IMMUNOLOGIC AND GENETIC MARKERS IN PATIENTS WITH IDIOPATHIC OCULAR INFLAMMATION AND A FAMILY HISTORY OF INFLAMMATORY BOWEL DISEASE

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Short Title: Ocular inflammation and a family history of bowel disease
Introduction

Ocular inflammation is seen in 6-14% of patients with inflammatory bowel disease.\textsuperscript{1-3} Ocular involvement can include conjunctivitis, keratitis, scleritis, episcleritis, uveitis, optic neuritis and, less commonly, retinal vasculitis\textsuperscript{2,4} and can precede gastrointestinal disease.\textsuperscript{5} Uveitis is the most common ocular finding in patients with inflammatory bowel disease and is typically classified as chronic anterior uveitis.\textsuperscript{4}

We have previously reported that the prevalence of a family history of inflammatory bowel disease is three to fifteen-fold higher in patients with ocular inflammation than in the general population.\textsuperscript{6} Additionally, seventy four percent of these patients were HLA-B27 negative, suggesting that HLA-B27 is not an adequate diagnostic marker for patients with only a family history of inflammatory bowel disease and idiopathic uveitis.\textsuperscript{6}

This study was conducted in order to identify immunologic markers specific to inflammatory bowel disease in a cohort of patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease. Additionally, we assessed the presence or absence of single nucleotide polymorphisms in the NOD2 gene (also known as CARD15), which is known to be involved in the heritable aspect of Crohn disease.\textsuperscript{7-8}
Methods:

This study was designed as a matched case-control study of patients diagnosed with idiopathic uveitis at the department of ophthalmology and visual sciences at the University of Illinois at Chicago (UIC). A retrospective chart review encompassing all patients seen at the UIC uveitis clinic between 1995 and 2010 was conducted to identify patients with a history of idiopathic uveitis. A diagnosis of idiopathic uveitis was made in patients with ocular inflammation in which the diagnostic workup did not result in a known etiology. Workup included rapid plasma reagin (RPR), Fluorescent Treponemal Antibody absorption test (FTA-abs), chest radiograph, angiotensin converting enzyme, and serum lysozyme, as well as other testing based on the patient’s history, review of systems and examination findings. Patients with acute anterior uveitis were also tested for HLA-B27. Those with a positive result were excluded from the study.

Patients with idiopathic ocular inflammation and a family history of a first, second, or third degree relative with inflammatory bowel disease without a personal history of inflammatory bowel disease were recruited and enrolled in this study. First degree relatives were defined as parents, siblings, or children; second degree relatives included aunts and uncles, and third degree relatives included cousins or grandparents. Age, gender and race matched control patients were recruited with a diagnosis of idiopathic uveitis without a personal or family history of inflammatory bowel disease. Age match was defined as chronological age within 10 years of a test subject. Informed consent was obtained from all recruited participants. Clinical evaluation included a medical history, review of systems and ophthalmic examination. Patients underwent standard venipuncture phlebotomy to obtain serum and anti-coagulated whole blood samples.

Sample size estimates were based on prevalence studies of p-Anti-Neutrophil Cytoplasmic Antibodies (p-ANCA) and anti-Saccharomyces antibodies in inflammatory bowel disease patients. Studies report a range of 40-80% of ulcerative colitis patients testing positive for p-ANCA, while 60-70% of tested Crohn disease patients had elevated anti-Saccharomyces titers. First-degree relatives of those with ulcerative colitis have been reported to have p-ANCA prevalence rates of 15-30%, while anti-Saccharomyces antibody prevalence amongst first-degree relatives with Crohn disease ranges from 20-25%. Normal population controls show rates of 0-5%, while studies of “diseased” controls with undefined colitis reveal rates up to 10% for both p-ANCA and anti-Saccharomyces antibodies. As this study uses diseased controls, sample size calculations were based on an assumption of 10% prevalence amongst controls without a positive family history and assumed a prevalence of 25% amongst cases with positive family histories. Sample size calculations to detect an odds ratio of 5 with a study powered to 80% with a one-way level of significance of 5% required 31 pairs (12 discordant pairs). However, given limited recruitment due to low disease prevalence, the study as presented was powered at 80% with a one-way level of significance of 5% to detect an odds ratio of 8 based on the above assumptions of prevalence.

