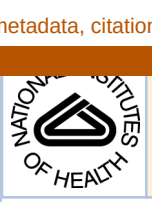

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NOD2 mutations and anti-*Saccharomyces cerevisiae* antibodies are risk factors for Crohn's disease in African Americans

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CONFLICT OF INTEREST

Guarantor of the article: Steven R. Brant

Specific author contributions: Themistocles Dassopoulos was involved in study conception, design and supervision; patient recruitment; data analysis and interpretation; and writing of the first draft. Geoffrey Nguyen was involved in study design, statistical analysis and data interpretation. Monica Vladut Talor and Carol L. Burek performed the ASCA serologies. Lisa Wu Datta performed the genotype analysis and maintained the database. Kim L. Isaacs, James D. Lewis, Michael S. Gold, John F. Valentine, Duane T. Smoot, Mary L. Harris, Maria Oliva-Hemker, and Theodore M. Bayless were involved in patient recruitment. Steven R. Brant was involved in the conception, design, supervision, administrative support and funding support of the study; patient recruitment; and data analysis and interpretation. All authors were involved in critical revision of the paper for important intellectual content.

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The other authors have no potential competing interests.

Abstract

Background—*NOD2* mutations and anti-*Saccharomyces cerevisiae* antibodies (ASCA) are associated with Crohn's disease (CD), ileal involvement and complicated disease behavior in whites. ASCA and the three common *NOD2* mutations have not been assessed in African American (AA) adults with CD.

Methods—AA patients with CD and controls were recruited by the Mid-Atlantic African American IBD Study (Johns Hopkins Hospital and satellite centers at Howard University, University of Florida, University of North Carolina, University of Pennsylvania, and the Washington Hospital Center, Washington, DC) as part of the NIDDK IBD Genetics Consortium. Genotyping for the three common CD associated *NOD2* mutations (Leu1007fsinsC, G908R/2722g>c, and R702W/2104c>t) and ASCA ELISA assays were performed in 183 AA CD patients and 143 controls. Positive ASCA was based on either IgA or IgG above threshold. CD phenotyping was performed using the NIDDK IBD Genetics Consortium guidelines. Logistic regression was used to calculate adjusted odds ratios (OR) for the association between ASCA and disease phenotype.

Results—ASCA sensitivity and specificity in this AA population were 70.5% and 70.4% respectively. On univariate analysis, ASCA was associated with younger mean age at diagnosis (25.0±11.8 vs. 32.1±14.2 yrs, p<0.001), ileal involvement (73.0% vs. 48.0%, p=0.002), and complicated (stricturing/ penetrating) behavior (60.3% vs. 41.7%, p=0.03). On multivariate analysis, ASCA titer (/25U) was associated with ileal involvement (OR 1.18, 95% CI 1.04-1.34), complicated behavior (OR 1.13, 95% CI 1.01-1.28) and surgery (hazard ratio 1.11, 95% CI 1.02-1.21). Risks for surgery also included smoking (hazard ratio 1.50, 95% CI 1.14-1.99) and CD family history (hazard ratio 2.39, 95% CI 1.11-5.14). *NOD2* carriers (all heterozygotes) were more common among CD cases than controls (8.2 vs. 2.1%; OR 4.17, 95% CI: 1.18 - 14.69). *NOD2* mutation population attributable risk was 6.2%.

Conclusions—In comparison to whites, ASCA in AAs has a similar sensitivity but a lower specificity for CD. ASCA is associated with ileal involvement, complicated behavior and surgery in AAs with CD. *NOD2* is a risk gene for AA CD, although mutation frequency and population attributable risk are much lower than in whites.

INTRODUCTION

Crohn's disease (CD) is a chronic, inflammatory bowel disease (IBD) that primarily involves the ileum and/or colon. Underscoring the phenotypic heterogeneity of CD, previous studies have identified CD phenotypes with distinct clinical features (age at diagnosis, site of involvement, and family history), complications (inflammatory, stricturing and/or penetrating behavior) and need for surgery (1-4).

