Novel mutations in NCF4 gene confer non-classic chronic granulomatous disease with disseminated histoplasmosis in a Colombian child

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Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by susceptibility to early-onset life-threatening bacterial and fungal infections as well as dysregulated chronic inflammation. CGD results from mutations in one of the components of the phagocyte NADPH oxidase, a multimeric complex that consists of two membrane-bound components (gp91phox and p22phox) and 3 cytoplasmic subunits (p40phox, p47phox y p67phox) that function to induce reactive O2 species (ROS) in phagocytic cells to induce microbial killing. To date, only a single patient with granulomatous colitis and compound heterozygous mutations in NCF4 encoding p40phox has been reported as a genetic subgroup of CGD.

Method: We performed whole exome-sequencing in a patient with early-onset systemic histoplasmosis. Functional testing to Investigate phagocyte NADPH oxidase included dihydrorhodamine oxidation assay as well as amplex red and luminol. Protein expression was assessed by FACS and immunoblotting.

Results: We found a missense homozygous variation in NCF4 within the phox homology (PX) domain, predicted to be damaging by polyphen and SIFT2 with a CADD score of 35. RT-PCR and immunoblotting demonstrated decreased p40phox protein expression protein both in neutrophils and
EBV-transformed B cells from the patient, but not from controls. In addition, intracellular (IC) ROS production was significantly impaired after physiological stimulation with fMLP, *Histoplasma capsulatum* and *Candida albicans* on neutrophils and EBV-B, but not with phorbol 12-myristate 13-acetate (PMA).

**Conclusions:** We report a novel homozygous mutation in *NCF4* selectively impairing IC ROS production in a Colombian child. Remarkably, systemic histoplasmosis has not been previously reported in association with classical CGD, therefore our results expand the spectrum of genetic and infectious diseases underlying CGD in humans.

**Keywords:** NADPH; Whole exome sequencing; NCF4; Chronic granulomatous diseases