Immunologic Studies

A single vial of serum was sent to Prometheus Laboratories (San Diego, CA) for analysis using the Prometheus IBD Serology 7®. Prometheus IBD Serology 7®
assesses the following immunologic markers by Enzyme-Linked Immunosorbent Assay (ELISA): anti-Saccharomyces IgA, anti-Saccharomyces IgG, anti-OmpC IgA, anti-CBir1 antibodies, and p-ANCA antibodies with additional testing for DNAse sensitivity and indirect immunofluorescence (IFA) perinuclear staining pattern. Inflammatory bowel disease-specific p-ANCA is determined by DNAse sensitivity and indirect immunofluorescence (IFA) perinuclear staining.

The results of these assays are imputed into a proprietary diagnostic algorithm allowing for a “laboratory predicted serology” versus “laboratory non-predicted serology” result, assessing the risk of having either ulcerative colitis or Crohn disease. Prometheus IBD serology 7® is marketed as having 74% sensitivity and 86% specificity for use as a diagnostic test for inflammatory bowel disease. The specificity for each individual bowel disease, ulcerative colitis and Crohn disease, is slightly higher at 93% and 92%, respectively (unpublished internal data, Prometheus Labs, San Diego, CA).

Sequencing Analysis
Fresh, anti-coagulated whole blood was sent to Oregon Health & Science University (OHSU) where it was processed to extract genomic DNA by standard procedures. Polymerase chain reaction (PCR) was performed using primers designed to amplify appropriate regions of exons 4, 8 and 11 of NOD2. Direct sequencing of the PCR products was obtained in both directions. Four SNPs were genotyped: rs2066842 (C>T, encoding P268S, single-letter amino acid abbreviations and position), rs2066844 (C>T, encoding R702W), rs2066845 (G>C, encoding G908R), and rs5743293 (a C insertion causing a frame-shift resulting in premature termination: 1007fs).

Statistical Analysis
A matched analysis was performed using McNemar’s testing with exact P values calculated. One-sided P value testing was performed as the hypothesis tested was greater correlation of inflammatory bowel disease markers with those patients with a family history of disease. One-sided analysis was chosen a priori based on published data supporting the association of a family history of inflammatory bowel disease with the risk of development of ocular inflammation. Comparisons of proportions and matching characteristics were evaluated by Fisher exact testing. Analyses of NOD2 risk alleles comparing test vs. control patient groups were performed with the Fisher exact test and the reported P values are uncorrected for multiple comparisons.
Results:

Fifteen patients with a diagnosis of idiopathic uveitis and a family history of inflammatory bowel disease without a personal history of inflammatory bowel disease were included. Another 15 patients diagnosed with idiopathic uveitis without a family or personal history of inflammatory bowel disease were used as controls. Demographics of both groups are summarized in Table 1. Fourteen patients in each group were of Caucasian descent and one patient of African-American ancestry. The mean age of our test and control groups was similar at 35.8 years (range 8-61) and 35.4 years (range 16-58), respectively. The majority of patients in the test group had an inflammatory bowel disease affected first degree relative (12 out of 15).

The type of uveitis represented in each group is presented in Table 2. Although subjects were not matched as to disease location, there were no statistically significant differences between groups (Table 2).

Of the fifteen test patients, nine patients (60%) had a family history of ulcerative colitis, while six (40%) had a family history of Crohn disease. Four patients (27%) had serology predicting inflammatory bowel disease on the Prometheus IBD Serology 7® as shown in Table 3. All four of these test patients were “UC-predicted” meaning their results indicated positive ulcerative colitis immunologic markers. None of the control subjects had positive Prometheus IBD serology 7®; thus, an odds ratio could not be calculated. However, McNemar’s testing demonstrated a possible association (one-sided P=0.063). Interestingly, two of the patients with predicted ulcerative colitis had a first or second degree relative with Crohn disease while the other two had a first or third degree relative with ulcerative colitis. No patient developed ulcerative colitis during follow-up, although three of the four “UC predicted” patients had follow-up of less than one year, while one patient has been followed for five years. None of the patients in this series developed Crohn disease, with follow-up ranging from less than 1 month to 5 years (median 1.75 years).