Three *NOD2* mutations (Leu1007fs/3020insC [Leu1007fsinsC], G908R/2722g>c, and R702W/2104c>t), found commonly in all European ancestry populations (7 to 19% carriers in controls vs. 16 – 45% in CD cases in hospital based studies) were the first identified and remain the highest penetrance genetic risk factor for CD in white populations (5-7). *NOD2*-associated overall risk was 2.4-fold for simple heterozygotes and 17-fold for homozygote/compound heterozygote mutation carriers(7). *NOD2* mutations have also been associated with specific CD phenotypes: younger age at disease onset (8-11), ileal involvement (8, 9, 11-15), and complicated (stricturing and penetrating) behavior (8, 10-12, 16, 17). Interestingly, expression of antibodies to *Saccharomyces cerevisiae* (ASCA) has been associated with a CD phenotype that is remarkably similar to that of patients with *NOD2* polymorphisms. ASCA seropositive patients present at a younger age (18-23) and are more

likely to have ileal disease (18-25). They are also at greater risk for complicated disease behavior (18, 21, 24-27) and require surgery more frequently (21, 24, 26, 28).

Our group reported a dose-response between the number of *NOD2* mutations and the prevalence and titers of ASCA (18), a finding that was also reported in studies from Italy (11) and Hungary (29). Importantly, we also found that ASCA titer was associated with ileal involvement and with complicated behavior, *independently* of *NOD2* status (18).

To date, there have been no studies of ASCA in the African-American (AA) population. The only study of *NOD2* in AAs was in children and found that *NOD2* alleles were infrequent in AA children with CD in comparison to white children with CD (allele frequency 1.6% vs. 25.7%, $p < 0.0001$) (30). *NOD2* mutations showed equal frequencies among 58 AA children with CD relative to 124 controls (heterozygote carriers 3.8% vs. 4.3%, respectively). However, the study was not adequately powered to assess *NOD2* risk given the low frequency of *NOD2* mutations and low risk of heterozygotes, and controls were taken from newborn screening dried blood spots. We and others have observed that AA patients with CD have lower frequencies of an IBD family history (30-34) and ileal involvement (31, 35) as compared to white CD patients. In view of the biologic and genetic heterogeneity of CD and the differences in CD phenotype in AA patients, we performed a study to characterize ASCA and the three common *NOD2* mutations in CD in the AA population.

METHODS

Patient Population

The study population consisted of 334 individuals, self-identified as AA and recruited between 2003-2007 by the Mid-Atlantic African-American Inflammatory Bowel Disease Study (MAAAIS), a sub-study of the Inflammatory Bowel Disease Genetics Consortium (IBDGC)(31). There were 190 unrelated patients with that carried a diagnosis of CD, and 144 matched, healthy controls. Blood for DNA purification and sera were successfully collected from all study subjects. We included all study subjects available at this time. MAAAIS is coordinated through the Meyerhoff IBD Center at Johns Hopkins University with recruitment from the Meyerhoff IBD Center clinics, the Johns Hopkins Hospital and from satellite recruitment at the IBD centers at Howard University, University of Florida, University of North Carolina, University of Pennsylvania, and the Washington Hospital Center, Washington, DC. Subjects were also referred to the Johns Hopkins coordinating site by their gastroenterologists in the state of Maryland or by the Maryland, Washington and Northern Virginia Chapter of the Crohn's and Colitis Foundation of America (CCFA), or were self-referred after learning about the study from IRB-approved advertisements for AA patients and AA healthy controls in Baltimore area newspapers. Healthy controls were the non-genetically related spouse, partner or friend of the case, and were without a known personal or family history of IBD. The controls were recruited by each center primarily by referral from cases. When cases were unable to refer a control, population controls living in the same geographic region (i.e. identical first 3 digits of mail "zip" code) and matched by age and sex were also recruited. An additional 26 CD cases and 13 controls were also included that were unrelated AA participants of the Meyerhoff IBD Center genetics research studies between 1997 and 2003 and that had both sera and DNA.

