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

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Abstract

PURPOSE: To evaluate the prevalence of immunologic and genetic markers in patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease.

DESIGN: Prospective, matched case-control study.

METHODS: Patients with a diagnosis of idiopathic ocular inflammation and family history of inflammatory bowel disease (IBD) who did not have IBD themselves were identified and matched to control patients with idiopathic ocular inflammation without a personal or family history of IBD. Serum was evaluated for immunologic markers using Prometheus IBD 7 Serology[®]. Genomic DNA was analyzed for single nucleotide polymorphisms (SNP) of the NOD2 gene associated with Crohn's disease.

RESULTS: Fifteen patients with idiopathic ocular inflammation and family history of inflammatory bowel disease were matched to fifteen control patients based on age, gender, and race. 8/15 (53%) patients with a family history of IBD had elevated p-ANCA antibody levels compared to 3/15 (20%) of controls (one sided P=0.04) with a matched analysis OR of 6.0 [one-sided P=0.06]. 4/15 (27%) patients with family history of IBD tested positive for immunologic markers predicting ulcerative colitis, while no control patients tested positive [one-sided P=0.06]. Carrier rates of NOD2 SNPs did not differ significantly between the test and control groups.

CONCLUSIONS: One quarter of patients with idiopathic ocular inflammation and a family history of inflammatory bowel disease had immunologic markers predicting IBD, and one half had elevated p-ANCA levels. IBD serology may be useful in the evaluation of selected patients with unexplained uveitis.

Keywords

idiopathic intraocular inflammation; inflammatory bowel disease

Introduction

Ocular inflammation is seen in 6-14% of patients with inflammatory bowel disease (IBD).¹⁻³ Ocular involvement can include conjunctivitis, keratitis, scleritis, episcleritis, uveitis, optic neuritis and, less commonly, retinal vasculitis^{2,4} and can precede

gastrointestinal disease.⁵ Uveitis is the most common ocular finding in patients with inflammatory bowel disease and is typically classified as chronic anterior uveitis.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.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 IBD and idiopathic uveitis.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 IBD. 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's disease.⁷⁻⁸

Methods

This study was designed as a prospective, 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 regain (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 (IBD) without a personal history of IBD were recruited. 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 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-ANCA and anti-Saccharomyces (anti-ASCA) antibodies in IBD patients. Studies report a range of 40-80% of UC patients testing positive for p-ANCA, while 60-70% of tested Crohn's patients had elevated anti-ASCA titers.^{9,10} First-degree relatives of those with UC have been reported to have p-ANCA prevalence rates of 15-30%, while anti-Saccharomyces antibody prevalence amongst first-degree relatives with Crohn's disease ranges from 20-25%.^{11,12} 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-ASCA antibodies.^{11,12} As this study uses diseased controls, sample size calculations were based off 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 IBD Serology 7[®]. Prometheus IBD Serology 7 assesses the following immunologic markers by Enzyme-Linked Immunosorbent Assay (ELISA): ASCA IgA, ASCA IgG, anti-OmpC IgA, anti-CBir1 antibodies, and p-ANCA antibodies with additional testing for DNase sensitivity and IFA perinuclear staining pattern. IBD-specific p-ANCA is determined by DNase sensitivity and IFA perinuclear staining.

The results of these assays are imputed into a proprietary diagnostic algorithm allowing for a “predicted” versus “non-predicted” result, assessing the risk of having either ulcerative colitis (UC) or Crohn’s Disease (CD). IBD 7 serology 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’s 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 higher correlation of IBD markers with those patients with a family history of disease. Comparisons of proportions and matching characteristics were evaluated by Fischer’s Exact-testing.

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 IBD were recruited 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 IBD-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’s disease. Four patients (27%) had a positive

predictive result 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 showed a high probability of association (one-sided $P=0.063$). Interestingly, two of the patients with predicted ulcerative colitis had a first or second degree relative with Crohn’s 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.

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 UC predicted results were positive for IBD-specific p-ANCA, as determined by DNase sensitivity and IFA perinuclear staining. No controls had positive IBD specific p-ANCA (Table 3).