Further analysis of individual immune markers revealed an association with p-ANCA antibodies. Eight test patients (53%) had an elevated p-ANCA antibody ELISA compared to three control patients (20%) (one-sided P=0.040). Matched analysis resulted in an OR=6.0 (one-sided P=0.063). All four test patients with ulcerative colitis predicted results were positive for inflammatory bowel disease-specific p-ANCA, as determined by DNase sensitivity and IFA perinuclear staining. No controls had positive inflammatory bowel disease specific p-ANCA (Table 3).

Anti- Saccharomyces serologies were negative in all test patients and were observed to be positive in only one control patient.

Ten test patients and twelve control patients were evaluated for four SNPs of the NOD2 gene. Three of these SNPs (R702W, G908R, and 1007fs) are highly associated with Crohn disease and one (P268S) has some evidence of disease association. All four variants encode changes to the resultant peptide. Our test population and control populations each demonstrated one individual who was positive for the R702W
substitution, with all other patients exhibiting the major allele. Similarly, one test patient exhibited the SNP encoding G908R, while no control patients carried this variant. While not considered a major risk allele for Crohn disease, the SNP encoding P268S was also examined and found to be present in 4 individuals of the test cohort (one was homozygous) and in 6 individuals of the control cohort. The distribution of each NOD2 SNP was not statistically different between the test and control groups (Table 4). Furthermore, the overall minor allele frequencies of each SNP in the uveitis patients in this study are within reported ranges of other Caucasian cohorts (data not shown), so a skewed association with the uveitis phenotype appears to be unlikely.
Discussion

While the etiology of inflammatory bowel disease is still largely unknown, the currently accepted view centers around a combination of environmental, genetic and immunologic factors which results in intestinal inflammation in pre-disposed individuals. An imbalance in these factors results in an inappropriate immune response to normal intestinal microflora present in the gut lumen.

Ocular inflammation is seen in approximately 4% of ulcerative colitis patients and up to 6-13% of Crohn disease patients. Vavricka et al. examined a cohort of 950 patients with inflammatory bowel disease and found that uveitis could be present in the absence of active intestinal disease. It is also known that ocular inflammation can present as an extra-intestinal manifestation of inflammatory bowel disease prior to its diagnosis.

Banares et al. performed ileocolonoscopy on 27 consecutive patients with anterior uveitis to assess for microscopic evidence of bowel inflammation. Histologic examination of biopsy specimens from the gut lumen revealed chronic intestinal inflammation in 66% of patients with uveitis, the majority of whom were asymptomatic for bowel complaints. The risk of intestinal inflammation was further observed to be higher in patients with HLA-B27 negative anterior uveitis, although this increased risk was based on a small number of patients. Other studies involving patients with ankylosing spondylitis, reactive arthritis and undifferentiated spondyloarthropathy have also demonstrated subclinical pathologic bowel inflammation in the absence of gastrointestinal symptoms.

Banares proposed two theories to explain these findings: one hypothesis centered on a unique underlying systemic process while the other involved activated antigens presenting from gut lesions triggering joint and ocular inflammation. The loss of immune tolerance to resident bacterial flora has become one of the major theories in the pathogenesis of Crohn disease. Further, it has been proposed that spondyloarthropathies are extra-intestinal manifestations of inflammatory bowel disease secondary to the activation of T cells in the gut epithelium that can target joint synovium antigens, become re-activated, and cause joint inflammation. This relationship is also plausible to explain ocular inflammation.

Our findings of a higher rate of p-ANCA and other associated immune markers in patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease are consistent with the observation of a relationship between uveitis and gut inflammation. P-ANCA has been associated with ulcerative colitis and ulcerative colitis-like Crohn disease and, in our cohort, half of test patients had a positive p-ANCA, and half of those were "UC-predicted" based on Prometheus IBD Serology testing.