AA ethnicity was self-defined. In addition, as part of a separate study, we had genotyped 96 African/European admixture informative markers with comparison to West African and European controls, and average ethnicity was identical between cases and controls (78.9% vs. 80.2% average West African ancestry, respectively)(36). One of the controls had 98% European ancestry and was excluded. None of the remaining participants had less than 28%

West African ancestry. Diagnosis of CD was confirmed by chart review, in accordance with the NIDDK IBDGC requirements (31).

DNA samples were successfully isolated in 100% of study subjects. DNA was purified from blood by use of a Gentra Puregene kit (Qiagen, Valencia, CA). Samples from cases and controls were plated for genotyping or serotyping together, and laboratory personnel were blinded as to disease status. In each genotyping plate, water controls were used to control for cross sample contamination and samples of known genotypes and duplicate samples (placed in wells on two different plates), were placed as negative and positive controls.

Phenotyping

CD phenotyping was performed using a previously validated protocol and in accordance with the Montreal classification(37, 38). Age at diagnosis was determined by chart review and if necessary by patient questionnaire. For disease location, patients were classified into one of four mutually exclusive groups: 1) L1: ileum involvement only; 2) L2: colonic involvement only; 3) L3: ileocolonic involvement; and 4) L4: isolated upper gastrointestinal involvement. Disease location was determined as maximal extent of the disease. As specified in the Montreal classification, perianal and rectovaginal fistulae did not, by themselves, constitute penetrating disease. History of surgery was confirmed bowel resection or diversion for treatment of CD or complications. History of immunomodulator use was defined as any history, captured in the medical records or reported by the patient, of azathioprine, 6-mercaptopurine or methotrexate use. History of infliximab use was infliximab therapy as reported in the medical records or by the patient. Family history of CD was defined as CD in a 1st or 2nd degree relative (e.g., parent, sibling, grandparent, or avuncular relative). Current smoking was defined as consumption of a minimum of 7 cigarettes per week for at least 1 year.

NOD2 Genotyping

Patients were genotyped for Leu1007fsinsC and G908R/2722g>c polymorphisms using the TaqMan method as described (39). R702W/2104c>t status was determined by PCR amplification of *NOD2* exon 4, column purification and direct sequencing.

ASCA

Sera were analyzed for the presence of *S. cerevisiae* IgG and IgA antibodies by a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications (QUANTA Lite, INOVA Diagnostics, San Diego, CA). The test result is calculated by multiplying the ratio of sample to control optical density (OD) with the number of units assigned to the control by the manufacturer. Reactivity is related to the quantity of antibody present in a nonlinear fashion. Results are classified as negative (0.0 –20.0 Units, U), equivocal (20.1–24.9 U), and positive (≥25 U). Our ASCA assay correlates well with the assay of Prometheus Laboratories (San Diego, CA), which is the most commonly used assay in the United States (40).

Statistical analysis

Statistical analysis was performed with the Stata 10 (College Station, Texas, USA). Patients were considered $ASCA^+$ if they were ASCA IgA-positive ($ASCA\ IgA^+$) and/or ASCA IgG-positive ($ASCA\ IgG^+$). Since stricturing and penetrating behaviors represent more complicated disease that almost always necessitates surgery, we analyzed jointly these patients, as we also did in our prior study of white CD patients (18).

Patient genotypes were divided into three groups: "MM," patients carrying two *NOD2 mutant* alleles (homozygotes or compound heterozygotes); "MW," those carrying a single

NOD2 mutant allele and a single wild type allele; and "WW," those carrying only wild type alleles.

