Case Report

Variant of X-Linked Chronic Granulomatous Disease Revealed by a Severe *Burkholderia cepacia* Invasive Infection in an Infant

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by increased susceptibility to bacteria and fungi since early in life, caused by mutations in any of the five genes coding for protein subunits in NADPH oxidase. X-linked variant CGD can be missed during routine evaluation or present later in life due to hypomorphic mutations and a residual superoxide production. The case of a 10-month-old boy who died of pneumonia is reported. The isolation of *Burkholderia cepacia* from his lung, together with a marginally low nitroblue tetrazolium reduction assay (NBT), made us suspect and pursue the molecular diagnosis of CGD. A postmortem genetic analysis finally demonstrated CGD caused by a hypomorphic missense mutation with normal gp91phox expression. In a patient being investigated for unusually severe or recurrent infection, a high index of suspicion of immunodeficiency must be maintained.

1. Introduction

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency that affects microbial killing by phagocytes, resulting in bacterial, fungal, and/or mycobacterial infections since early life [1, 2]. The superoxide production by NADPH oxidase is markedly reduced or absent due to mutations in any of the five genes coding for protein subunits of the enzymatic complex [3]. Mutations in CYBB, coding for gp91phox, result in the most common X-linked CGD (65%–70% of all cases) [4]. Hypomorphic mutations (Xgp91+ and Xgp91−) may result in X-linked variant CGD [5, 6]. Patients with variant CGD express the gp91phox protein and produce decreased but detectable superoxide, which allow the defect to manifest later in life with a milder history of infections. By far, the most common micro-organisms causing infections in CGD are *Staphylococcus aureus* and *Aspergillus* species; other agents include *Pseudomonas, Serratia, Salmonella,* and *Candida* species. *Burkholderia cepacia* infection is frequently associated to CGD diagnosis (6–8). Here, we present the case of a patient who died of *Burkholderia cepacia* lung infection, in whom the diagnosis of X-CGD could only be attained postmortem due to residual superoxide production and normal protein expression.
2. Case Report

A 10-month-old boy, the first child of nonconsanguineous parents living in the Tahiti archipelago (French Polynesia), was referred for severe pneumonia. The father is from Europe and the mother is from Oceania; there was no relevant family history. During the first months of life, the patient had experienced some infections, mostly of the upper airways, as well as bronchitis and diarrhea. He received all the immunizations according to his age (including BCG) with no adverse events. He developed a failure to thrive at the age of 3 months. One month before admission he had a severe lung infection with fever, cough, dyspnea, and diarrhea, unresponsive to an empiric oral macrolide (josamycin). Upon admission to his local hospital, he had fever (39.5°C), mild respiratory distress, and crackles on auscultation. Oxygen saturation was 95% in room air. Complete blood count (CBC) reported marked leukocytosis (36,600/mL) with neutrophilia (29,000 polymorphonuclear cells (PMN)/mL) and anemia (Hb = 7.6 g/dL); serum immunoglobulin levels were as follows: IgG = 1,900 mg/dL (reference value for 7–12 months: 661 ± 219 mg/dL), IgA = 166 mg/dL (37 ± 18), IgM = 220 mg/dL (54 ± 23), and IgE 43 IU/mL (normal < 20 IU/mL). Chloride sweat test and tuberculin skin test were negative. Chest X-ray and computed tomography scan (CT) revealed bilateral pneumatosis with multiple bullous lesions and opacification of the left lung; CT scan revealed extensive destruction of the lungs with involvement of the right lung, with hyperaeration, and consolidation and ground glass opacities. Intravenous (IV) rifamycin and trimethoprim/sulfamethoxazole was started, followed by fMLF (formyl-methionyl-leucyl-phenylalanine) stimulation. PMNs from the patient produced detectable but low H2O2 (Figure 1(c)).