Nevertheless, the question remains whether the elevation in p-ANCA implies a genetic susceptibility or whether these antibodies develop in response to an environmental exposure such as a gut pathogen. The former is supported by the fact
that family members of inflammatory bowel disease patients are predisposed to the
disease with an increased relative risk and prevalence of these antibodies, and that p-
ANCA is elevated in a host of autoimmune diseases and is not limited to inflammatory
bowel disease.23,27 Supporting the latter hypothesis is the fact that, while p-ANCA and
anti- Saccharomyces antibodies are elevated in ulcerative colitis and Crohn disease
patients, respectively, before the onset of disease, the majority of patients develop the
antibodies after childhood, yet antecedent to the disease.27 Additionally, the frequency
of the development of anti-Saccharomyces antibodies have been demonstrated to peak
36 months prior to the development of Crohn disease.27 Nevertheless, levels of both of
these antibodies have been shown to be stable throughout the disease course and do
not respond to treatment in inflammatory bowel disease, in contrast to other
autoimmune conditions.23 Thus, it is unknown whether these antibodies are secondary
markers of an autoimmune process or truly represent a form of subclinical disease.

This leaves a gap in knowledge in regards to genetic susceptibility. In this study,
two patients with ulcerative colitis-predictive Prometheus IBD Serology had first
degree and third degree relatives with ulcerative colitis, while two patients had first
degree and second degree relatives affected with Crohn disease with negative anti-
Saccharomyces antibody levels. It is to still be determined which genes confer
susceptibility.

This study was also designed to compare carrier rates of NOD2 SNPs between
the test and control groups. The NOD2 protein is involved in bacterial recognition by
immune cells and, a decade ago, polymorphisms in the gene were found to be
associated with inflammatory bowel disease. Numerous studies in both sporadic and
familial cases of Crohn disease have confirmed three polymorphisms, encoding
R702W, G908R and 1000fs, to be independently associated with Crohn disease
susceptibility.23 Ulcerative colitis patients have been examined as well for the above
mutations, but these SNPs were not associated with ulcerative colitis nor were any
other mutations screened on the NOD2 gene. Our study failed to find an association
of uveitis and a family history of inflammatory bowel disease with any of the tested
NOD2 SNPs. However, as these variants are known to be associated with Crohn
disease, not ulcerative colitis, and our immunologic marker testing predicted ulcerative
colitis, not Crohn disease, this result is not surprising. In recent years there have been
several genetic studies which have identified additional genes involved in the genetic
susceptibility to inflammatory bowel disease. We hope to analyze our uveitis
patient population to see if there is an association with any of these more recently
identified mutations.

This study is limited by its number of patients. However, while the sample size
was small, the study was powered to find a large effect size. The odds ratio of 6.0 for
elevated p-ANCA antibodies in the case population is at the borderline of this study’s
power. However, given the large effect size, frequency differential between cases and
controls, level of significance, and biologic plausibility we believe this result to represent
a true association and not one found by chance. Nevertheless, these results should be
interpreted with caution given the small sample size,
Another limitation of the study is that, while patients were matched by age, sex, and race, they were not matched according to the location/type of uveitis, which may leave other confounding factors unaccounted. However, since there were no statistically significant differences between cases and controls with regard to location and type of inflammation (Table 2), we do not think that this is a major limitation.

In conclusion, the aim of this study was to identify immunologic or genetic markers associated with inflammatory bowel disease in our patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease. Our immunologic analysis suggests that 27% of uveitis patients with a family history of inflammatory bowel disease and none of the controls were “predicted” to develop ulcerative colitis, despite an absence of clinically detectable bowel disease. These data suggest that some patients with uveitis previously defined as “idiopathic” may, in fact, have uveitis related to clinically undiagnosed ulcerative colitis, or associated with markers that correlate both with inflammatory bowel disease and isolated uveitis. It is also possible that some of these patients will go on to develop bowel disease consistent with ulcerative colitis, and that the uveitis represents an early extra-intestinal manifestation. Inflammatory bowel disease serologic testing may be useful in the evaluation of selected patients with unexplained uveitis.

Genetic analysis did not reveal any statistical differences in NOD2 variants known to be associated with Crohn disease. Future studies on idiopathic uveitis patients with a family history of inflammatory bowel disease should include additional gene risk studies, including those which are specifically associated with ulcerative colitis.
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Approval for a prospective study involving patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease was obtained from the Institutional Review Boards (IRB) at both the University of Illinois at Chicago and Oregon Health & Science University and was in accordance with HIPAA regulations. We also thank the following research personnel from Oregon Health & Sciences University: Kelley Goodwin and Trudy Doyle for expert technical assistance with NOD2 SNP genotyping, and Carrie Austin for study coordination between the two centers.
References