The prevalences of ASCA in the CD and control populations were compared using the chi-square statistic. Characteristics of $ASCA^+$ and $ASCA^-$ patients were compared using the Wilcoxon ranksum test for continuous variables, and the chi-square or Fisher's exact test for nominal categorical variables. The prevalences of *NOD2* mutations and *NOD2* allele frequencies in CD cases and controls were compared using the Fisher exact test. Population attributable risk (PAR) in AA was calculated as per Schlesselman (41): PAR = [Pe*(OR-1)] / [Pe*(OR-1) + 1], where Pe is the prevalence of exposure (*NOD2* mutation) in the population, and OR is the odds ratio for CD and any *NOD2* mutation..

The independent association of ASCA titer and other factors with the presence of ileal involvement and stricturing/penetrating behavior was evaluated with logistic regression adjusting for disease duration, age at diagnosis, family history, and smoking at time of diagnosis. We used Cox proportional hazards to assess the association between ASCA titer and time to surgery while adjusting for the same covariates used for the logistic regression models. Disease behavior was not included in the model for disease site, since disease behavior evolves over time. Disease behavior was also not included in the model for time to surgery, since disease behavior is frequently determined near or at the time of surgery.

Ethical Considerations

The Institutional Review Boards of the Johns Hopkins Medical Institutions, the University of North Carolina, the University of Pennsylvania, Washington Hospital Center, the University of Florida and Howard University approved the study and informed consent was obtained from all patients.

RESULTS

Patient characteristics

Among the 190 unrelated AA patients recruited who carried a diagnosis of CD based on their physicians' assessment, our review of primary medical records confirmed a diagnosis of CD in 183 cases (with 1 case reclassified as indeterminate colitis). After exclusion of one control who had less than 15% West African ancestry, there were 143 controls. The characteristics of these studied CD cases and controls are presented in Table 1. Mean (SD) age of patients at diagnosis was 27.1 (12.9) years. A family history of IBD was observed in 11.5% of patients. Ileal involvement (isolated ileal or ileocolonic) was seen in 66.0%. Disease behavior was inflammatory in 45.0%, stricturing in 30.8%, and penetrating in 24.2%. Immunomodulators and infliximab were used in 62.8% and 42.8% of patients respectively.

ASCA

Among the 183 AA patients with CD, 129 (70.5%) were $ASCA^+$. ASCA data were available for 142 controls. Among the controls, 100 were $ASCA^-$, yielding an ASCA specificity for CD of 70.4%. Age at diagnosis was significantly lower in $ASCA^+$ patients (25.0±11.8 vs. 32.1±14.2, $p<0.001$) (Table 2). On univariate analysis, $ASCA^+$ status was also associated with ileal involvement (73.0% vs. 48.0%, $p=0.002$), complicated (stricturing or penetrating) behavior (60.3% vs. 41.7%, $p=0.03$), and surgery (63.0% vs. 47.2% $p=0.05$). $ASCA^+$ and $ASCA^-$ patients did not differ in terms of gender, family history of IBD, tobacco use, and immunomodulator and infliximab therapy.

NOD2

NOD2 data were available for all cases and controls. All individuals carrying *NOD2* mutations were simple heterozygotes and carriage of any *NOD2* mutation was 8.2% (15/183) among patients, compared to 2.1% (3/143) in controls (p=0.03) (Table 1). The OR for CD and any *NOD2* SNP was 4.17 (95% confidence interval (CI): 1.18 - 14.69) with a population attributable risk of 6.2%. Of the three alleles investigated, only Leu1007fsinsC was significantly more frequent in patients than controls (0.019 vs. 0.000; p=0.02) (Table 1). The MW genotype was seen among 7.0% and 11.1% of $ASCA^+$ and $ASCA^-$ cases, respectively (Table 2).

We found no association between any *NOD2* mutation and ileal involvement or CD behavior. In addition, no association was seen between individual mutations and any phenotypic characteristics (data not shown). However, with so few *NOD2* cases, we had limited power to identify very strong effects.