Genomic sequencing of CYBB revealed a hemizygous A > G substitution in exon 9, generating the replacement of a histidine by an arginine residue (H338R) in the FAD binding domain (FADB), a probably damaging substitution according to the PolyPhen-2 prediction website (http://genetics.bwh.harvard.edu/pph2). The patient’s mother was heterozygous, and his brother (born after the patient’s death) was hemizygous for the mutation. The mutation was confirmed also in cDNA from the patient (c.1013A > G). We investigated the molecular basis of the germline H338R mutation through detection of flavocytochrome b558 expression by flow cytometry, using the monoclonal antibody 7D5 (MLB, Nagasaki, Japan), which recognizes residues166IKNF163 and 226 RIVRC230 on gp91phox in the presence of p22phox. Protein expression in Epstein-Barr virus transformed B cells (EBV-B cells) from the patient was similar to the healthy control (Figure 2).

3. Discussion

The isolation of Burkholderia cepacia from lung secretion or blood of a previously healthy patient is strongly suggestive of CGD. Aside from it, lung infections caused by Burkholderia species can be seen in patients with existing bronchiectasis (lung epithelial damage is a prerequisite for Burkholderia invasiveness), including notably patients with cystic fibrosis [7] and in some immunocompromised and hospitalised patients [8, 9]. In a child being investigated for recurrent infections, isolation of Burkholderia should always raise the suspicion of CGD [10–12]. For some patients with normal gp91phox expression and residual superoxide production as measured by conventional assays, a milder activation assay with FMLF might be needed to demonstrate low ROS production.
Figure 1: Continued.
Figure 1: NADPH oxidase activity evaluation in PMNs. (a) Superoxide generation was measured by assaying superoxide dismutase-inhibitable cytochrome-c reduction in PMNs after adding three doses of PMA (4, 40, and 400 ng/mL), for healthy controls (C+), CGD patient (C−), and our patient (P). (b) Histograms for the flow cytometric analysis of intracellular H₂O₂ production, using the fluorescent 123DHR probe in PMNs from a healthy control (C+), an X-linked CGD patient (C−), the proband (P), and the mother (H), before (NS) and after stimulation with PMA (4, 40, and 400 ng/mL). (c) PMNs from C+, C−, and P were left untreated or treated with TNF-α, IL-1β, and cytochalasin b and then stimulated with fMLF. The results shown are representative of two independent experiments.

Figure 2: Expression of gp91phox in a patient with the H338R CYBB mutation. Immunostaining of cytochrome b₅₅₈ in EBV-B cells from a healthy control (C+), an X-linked CGD patient (C−), and the patient (P). Cell surface staining with mAb7D5 (an antibody specific for the extracellular epitope of gp91phox; solid lines); an isotype IgG1 (dotted lines) followed by staining with an Alexa Fluor 488 goat anti-mouse Ig secondary antibody. The results shown are representative of two independent experiments.

Missense mutations beyond amino acid 309 of gp91phox usually allow normal protein expression but result in null superoxide production. The patient’s residual ROS generation is thus different from the thorough survival analysis by Kuhns et al. [3]. Also, given this infant’s residual superoxide production, a severe course with early demise is surprising.

In conclusion, we identified postmortem a point mutation in a CGD causing gene from a 10-month-old boy who presented with a Burkholderia spp. overwhelming lung infection. X-CGD diagnosis was delayed because of initial normal results. A high index of suspicion for CGD must be maintained in patients with Burkholderia isolates and close to normal values of usual CGD diagnostic tests such as NBT.
An early and accurate diagnosis can lead to genetic counselling, to family screening, and to a timely intervention.

**Abbreviations**

CGD: Chronic granulomatous disease  
PID: Primary immune deficiency  
NADPH: Nicotinamide adenine dinucleotide phosphate hydrogen  
BCG: Bacillus Calmette-Guérin.

**Conflict of Interests**

The authors declare no conflicts of interest.

**Authors’ Contributions**

Saul Oswaldo Lugo Reyes and Nizar Mahlaoui equally contributed to this work.

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