

STAT3 Risk Allele Associated with Increased Activation of STAT3 Pathway in EBV-transformed B Lymphocytes from Pediatric IBD Patients

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Introduction: Genome-wide association studies have identified an intronic STAT3 "A" allele linked to increased risk of inflammatory bowel disease (IBD). The STAT3 risk allele is further associated with more severe disease symptoms, decreased time to first interventional surgery and increased incidence of intestinal stricturing and penetrating disease.

Aims: Preliminary studies in the Denson Lab utilizing circulating T cells have determined that the STAT3 risk allele is associated with increased STAT3 activation following IL-6 stimulation and increased levels of GM-CSF auto-antibodies. Our primary aim in this study was to determine the degree of IL-6 mediated STAT3 activation in EBV-transformed B cells from pediatric IBD patients, comparing STAT3 "A/A" risk allele genotype to STAT3 "G/G" wildtype. Additional aims included identifying changes in protein levels associated with the STAT3 pathway (JAK2, GP130, IL6R, SOCS3, SHP-1), STAT1 activation and antibody production (total IgG and GM-CSFab).

Methods: Cytosolic and nuclear proteins were isolated from EBV-transformed B cell lines from 18 pediatric IBD patients (STAT3 risk allele group, "A/A" genotype, n=10; STAT3 wildtype group, "G/G" genotype, n=8). Western blot analysis was performed on cytosolic proteins (STAT3, JAK2, GP130, IL-6R, SOCS3, SHP-1, STAT1) and nuclear proteins (pSTAT3, pSTAT1, SHP-1) using rabbit primary Abs and goat anti-rabbit HRP secondary antibody. β -actin was used as a cytosolic control and TFIIB was used as a nuclear control. Chemoluminescence units were measured using a Fujifilm LAS-4000 Luminescent Image Analyzer. Total IgG and GM-CSFab levels were also measured by core lab facilities at CCHMC from 3 day supernatants from 12-well plates seeded with 10^6 cells.

Results: Western blot analysis: There was a significant increase in activated pSTAT3 in STAT3 "A/A" group vs. STAT3 "G/G" ($p < .05$) following IL-6 stimulation. Levels of pSTAT3 *prior to* stimulation were equivalent in both groups. The STAT3 "A/A" group also showed decreased levels of IL-6 activated pSTAT1 ($p < .01$) and unstimulated cytosolic JAK2 ($p < .05$) when compared to STAT3 "G/G" group. Levels of cytosolic proteins STAT3, STAT1, GP130, IL-6R, SOCS3, and SHP-1 and nuclear protein SHP-1 were equivalent in both groups. **Supernatant antibody titers:** Total IgG and GM-CSFab were equivalent in both groups.

Conclusions: The STAT3 risk allele is associated with preferential activation of the STAT3 (an oncogene) pathway over the STAT1 (a tumor suppressor) pathway. In B cells this may enhance proliferation and maturation creating hyperactive humoral responses to changes in T cell tolerance in IBD patients.

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