Multivariate analysis

We evaluated the independent association of ASCA titer as a continuous variable, to ileal (L1 or L3) involvement using multiple logistic regression (Table 3). Ileal involvement was independently associated with ASCA titer (OR 1.18 per 25 unit increase, 95% CI 1.04–1.34, p=0.01) after adjustment for age at diagnosis, disease duration, family history, and smoking.

Stricturing and penetrating behaviors are encountered more frequently in patients with ileal disease. We therefore investigated whether the association between ASCA and complicated behavior persisted after accounting for the contribution of ileal involvement. On multiple logistic regression (Table 4), ASCA titer was associated with complicated behavior (OR 1.13 per 25 unit increase, 95% CI 1.01 – 1.27). Additionally, complicated disease behavior was independently associated with ileal involvement (OR: 3.97, 95% CI: 1.84 – 8.54) and longer disease duration (OR: 1.75 per 10 years, 95% CI: 1.11–2.75).

Table 5 gives the results of the analysis of time to surgery. Shorter time to surgery was associated with ASCA titer (hazard ratio (HR) 1.11 per 25 unit increase, 95% CI 1.02 – 1.22), family history of CD (HR: 2.39, 95% CI: 1.11–5.14) and smoking (HR 1.50; 95% CI 1.14-1.99).

DISCUSSION

Our study is the first to assess ASCA and the three common *NOD2* mutations in AA adults with CD. We report the following findings: 1) The sensitivity of ASCA for CD in AAs was 70.5%; 2) The specificity of ASCA for CD was 70.4%; 3) ASCA was independently associated with earlier disease onset, ileal involvement, complicated behavior, and surgery; 4) Although less common than in whites, the presence of a *NOD2* mutation, and specifically Leu1007fsinsC, was for the first time shown to be a risk factor for CD in African Americans.

In a previous study (31), we found that AA adult patients with CD were significantly more likely than whites to develop upper GI disease (OR, 2.8), colorectal involvement (OR, 1.9), perianal disease (OR, 1.7), and particularly extra-intestinal manifestions of uveitis and sacroileitis (OR 5.5 and 4.0, respectively). Conversely, AA patients were less likely to have ileal involvement (OR, 0.55) or internal penetrating disease (OR, 0.59). A family history of IBD was present less frequently in AA than their white counterparts (20 vs. 31%, p<0.05). Other studies have also found differences in CD family history and phenotypic manifestations by race (30, 32-35). Differences between white and AA CD patients in

family history, disease location, and extraintestinal manifestations suggest potential genetic and environmental differences.

Numerous studies have linked CD phenotypes with genetic polymorphisms. *NOD2* mutations have been associated with ileal involvement and complicated behavior (8-10, 12-17). Although *NOD2* is a confirmed CD susceptibility gene in white populations, *NOD2* mutations are rare or absent among other ethnic groups (42-44). To date, only one study in a population from Wisconsin, USA, has assessed *NOD2* mutations in the AA population (30). In that report, *NOD2* allele frequencies were markedly lower in AA vs. white children, both among healthy controls (2.1 vs. 9.1%, $p<0.01$) and among CD patients (1.6% vs. 25.7%, $p<0.0001$). There was no observed association between *NOD2* and CD in the AA children. Yet, given the small size (58 cases and 124 controls) and the low *NOD2* mutation frequency in the AA population, the study was underpowered to observe the overall 3-fold risk seen for *NOD2* mutation carriers seen in European ancestry studies (7). For example, a meta-analysis of 29 non-Jewish, white, hospital-based studies reported a *NOD2* risk of 3.2 (95% CI: 2.8 – 3.6) for all *NOD2* carriers, with OR 2.4 (95% CI 2.0 – 2.9) for simple heterozygotes (MW) and 17.1 (95% CI 10.7 – 27.2) for two mutant allele carriers (MM) (7). *NOD2* risk in our study (OR for heterozygotes 4.17, 95% CI: 1.18 - 14.69) was therefore within the same range. Nonetheless, the lower frequency of *NOD2* in the AA population relative to white populations results in a much smaller population attributable risk, only 6.2% in AA vs. 22% in non-Jewish whites (7).

