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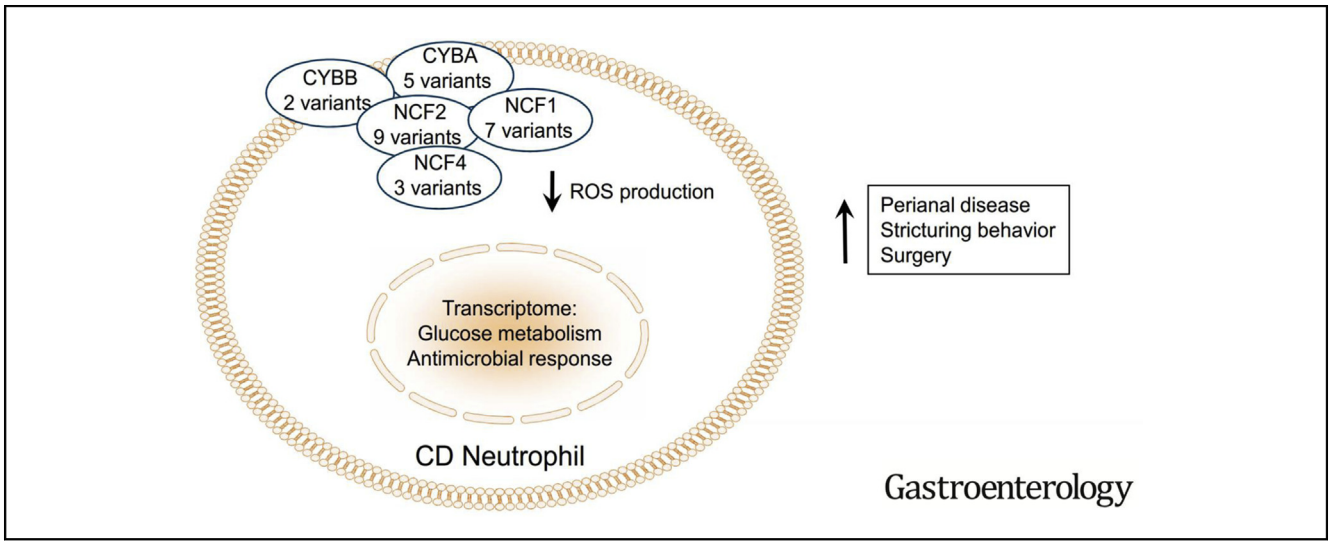
Clinical and Genomic Correlates of Neutrophil Reactive Oxygen Species Production in Pediatric Patients With Crohn's Disease

Lee A. Denson,[1] Ingrid Jurickova,[1] Rebekah Karns,[1] Kelly A. Shaw,[2] David J. Cutler,[2] David T. Okou,[3] Anne Dodd,[3] Kathryn Quinn,[4] Kajari Mondal,[3] Bruce J. Aronow,[5] Yael Haberman,[1] Aaron Linn,[1] Adam Price,[1] Ramona Bezold,[1] Kathleen Lake,[1] Kimberly Jackson,[1] Thomas D. Walters,[6] Anne Griffiths,[6] Robert N. Baldassano,[7] Joshua D. Noe,[8] Jeffrey S. Hyams,[9] Wallace V. Crandall,[10] Barbara S. Kirschner,[11] Melvin B. Heyman,[12] Scott Snapper,[13] Stephen L. Guthery,[14] Marla C. Dubinsky,[15] Neal S. Leleiko,[16] Anthony R. Otley,[17] Ramnik J. Xavier,[18] Christine Stevens,[18] Mark J. Daly,[18] Michael E. Zwick,[2] and Subra Kugathasan[3]

[1]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, University of Cincinnati College of Medicine and the Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; [2]Department of Human Genetics, Emory University, Atlanta, Georgia; [3]Department of Pediatrics, Emory University, Atlanta, Georgia; [4]Cancer and Blood Disease Institute, Department of Pediatrics, University of Cincinnati College of Medicine and the Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; [5]Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; [6]Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Canada; [7]Department of Pediatrics, University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; [8]Department of Pediatric Gastroenterology, Hepatology, and Nutrition, Medical College of Wisconsin, Milwaukee, Wisconsin; [9]Division of Digestive Diseases, Hepatology, and Nutrition, Connecticut Children's Medical Center, Hartford, Connecticut; [10]Department of Pediatric Gastroenterology, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, Ohio; [11]Department of Pediatrics, The University of Chicago, Chicago, Illinois; [12]Department of Pediatrics, University of California at San Francisco, San Francisco, California; [13]Department of Gastroenterology and Nutrition, Boston Children's Hospital, Boston, Massachusetts; [14]Department of Pediatrics, University of Utah, Salt Lake City, Utah; [15]Department of Pediatrics, Mount Sinai Hospital, New York, New York; [16]Department of Pediatrics, Hasbro Children's Hospital, Providence, Rhode Island; [17]Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada; and [18]The Broad Institute of MIT and Harvard, Cambridge, Massachusetts

This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e27. Learning Objective: Upon completion of this CME activity, successful learners will be able to summarize clinical outcomes and differences in neutrophil function associated with carriage of rare NADPH oxidase gene mutations in children with Crohn's disease (CD).



