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LETTER TO EDITOR

Disseminated Mycobacterial Disease in a Patient with 22q11.2 Deletion Syndrome: Case Report and Review of the Literature

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To the Editor:

The chromosome 22q11.2 deletion syndrome (Ch22q11.2DS) consists of a heterogeneous group of disorders with overlapping phenotypes that constitute a severity continuum. The most severe form of the disorder, complete DiGeorge syndrome (cDGS), presents with thymic aplasia with immunodeficiency, conotruncal congenital heart defects, and hypoparathyroidism with hypocalcemia [1]. Although considered a hallmark of the disease, thymic aplasia with severe combined immunodeficiency (SCID)-like phenotype is present in less than 1% of the patients affected by Ch22q11.2DS. Most patients exhibit only a mild to moderate decrease in circulating T cells, which respond normally to mitogen stimulation. They are diagnosed with partial or incomplete DiGeorge syndrome (pDGS) [1]. Most patients with cDGS are not diagnosed because of recurrent or severe infections, but due to congenital heart disease or persistent hypocalcemia during the newborn period; however, severe or recurrent infections may become an important health problem a few weeks or months later, and can include opportunistic infections by viruses (e.g., cytomegalovirus), bacteria (e.g., *Pseudomona aeruginosa*), or fungi (e.g., *Pneumocystis jirovecii*) [2]. Patients with pDGS typically do not display severe or recurrent infections.

Despite their predisposition to severe infections, mycobacteria rarely affect patients with cDGS and only a few cases have been reported in the literature to date [3, 4]. On the other hand, several primary immunodeficiencies (PID) affecting cellular immunity confer susceptibility to mycobacterial infections; if in the presence of normal T cell counts, defects of interferon-gamma (IFN-γ) immunity should be considered. Monogenic defects affecting this circuit underlie a group of PID called Mendelian susceptibility to mycobacterial infections (MSMD). MSMD encompass rare clinical conditions characterized by predisposition to infection by weakly virulent mycobacteria, like bacillus Calmette-Guérin (BCG), and non-tuberculous environmental mycobacteria in otherwise healthy individuals [5, 6]. Twelve genes causing MSMD have been described to date, which include 10 autosomal genes (*IFNGR1*, *IFNGR2*, *STAT1*, *IL12B*, *IL12RB1*,

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IL12RB2, *IL23R*, *SPPL2A*, and *IRF8*) and two X-linked genes (*NEMO* and *CYBB*) [5–9]. Other genes, such as *JAK1*, *ISG15*, *TYK2*, and *RORC*, are responsible for syndromic MSMD [6]. The high level of allelic heterogeneity within this group of PID has led to the description of 29 different allelic forms [5, 7, 9].

We present a 12-year-old boy, first born to non-consanguineous Chilean parents, with a past medical history of complete atrioventricular block without congenital heart malformations. He received BCG vaccine at birth without secondary effects. At 4 years of age, he developed coombs-positive autoimmune hemolytic anemia (AIHA), which was possibly drug-induced by clarithromycin, that responded to intravenous immunoglobulin and corticosteroids. Immune work-up at that age showed negative antinuclear antibodies, normal immunoglobulins and T, B, and NK cells. Two years later, he developed chronic immune thrombocytopenic purpura. He had no history of severe or recurrent infectious diseases. Family history was notable for ulcerative colitis, arthritis, psoriasis, and hypothyroidism in his mother, and chronic urticaria in his father.

At age 8 years, he was admitted for fever, pancytopenia, diffuse adenopathy, and hepatosplenomegaly. Spleen ultrasound also revealed multiple small hypoechogenic splenic lesions. Fludeoxyglucose positron emission tomography-computerized tomography (FDG-PET/CT) showed increased FDG uptake in mediastinal adenopathy and hepatosplenomegaly. A bone marrow biopsy showed tuberculous granulomas with acid-fast bacilli (AFB) (Fig. 1). QuantiFERON-TB Gold testing as well as polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* complex and mycobacterial cultures of the bone marrow was negative. Sputum, urine, and gastric aspiration smears and mycobacterial cultures were negative. Tuberculin skin test was not performed due to nationwide stock shortage of PPD at the time of diagnosis. Lymph node biopsy ruled out lymphoma and did not show evidence of mycobacteria. The source of the mycobacteria could not be identified by epidemiologic evaluation and there were no close contacts affected by tuberculosis; thus, we suspected the infection was probably due to non-tuberculous mycobacteria. In addition, despite having the clinical criteria and cytopenias, the patient did not fulfill the criteria for hemophagocytic lymphohistiocytosis. Immunological evaluation showed normal IgG, with low IgA and IgM, undetectable IgE, normal anti-tetanus toxoid titers, and low response to pneumococcal polysaccharide vaccine (40% of serotypes at protective titers). Immunophenotyping revealed normal CD4+ and CD8+ T cell and B cell numbers, reduced naïve CD3+ T cells and increased memory CD3+ T cells, decreased switched and unswitched memory B cells, mildly decreased NK cells, normal PHA-induced CD25+ activation and proliferation, and normal respiratory burst (supplementary Table 1).