We did not observe any *NOD2* mutant homozygotes or compound heterozygotes and given the low *NOD2* mutation allele frequency in both ours and the Wisconsin AA controls, (assuming Hardy-Weinberg equilibrium) only 0.01 to 0.04% of AA individuals would be expected to be *NOD2* homozygotes, as compared to the 0.5% of whites observed in a large study (45). It should be noted that the three NOD2 mutations are assumed to arise only from European admixture. R702W and G908R mutations were not present in 118 chromosomes genotyped in the Yoruban population (HapMap YRI database). Gasche et al. recently genotyped the three NOD2 mutations in DNA from 7 sub-Saharan African populations (including 25 Yorubans) and did not identify any *NOD2* mutations, including Leu1007fsinsC. (46). In a study of 3,575 healthy whites from around the world, *NOD2* allele frequency was 7.8% (45). European-African admixture among AA population has been considered as a continual process over four centuries, and the presence of *NOD2* mutations may be affected by survival advantages and disadvantages. For example, *NOD2* mutations may produce a survival advantage to exposure to certain infectious pathogens (46, 47). The 1.1% allele frequency we observed among AA controls is lower but within error of that expected from the 20% overall white admixture in AAs. It will be important to perform larger studies of *NOD2* in AA CD cases and healthy controls to 1) better define the overall AA population risk of *NOD2*; 2) assess whether *NOD2* in the AA population is below that expected by overall white admixture; and 3) determine whether the low rate of *NOD2* indeed explains the lower frequency of ileal CD in AAs.

With regard to individual mutations, only the Leu1007fsinsC was significantly associated with CD. The frequency of the Cins allele was 1.9% in patients vs. 0% in controls ($p=0.02$). In a meta-analysis of European ancestry studies, Economou and colleagues also observed that carriers of the Cins allele had a risk (OR 4.1; 95% CI 3.2 – 5.2) significantly greater than carriers of the R702W allele (OR 2.2; 95% CI 1.8 – 2.6), and a trend towards a greater risk than carriers of the G908R allele (OR 3.0; 95% CI 2.4 – 3.7) (7). As in nearly all *NOD2* studies, we observed that the G908R and R702W allele frequencies were numerically greater in CD vs. controls (R702W: 1.6% vs. 1.1%; G908R: 0.5% vs. 0.0%), but the differences did not reach statistical significance. Note that we had only 30% power to observe a significant association for the presence of any *NOD2* mutation, given the overall

mutant allele frequency of 0.010 in AA controls, and assuming the meta-analysis *NOD2* risks observed in whites. Therefore, much larger studies will be needed to assess the risk of the individual alleles. *NOD2* mutations were not a risk factor for ileal disease or complicated disease in the AA population, but again the study was underpowered.

ASCA expression has been associated in white populations with ileal involvement, earlier age at onset, stricturing and penetrating CD, and need for surgery (18-28). Furthermore, we found that the ASCA *titer* was associated with complicated behavior *independent* of *NOD2*status and ileal involvement. However, it is not known whether ASCA retains its phenotypic associations in AAs with CD, a population with a lower frequency of *NOD2* mutations and, probably, a different disease phenotype. Using the cutoff values determined by the manufacturer, the sensitivity and specificity values of our ASCA assay (QUANTA Lite, INOVA Diagnostics, San Diego, CA) in the AA CD population were 70.5% and 70.4% respectively. In comparison, the sensitivity of the QUANTA Lite assay in whites has ranged between 48%-76% (18, 22, 48, 49). Assay specificity was somewhat lower than the values observed in whites (86%-89%) (40, 48, 49). These differences should be placed in perspective by recognizing that the diagnostic accuracy of a test depends on the spectrum of diseased and non-diseased individuals comprising the study populations. As in whites, ASCA was associated with earlier disease onset, ileal involvement, complicated behavior and surgery in AA patients as well. On multivariate analysis, ASCA titer remained associated with ileal involvement, complicated behavior, and surgery. In this study, ASCA was not associated with *NOD2* mutations in AA, but there was a small number of *NOD2* carriers. ASCA seroprevalence is similar in whites and AAs, whereas *NOD2* mutations are much less common in AAs compared to whites. Therefore, other genes may be associated with the generation of ASCA in AAs .