BACKGROUND & AIMS: Individuals with monogenic disorders of phagocyte function develop chronic colitis that resembles Crohn's disease (CD). We tested for associations between mutations in genes encoding reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, neutrophil function, and phenotypes of CD in pediatric patients. **METHODS:** We

performed whole-exome sequence analysis to identify mutations in genes encoding NADPH oxidases (such as *CYBA, CYBB, NCF1, NCF2, NCF4, RAC1,* and *RAC2*) using DNA from 543 pediatric patients with inflammatory bowel diseases. Blood samples were collected from an additional 129 pediatric patients with CD and 26 children without IBD (controls); we performed assays for neutrophil activation, reactive oxygen species (ROS) production, and bacteria uptake and killing. Whole-exome sequence analysis was performed using DNA from 46 of the children with CD to examine associations with NADPH gene mutations; RNA sequence analyses were performed using blood cells from 46 children with CD to test for variations in neutrophil gene expression associated with ROS production. **RESULTS:** We identified 26 missense mutations in *CYBA, CYBB, NCF1, NCF2,* and *NCF4.* Patients with CD who carried mutations in these genes were 3-fold more likely to have perianal disease ($P = .0008$) and stricturing complications ($P = .002$) than children with CD without these mutations. Among patients with CD with none of these mutations, 9% had undergone abdominal surgery; among patients with mutations in these NADPH oxidase genes, 31% had undergone abdominal surgery ($P = .0004$). A higher proportion of neutrophils from children with CD had low ROS production (47%) than from controls (15%) among the 129 patients tested for ROS ($P = .002$). Minor alleles of the NADPH genes were detected in 7% of children with CD whose neutrophils produced normal levels of ROS vs 38% of children whose neutrophils produced low levels of ROS ($P = .009$). Neutrophils that produced low levels of ROS had specific alterations in genes that regulate glucose metabolism and antimicrobial responses. **CONCLUSIONS:** We identified missense mutations in genes that encode NADPH oxidases in children with CD; these were associated with a more aggressive disease course and reduced ROS production by neutrophils from the patients.

Keywords: WES; IBD; Neutrophil Oxidative Burst; Genetic Variant.

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Mutations in genes encoding NADPH oxidases have been linked to neutrophil function and clinical phenotypes in children with very early onset (VEO) IBD. The potential significance of these constituents in older pediatric patients was not known.

NEW FINDINGS

Missense mutations in genes that encode NADPH oxidases were identified in older children with IBD. In children with CD, these were associated with reduced production of ROS by neutrophils and a more aggressive course of disease.

LIMITATIONS

Only a small number of CD patients with mutations in genes that encode NADPH oxidases had neutrophil function studies completed.

IMPACT

This study shows that mutations in genes encoding NADPH oxidases previously linked to VEO IBD also play a role in neutrophil function and disease severity in older pediatric patients.

The development of chronic intestinal inflammation similar to Crohn's disease (CD) during the first decade of life in children with chronic granulomatous disease (CGD) has suggested that loss of function in phagocyte reactive oxygen species (ROS) production is likely to be a fundamental mechanism of pediatric CD pathogenesis.[1–3] CGD patients are notable for predominantly distal colonic and perianal disease, which is often refractory to standard medical approaches.[4,5] Genome-wide association studies of CD have linked pathways including autophagy and ROS production to disease pathogenesis.[6–8] Rare missense mutations in the *CYBB*, *NCF1*, *NCF2,* and *NCF4* genes, which regulate neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function and ROS production, have been reported in children with very early-onset (VEO) (<age 6 years) inflammatory bowel disease (IBD).[9,10] However, to our knowledge, an assessment of the association between NADPH oxidase gene mutations, neutrophil ROS production and gene expression, and clinical phenotype in an older cohort of pediatric-onset IBD patients has not been performed.

A variety of defects in neutrophil function have been described in both pediatric- and adult-onset CD.[9–12] These include reduced adhesion, chemotaxis, phagocytosis, and ROS production. Recent studies have defined the fundamental role that cellular metabolism plays in immune cell function.[13] Neutrophils have been shown to rely critically on glycolysis to mount effective antimicrobial responses.[14–16] Conversely, defects in ROS production in response to immune stimuli have, in turn, been shown to result in impaired glucose metabolism and overall cell function. Although the global pattern of gene expression has recently been defined in circulating neutrophils isolated from healthy adults, to our knowledge this has not been defined relative to neutrophil function, including ROS production in children with IBD.[17]

We hypothesized that rare genetic variants cause neutrophil dysfunction and thereby contribute to disease pathogenesis in pediatric CD. In the current study, we tested for associations between neutrophil ROS production, clinical phenotypes, and rare missense mutations in the core genes that comprise the NADPH oxidase complex. Whole-exome sequencing was performed in pediatric IBD patients, and

Abbreviations used in this paper: **ATP, adenosine triphosphate; CADD, Combined Annotation-Dependent Depletion; CD, Crohn's disease; CGD, chronic granulomatous disease; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; IBD, inflammatory bowel disease; IQR, interquartile range; MAF, minor allele frequency; MFI, mean fluorescence intensity; NADPH, reduced nicotinamide adenine dinucleotide phosphate; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; TNF, tumor necrosis factor; VEO, very early onset; WES, whole-exome sequencing.**

Most current article

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