Functional studies of the IFN-γ immunity and whole exome sequencing (WES) were performed as described to evaluate for MSMD [10]. Whole blood activation assays showed that IFN-γ production was mildly decreased in response to stimulation with BCG, although not significantly different from healthy controls. This was partially restored by co-stimulation with BCG and IL-12. IFN-γ production induced by PMA/ionomycin was normal. Production of IL-12p40 by patient cells was normal after stimulation with BCG and significantly increased in response to BCG + IFN-γ stimulation (Fig. 1). WES did not reveal any single mutations or copy number variations (CNVs) in genes known to cause PID including isolated or syndromic MSMD. The WES data was further analyzed with the HMZDelFinder algorithm to identify CNVs. Although this algorithm has high sensitivity for homozygous CNVs, it also has high false positive and false negative rates for heterozygous CNVs [11]. During his hospitalization, reevaluation by geneticist prompted suspicion of Ch22q11.2DS due to unilateral hypoplasia of depressor anguli oris, despite atypical immune and cardiac phenotypes. A heterozygous 3 Mb deletion affecting the 22q11.2 chromosome region, not revealed by WES, was detected by multiplex ligation-dependent probe amplification (MLPA).

Mycobacterial infection was suspected and the patient was treated with ethambutol, rifampin, and ciprofloxacin for three months and started on monthly 500 mg/kg intravenous immunoglobulin with complete regression of adenopathy and splenomegaly. Repeat bone marrow biopsies were normal and were negative for AFB or microbiological evidence of mycobacteria. The patient has continued to present intermittent AIHA, thrombocytopenia, and leukopenia, but has not developed any new severe infections.

We report the case of a patient with pDGS presenting with disseminated mycobacterial infection. His clinical and immune phenotype were also notable for hematologic autoimmunity and mild humoral immunodeficiency. Although the latter characteristics are common findings in Ch22q11.2DS, to date, only a few cases of disseminated mycobacterial infections in these patients have been reported, exclusively in those affected by cDGS or presenting with severe T cell lymphopenia [3, 4]. There is a wide spectrum of immune status in Ch22q11.2DS patients, ranging from normal to the more severe clinical phenotypes affecting those with severe lymphopenia and abnormal proliferative responses to mitogens. In line with current literature showing the occurrence of digenic PID, and that opportunistic mycobacterial infections do not commonly affect non-lymphopenic patients with Ch22q11.2DS, we evaluated the presence of additional genetic defects in our patient by WES, which did not show any mutations in the genes commonly involved in MSMD or other potentially suspicious new candidate genes. In addition, this case highlights the limitation of standard WES analysis for detecting CNVs, and reveals the need for new analyses to

