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Variants in Nicotinamide Adenine Dinucleotide Phosphate Oxidase Complex Components Determine Susceptibility to Very Early Onset Inflammatory Bowel Disease

Sandeep S. Dhillon,[1,2] Ramzi Fattouh,[1] Abdul Elkadri,[1,2,3] Wei Xu,[4] Ryan Murchie,[1] Thomas Walters,[1,3] Conghui Guo,[1] David Mack,[5] Hien Q. Huynh,[6] Shairaz Baksh,[6] Mark S. Silverberg,[2,7] Anne M. Griffiths,[1,3] Scott B. Snapper,[8] John H. Brumell,[1,2,9] and Aleixo M. Muise[1,2,3]

[1]SickKids Inflammatory Bowel Disease Center and Cell Biology Program, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; [2]Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; [3]Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada; [4]Princess Margaret Hospital and Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; [5]Division of Pediatric Gastroenterology, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; [6] Division of Pediatric Gastroenterology, Stollery Children's Hospital, Edmonton, Alberta, Canada; [7]Mount Sinai Hospital Inflammatory Bowel Disease Group, University of Toronto Group, Zane Cohen Center for Digestive Diseases, Toronto, Ontario, Canada; [8]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Children's Hospital Boston; Division of Gastroenterology and Hepatology, Brigham & Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts; and [9]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

BACKGROUND & AIMS: The colitis observed in patients with very early onset inflammatory bowel disease (VEOIBD; defined as onset of disease at younger than 6 years of age) often resembles that of chronic granulomatous disease (CGD) in extent and features of colonic inflammation observed by endoscopy and histology. CGD is a severe immunodeficiency caused by defects in the genes that encode components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. We investigated whether variants in genes that encode NADPH oxidase components affect susceptibility to VEOIBD using independent approaches. **METHODS:** We performed targeted exome sequencing of genes that encode components of NADPH oxidases (cytochrome b light chain and encodes $p22^{phox}$ protein; cytochrome b-245 or NADPH oxidase 2, and encodes Nox2 or $gp91^{phox}$; neutrophil cytosol factor 1 and encodes p47 ^{phox} protein; neutrophil cytosol factor 2 and encodes p67 ^{phox} protein; neutrophil cytosol factor 4 and encodes p40 ^{phox} protein; and Ras-related C3 botulinum toxin substrate 1 and 2) in 122 patients with VEOIBD diagnosed at The Hospital for Sick Children, University of Toronto, from 1994 through 2012. Gene variants were validated in an independent International Early Onset Pediatric IBD Cohort Study cohort of patients with VEOIBD. In a second approach, we examined Tag single nucleotide polymorphisms in a subset of patients with VEOIBD in which the NOX2 NADPH oxidase genes sequence had been previously analyzed. We then looked for single nucleotide polymorphisms associated with the disease in an independent International Early Onset Pediatric IBD Cohort Study cohort of patients. We analyzed the functional effects of variants associated with VEOIBD. **RESULTS:** Targeted exome sequencing and Tag single nucleotide polymorphism genotyping identified 11 variants associated with VEOIBD; the majority of patients were heterozygous for these variants. Expression of these variants in cells either reduced oxidative burst or altered interactions among proteins in the NADPH oxidase complex. Variants in the noncoding regulatory and splicing elements resulted in reduced levels of proteins, or expression of altered forms of the proteins, in blood cells from VEOIBD patients. **CONCLUSIONS:** We found that VEOIBD patients carry heterozygous functional hypomorphic variants in components of the NOX2 NADPH oxidase complex. These do not cause overt immunodeficiency, but instead determine susceptibility to VEOIBD. Specific approaches might be developed to treat individual patients based on their genetic variant.

Keywords: VEOIBD; CGD; Genetics; Phagocytes.

BASIC AND TRANSLATIONAL AT

Chronic granulomatous disease (CGD) is a severe immunodeficiency caused by genetic defects in components of the NOX2 nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex (Figure 1). The mutations identified in the NADPH oxidase gene in CGD patients mostly result in the complete loss of phagocytes' ability to mount the sufficient respiratory burst required to kill invading pathogens, leading to a severe immunodeficiency with

Abbreviations used in this paper: **CGD, chronic granulomatous disease; CYBA, cytochrome b light chain and encodes $p22^{phox}$ protein; CYBB, cytochrome b-245 or NADPH oxidase 2 and encodes Nox2 or $gp91^{phox}$; GFP, green fluorescent protein; IBD, inflammatory bowel disease; MSMD, Mendelian susceptibility to mycobacterial disease; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium; NCF1, neutrophil cytosol factor 1 and encodes p47 ^{phox} protein; NCF2, neutrophil cytosol factor 2 and encodes p67 ^{phox} protein; NCF4, neutrophil cytosol factor 4 and encodes p40 ^{phox} protein; PID, primary immunodeficiency; RAC1 and 2, Ras-related C3 botulinum toxin substrate 1 and 2; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; VEOIBD, very early onset inflammatory bowel disease; VEOUC, very early onset ulcerative colitis.**

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