THE ROLE AND ASSOCIATION OF THE NADPH OXIDASE COMPLEX WITH VERY EARLY-ONSET IBD

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Background: Growing evidence shows that innate immunity plays a significant role in the gastrointestinal tract and has been associated with the pathology of inflammatory bowel disease (IBD). Very early-onset IBD (VEO-IBD), characterized by onset before the age of 10 years, has similar features to the colitis in chronic granulomatous disease (CGD). CGD is caused by defects in the NADPH oxidase complex, resulting in phagocytes not adequately producing reactive oxygen species (ROS) for pathogen clearance. Recently, in a pilot study, our group identified a damaging variant in NCF2, a gene in the NADPH complex, as being uniquely associated with VEO-IBD and resulting in reduced superoxide production. These results demonstrated the importance of the NADPH complex in IBD and introduced the genetic distinction of VEO-IBD.
Purpose: We explored the role of NADPH oxidase genes in VEO-IBD. Variants in these genes were tested for association with this disease. Individual patients with disease-causing variants were identified and studied.
Hypothesis: NADPH oxidase gene variants contribute to the susceptibility to VEO-IBD because of altered oxidative burst mounted by phagocytes.
Methods: 159 VEO-IBD patients and 1000 healthy controls were genotyped for 85 single nucleotide polymorphisms (SNPs) in the NADPH oxidase genes (CYBA, CYBB, RAC1, RAC2, NCF2, NCF4). In addition, 59 VEO-IBD patients were exome sequenced for the NADPH oxidase genes. We found 10 potentially disease-causing SNPs, which were then genotyped in a separate VEO-IBD cohort for association. Replication in a separate VEO-IBD cohort is currently being undertaken. Neutrophils from patients with functional SNPs were studied for altered function.
Results: We found significant associations between a number of NADPH oxidase genes and VEO-IBD. Exploring 85 SNPs in the NADPH oxidase complex, we found RAC1 (rs10951982, $p<0.05$) and NCF4 (rs1883113, $p<0.01$) were associated with VEO-IBD. We also found two SNPs (rs739041, rs1476002) in the RAC2 gene associated with ulcerative colitis under the age of 10 years (<10 UC) ($p<0.01$). Additionally, from the exome sequencing, an intronic RAC1 SNP (rs35761891) was associated with VEO-IBD ($p<0.01$), a promoter SNP in CYBA (rs72550704) was associated with <6 Crohn's Disease ($p<0.01$), and a functional coding NCF2 SNP (rs35012521) was associated with <6 UC ($p<0.05$). A patient, diagnosed with severe UC at 2 years of age, was identified to be compound heterozygous for two damaging, coding SNPs in NCF2 (rs35012521, rs17849502); this patient's neutrophils were found to have highly reduced superoxide-dependent chemotaxis and decreased superoxide production, as measured by NBT.
Discussion: Finding variants in the NADPH oxidase complex associated with VEO-IBD helps characterize the pathogenesis of this disease. Identifying disease-causing variants in this pathway helps develop personalized therapies for patients with severe and complicated disease.