In summary, ASCA in AAs was associated with CD diagnosis, earlier disease onset, ileal involvement, complicated behavior, and surgery. In the AA population, the Leu1007fsinsC *NOD2* mutation was associated with CD diagnosis. However, *NOD2* mutations, the strongest genetic risk factors identified for CD, account for a much smaller proportion of CD among the AA population than among white populations. Nonetheless, the incidence of CD in both populations in the United States has been found to be similar (50). Therefore further studies are needed to assess the importance of other CD risk genes in AA and to identify specific novel genetic and potential environmental risk factors in this understudied American population.

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STUDY HIGHLIGHTS

1. WHAT IS CURRENT KNOWLEDGE

- Common *NOD2* mutations (R702W, G908R and Leu1007fsinsC) are genetic risk factors for Crohn's disease in whites.
- The presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) is associated with Crohn's disease in whites.
- Both *NOD2* mutations and ASCA are established risks for ileal involvement and complications of intestinal stricturing and internal fistulas in whites.
- *NOD2* is rare in African Americans (AA).

2. WHAT IS NEW HERE

- *NOD2* is a genetic risk factor for Crohn's disease in AAs with a similar risk for heterozygotes as that observed for whites, although the frequency of *NOD2* mutations and the population attributable risk are significantly lower than those observed in whites.
- *NOD2* mutation homozygotes and compound heterozygotes were not observed in either Crohn's disease cases or controls and are thus likely to be very uncommon.
- Among the three *NOD2* mutations, there was evidence of a significant Crohn's disease association for the Leu1007fsinsC mutation in AAs.
- ASCA sensitivity in AAs was 70.5%, similar to values observed in white. ASCA specificity was 70.4%, somewhat lower than values in whites.
- Similar to white populations, ASCA in AAs is a risk for younger onset Crohn's disease, ileal involvement,complicated (stricturing/internal penetrating) behavior, and surgery.
- In AA with CD, elevated ASCA titers, smoking and CD family history are independent risk factors for surgery.

TABLE 1

Demographic and phenotypic characteristics, ASCA serotypes and *NOD2* genotypes among cases and controls

	Cases (n=183)	Controls (n=143)	p-value
Age at diagnosis (SD) (n = 182 for cases)	27.1 (12.9)	N/A	
Age at study entry (SD)	38.2 (14.0)	39.6 (11.8)	0.17
Female	121 (66.1%)	88 (61.5%)	0.39
Family history of IBD	21 (11.5%)	0.0	<0.001
Smoking (at study entry) (171 cases, 141 controls)			0.04
Never	96 (56.1%)	70 (49.7%)	
Former smoker	35 (20.5%)	20 (14.2%)	
Current	40 (23.4%)	51 (36.2%)	
Smoking (at diagnosis) (171 cases)			
Never	111 (65.3%)	N/A	
Former	14 (8.2%)	N/A	
Yes	45 (26.5%)	N/A	
ASCA+ (142 controls)	129 (70.5%)	42 (29.6%)	<0.001
Any *NOD2* mutation	15 (8.2%)	3 (2.1%)	0.03
G908R	2 (1.1%)	0	0.51
Leu1007fsinsC	7 (3.8%)	0	0.02
R720W	6 (3.3%)	3 (2.1%)	0.74
NOD2 allele frequency	0.040	0.010	0.03
G908R	0.005	0	0.51
Leu1007fsinsC	0.019	0	0.02
R720W	0.016	0.010	0.74
NOD2 genotype			0.03
WW	168 (91.8%)	140 (97.9%)	
MW	15 (8.2%)	3 (2.1%)	
MM	0	0	
Disease location (n=174)[a]		N/A	
L1: Ileal	35 (20.1%)		
L2: Colonic	57 (32.8%)		
L3: Ileocolonic	80 (46.0%)		
L4: Isolated upper GI	2 (1.1%)		
Any ileal involvement (n=177)[b]	117 (66.1%)	N/A	
Behavior (n=169)		N/A	
Inflammatory	76 (45.0%)		