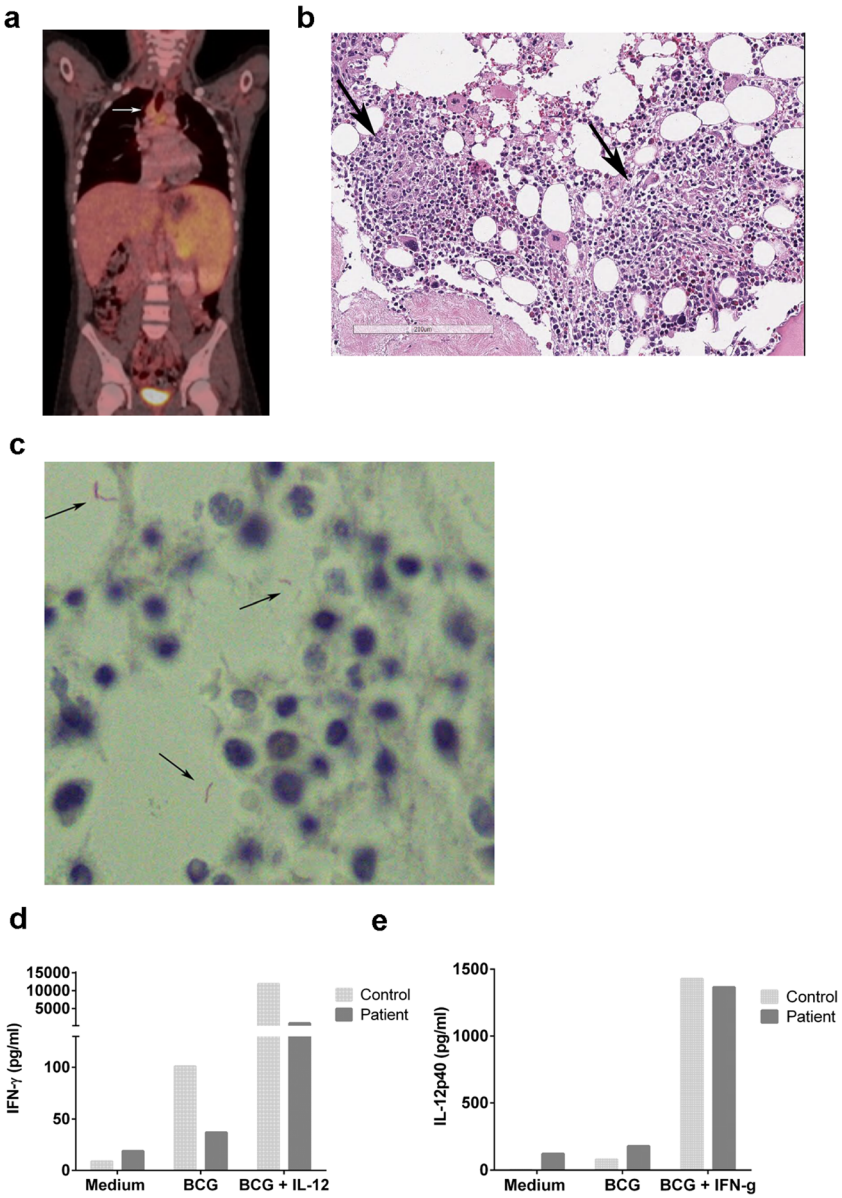

Fig. 1 **a** Fludeoxyglucose positron emission tomography-computerized tomography demonstrating increased FDG uptake in mediastinal adenopathy (white arrow) and hepatosplenomegaly. **b** Bone marrow biopsy (Hematoxylin and Eosin stain, × 20). Arrows indicate two poorly formed interstitial granulomas mainly composed of epitheloid histiocytes with no central necrosis. **c** Bone marrow Ziehl-Neelsen stain (× 100). Arrows indicate few scattered acid-fast bacilli. **d** IFN-γ production in patient and control after whole blood stimulation with BCG and BCG + IL-12. **e** IL-12p40 production in patient and control after whole blood stimulation with BCG and BCG + IFN-γ

detect these by WES, particularly in cases with heterozygous CNVs like our patient's. Combined analyses using other CNV-calling algorithms such as CANOES and CLAMMS may improve in some cases the sensitivity of detection of heterozygous CNVs in WES data [11].

Considering the importance of the integrity of IFN-γ immunity in the immune response against mycobacteria and having ruled out additional PID by sequencing, we functionally tested the patient's response to mycobacteria in vitro and we did not find significant impairment of IFN-γ production of whole blood in patient cells compared with controls. The production of IFN-γ after stimulation with mitogens was normal in our patient, as has been previously reported in Ch22q11.2DS children and adults [12]. Despite the mildly decreased in vitro IFN-γ production, in vivo, this defect seems to behave as a partial deficiency considering the late clinical presentation, the lack of complications following mandatory BCG vaccination, and that the defect can be rescued in vitro by IL-12. Further studies with a larger number of pDGS patients are needed in order to discriminate if this PID is characterized by defective *Mycobacterium*-specific IFN-γ production in Th cells, as we reported in IL-12Rβ1/IL-12Rβ2/IL-23R/ROR-γ deficient patients [9, 13].

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Compliance with Ethical Standards

Approval for the study was obtained from the Scientific Ethics Committee, School of Medicine, Pontificia Universidad Católica de Chile. Informed consent was provided according to the Declaration of Helsinki.

Conflict of Interest The authors declare that they have no conflict of interest.

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