ASCA 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’s disease and one (P268S) has some evidence of disease association.¹³ 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’s 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.¹⁴ An imbalance in these factors results in an inappropriate immune response to normal intestinal microflora present in the gut lumen.^{14,15}

Ocular inflammation is seen in approximately 4% of ulcerative colitis patients and up to 6-13% of Crohn’s disease patients.^{16,17} 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.¹⁶ It is also known that ocular inflammation can present as an extra-intestinal manifestation of IBD prior to its diagnosis.^{2,5}

Banares et al. performed ileocolonoscopy on 27 consecutive patients with anterior uveitis to assess for microscopic evidence of bowel inflammation.¹⁸ 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 complaints¹⁸. 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.¹⁸ 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.¹⁹⁻²²

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.¹⁸ The loss of immune tolerance to resident bacterial flora has become one of the major theories in the pathogenesis of Crohn's disease.²³ Further, it has been proposed that spondyloarthropathies are extra-intestinal manifestations of IBD secondary to the activation of T cells in the gut epithelium that can target joint synovium antigens, become re-activated, and cause joint inflammation.²⁴ 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 IBD are consistent with the observation of a relationship between uveitis and gut inflammation. P-ANCA has been associated with UC and UC-like CD^{25,26} and, in our cohort, half of test patients had a positive p-ANCA, and half of those were "UC-predicted" based on Prometheus 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.²³ The former is supported by the fact that family members of IBD patients are predisposed to the disease with an increased relative risk and prevalence of these antibodies,²⁸ and that p-ANCA is elevated in a host of autoimmune diseases and is not limited to IBD.^{23,27} Supporting the latter hypothesis is the fact that, while p-ANCA and ASCA antibodies are elevated in UC and Crohn's 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 ASCA antibodies have been demonstrated to peak 36 months prior to the development of Crohn's 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 IBD, 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 UC-predictive Prometheus serology had first degree and third degree relatives with UC, while two patients had first degree and second degree relatives affected with Crohn's disease with negative ASCA 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.⁷⁻⁸ Numerous studies in both sporadic and familial cases of Crohn's disease have confirmed three polymorphisms, encoding R702W, G908R and 1000fs, to be independently associated with Crohn's disease susceptibility.²⁹ Ulcerative colitis patients have been examined as well for the above mutations, but these SNPs were not associated with UC nor were any other mutations screened on the NOD2 gene.¹³ Our study failed to find an association of uveitis and a family history of IBD with any of the tested NOD2 SNPs. However, as these variants are known to be associated with Crohn's disease, not ulcerative colitis, and our immunologic marker testing predicted ulcerative colitis, not Crohn's disease, this result is not surprising. In recent years there have been several IBD

genetic studies which have identified additional genes involved in the genetic susceptibility to IBD^{15,30,31}. 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, and level of significance, we believe this result to represent a true association and not one found by chance. 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 IBD. Our immunologic analysis suggests that 27% of uveitis patients with a family history of IBD 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 of IBD. IBD 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's disease. Future studies on idiopathic uveitis patients with a family history of IBD should include additional IBD-risk genes, including those which are specifically associated with UC.

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Table 1

Demographics of test and control patients

	With IBD family history (Test)	Without IBD family history (Control)
Age	mean= 35.8 years (range 8-61)	mean= 35.4 years (range 16-58)
Sex	Males: females = 2:13	Males: females = 2:13
Race	Caucasian: African American: 14:1	Caucasian: African American: 14:1
First Degree Relative	n=12	
Second Degree Relative	n=2	
Third Degree relative	n=1	

Table 2

Type of uveitis in test and control subjects

	Test Patients	Control Patients	P value [§]
Acute Anterior Uveitis	n=4 (27 %)	n= 1 (6 %)	P= 0.16
Chronic Iridocyclitis	n= 3 (20%)	n= 7 (47%)	P = 0.12
Intermediate Uveitis	n= 6 (40%)	n= 2 (13%)	P= 0.11
Scleritis/Sclerokeratitis	n= 2(13%)	n= 4 (27)	P= 0.32
Posterior Uveitis	n=0	n=1 (6%)	P= 0.50

[§] two sided P value calculated using Fisher's exact test

Table 3

Prometheus test results in test and control patients.

	Test Patients	Control Patients	P value [†]
Predicted IBD-UC	n=4 (27%)	n=0	0.063
Predicted IBD-CD	n=0	n=0	
IBD specific pANCA	n=8	n=3	0.063
DNASE sensitivity	n=4	n=0	0.063
Anti-cBir-1	n=4	n=1	0.126
ASCA IgG OR IgA	n=0	n=1	0.999

[†] one-sided exact P-value using McNemar's test (matched analysis)

Table 4

Distribution of N0D2 SNP results in test and control patients.

N0D2 variant	Test Patients (n=10)	Control Patients (n=12)	P value
P268S	4 (40.0) *	6 (50.0)	0.6914
R702W	1 (10.0)	1 (8.3)	1.000
G908R	1 (10.0)	0	0.4545
1000fs	0	0	1.000
At least 1 main risk allele [#]	2 (20.0)	1 (8.3)	0.571

* Number of individuals (percentage); for P268S in the FHx IBD group, one individual was homozygous for the variant allele; for all other data, carriers were heterozygous for the variant allele

[#] R702W, G908R or 1000fs; there were no individuals who carried the main risk alleles as compound heterozygotes