had been reported as high as 5% annually for X-linked CGD and 2% annually for autosomal recessive CGD (Winkelstein et al. 2000).

Improvements in mortality are thought to be related to the use of oral antifungal medications, earlier disease recognition, better antibiotic prophylaxis, the use of interferon-gamma and treatment with hematopoietic stem cell transplantation (HSCT) (Kuhns et al. 2010). Studies have shown mixed results for the use of interferon-gamma supplementation for infection prophylaxis and its use varies worldwide (The International Chronic Granulomatous Disease Cooperative Study Group 1991; Marciano et al. 2004; Martire et al. 2008). The only curative treatment for CGD remains allogenic HSCT, with the potential to prevent inflammatory and infectious complications altogether. Many issues remain however, including graft failure, complications from infections developed prior to transplant and organ dysfunction (Arnold and Heimall 2017). Unfortunately, the median age of death continues to be around age 30–40 with worsening quality of life over time (Jones et al. 2008; Martire et al. 2008; van den Berg et al. 2009).

We report on 2 patients with novel mutations in the *CYBB* gene causing X-linked CGD who have undergone HSCT. To our knowledge these mutations have not been previously described in the literature.

Case report

Case 1

A 14-year-old male was diagnosed with X-linked CGD at 3 years of age when he was hospitalized for disseminated aspergillosis that initially presented as a left axillary lymphadenitis, left wrist abscess and likely pulmonary involvement with nodules. He is the product of an in vitro fertilization with a frozen embryo. He was found to have an abnormal neutrophil oxidative burst index (NOBI) of 1.04 (normal range 32–300) and suspicions for CGD were subsequently confirmed with genetic testing (results described below). He was started on antimicrobial and antifungal prophylaxis. The patient underwent allogenic HSCT at 4 years of age using an HLA-identical sibling donor with normalization of his NOBI at 300. His transplant course was relatively unremarkable.

From an infectious standpoint, prior to his aspergillus infection, he had a history of a salivary gland infection that did not respond well to antibiotic therapy requiring partial excision and long-term antibiotic therapy. He had otherwise been well.

At the age of 10 he developed worsening headaches and was found to have an intra-cranial rhabdomyosarcoma, embryonal subtype. He underwent surgical resection, radiation and chemotherapy. He developed hypothyroidism as a consequence of his cancer treatment and was started on levothyroxine replacement. He was noted to have multi-focal bronchiectasis on chest CT at the age of 13. Since then he has been well with no significant medical concerns. He remains free of cancer recurrence more than 6 years from diagnosis.

Genetic testing

Sequence analysis (*CYBB* Gene/X-linked CGD, GeneDx) revealed a novel mutation in the *CYBB* gene denoted as c.833_87dupGTGGT or p.Val296ValfsX19. Specifically a hemizygous duplication of 5 nucleotides was identified in exon 8 with a the following sequence GTGGT[dupGTGGT]CATC (duplicated bases in brackets). This duplication causes a frameshift mutation starting with codon Val296 and leads to a premature Stop codon at position 19 of the new reading frame denoted V296VfsX19. The presence of the c.883_87dupGTGGT mutation was felt to be consistent with the clinical diagnosis in the patient.

Case 2

A 4-year-old male was diagnosed with X-linked CGD at 4 months of age. He initially presented to hospital with a perianal abscess that required drainage. He was not hospitalized and did not require antibiotics at that time. At 2 months of age he developed a high fever with no clear focus. A full septic work-up showed Moraxella nonliquefaciens bacteremia and a positive nasal swab for Bocavirus and Rhinovirus. He had 2 abnormal NOBIs at the time. His mother's NOBI was suggestive of carrier status (65% normal activity). Both his full-brother and half-sister were found to have a normal NOBI. He was started on antifungal and antimicrobial prophylaxis. Genetic testing confirmed the suspected diagnosis (results described below).

A living unrelated 10/10 HLA matched donor was identified and he underwent HSCT at 18 months of age. His NOBI post-transplant was robust at 300. There were no unexpected complications during his transplant course. He has been well post-transplant with no significant issues and has otherwise been well.

Genetic testing

Sequence analysis (*CYBB* Gene/p91 Protein/X-linked CGD, GeneDx) showed a novel mutation in the *CYBB* gene denoted as c.941delA or p.Lys314ArgfsX29. Specifically, a frameshift mutation starting with codon Lysine 314 changes the amino acid to an Arginine residue and leads to a premature Stop codon at position 29 of the new reading frame (deleted sequence in braces AAGA{A}GAAG). This mutation is predicted to lead to loss of normal protein function by truncation of a protein or non-sense mediated mRNA decay. The presence of this mutation was thought to be consistent with the diagnosis of CGD in this patient.

Discussion

CGD is a primary immunodeficiency caused by a mutation in any of the 5 genes that encode the NADPH oxidase system subunits. CGD can present from infancy to adulthood which is reflective of the heterogenous nature of the disease. Patients with X-linked CGD were previously thought to present earlier, have a more aggressive disease course and earlier age of death than patients with autosomal recessive CGD. More recent publications, however, suggest that certain autosomal recessive forms may also have a relatively severe course (Vignesh et al. 2017). Residual NADPH

activity has been thought to be reflective of the clinical course and likelihood of survival for a patient (Roos and de Boer 2014).

Given the wide spectrum of disease, variability in clinical manifestations and different genes that can be implicated in CGD, a better understanding of the association between the underlying genetic mutations and phenotypic manifestations is important.

Conflict of interest

We have no conflict of interest to declare.

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