The Role of Inducible Nitric Oxide Synthase Variants in VEO-IBD

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Background: Nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) is expressed after induction by certain cytokines. Colon biopsies have revealed that patients with active IBD have higher amounts of iNOS mRNA and protein expression, compared to controls, in the intestine. iNOS, encoded by NOS2A, has antibacterial effects and is responsible for tissue damage when its expression is uncontrolled. Single nucleotide polymorphisms (SNPs) located in the NOS2A gene associated with increased susceptibility to Crohn's disease (CD) in a previous study. The precise contribution of SNPs in iNOS function has not previously been investigated. The aim of this study is to determine if polymorphisms in NOS2A are associated with very early-onset inflammatory bowel disease (VEO-IBD; diagnosed prior to 10 years of age) and their affect on iNOS activity.
Methods: Goldengate genotyping was used in the first cohort (159 patients with VEO-IBD and 913 healthy controls), followed by the use of Taqman genotyping in a replication cohort (178 patients with VEO-IBD and 478 controls). A NOS2A variant was created using site-directed mutagenesis of a pcDNA3.1 plasmid containing wildtype NOS2A. NO production was measured via Griess assay in Henle-407 cells following transient transfection (24hrs).
Results: Rs2297518 was the only SNP to associate with IBD, CD, and UC in patients under 10 years old. In the combined analysis of the first cohort and replication cohort (337 patients with VEO-IBD and 1391 healthy controls) we found a strong replicated association p (combined) = 3.45×10^{-7} and OR 3.5 (95% confidence interval 2.1 to 6.0).
Conclusions: The rs2297518 SNP may affect iNOS activity because it results in a Ser608Leu substitution which is located in the catalytic domain, suggesting a possible cause for the increased tissue damage and inflammation in patients with IBD. Functional data on this variant is currently being analysed.