	Cases (n=183)	Controls (n=143)	p-value
Stricturing	52 (30.8%)		
Penetrating	41 (24.2%)		
Any complicated (n=169)	93 (55.0%)	N/A	
Any perianal disease (n=172)	60 (34.0%)	N/A	
Immunomodulator use	115 (62.8%)	N/A	
Infliximab use (n=180)	77 (42.8%)	N/A	
Surgery (n=180)	105 (58.3%)	N/A	

[a] Included persons with complete information to determine both ileal and colonic involvement

[b] Included persons with complete information to determine ileal involvement

Abbreviations: SD, standard deviation IBD, inflammatory bowel disease ASCA, antibodies to *Saccharomyces cerevisiae* NS, not significant N/A, not applicable

TABLE 2

Demographic and phenotypic characteristics of cases according to ASCA status and *NOD2* status

	ASCA$^+$ (n=129)	**ASCA$^-$ (n=54)**	**p**	***NOD2*$^+$ (n=15)**	***NOD2*$^-$ (n=168)**	**p**
Age at diagnosis						
Mean (SD)	25.0 (11.8)	32.1 (14.2)	0.001	29.4 (13.9)	26.9 (12.8)	0.75
Age category			0.02			0.40
≤ 16 y	35 (27.3%)	11 (20.4%)		3 (20.0%)	43 (25.8%)	
17 – 40 y	80 (62.5%)	29 (53.7%)		8 (53.3%)	101 (60.5%)	
> 40 y	13 (10.2%)	14 (25.9%)		4 (26.7%)	23 (13.8%)	
Disease duration (SD)	11.2 (9.0)	10.6 (8.3)	0.68	8.6 (5.3)	11.2 (9.0)	0.05
Female	86 (66.7%)	35 (64.8%)	0.81	11 (73.3%)	110 (65.5%)	0.54
Family History IBD	16 (12.4%)	5 (9.3%)	0.54	1 (6.7%)	20 (11.9%)	0.54
Tobacco Use (ever)	47 (37.9%)	28 (54.9%)	0.04	6 (40.0%)	69 (44.2%)	0.79
Disease location			0.012			0.54
L1: Ileal	29 (23.0%)	6 (12.5%)		5 (33.3%)	30 (18.9%)	
L2: Colonic	33 (26.2%)	24 (50.0%)		5 (33.3%)	52 (32.7%)	
L3: Ileocolonic	63 (50.0%)	17 (35.4%)		5 (33.3%)	75 (47.2%)	
L4: Isolated upper GI	1 (0.8%)	1 (2.1%)		0%	2 (1.3%)	
Any ileal (L1 or L3)	92 (73.0%)	25 (49.0%)	0.003	10 (66.7)	107 (66.1)	1.0
Behavior			0.08			0.69
Inflammatory	48 (39.7%)	28 (58.3%)		6 (46.2%)	70 (44.9%)	
Stricturing	40 (33.1%)	12 (25.0%)		5 (38.5%)	47 (30.1%)	
Penetrating	33 (27.2%)	8 (16.7%)		2 (15.4%)	39 (25.0%)	
Any complicated	73 (60.3%)	20 (41.7%)	0.03	7 (53.9%)	86 (55.1 %)	0.84
Any perianal disease	44 (35.2%)	16 (34.0%)	0.89	6 (40.0%)	54 (34.4%)	0.66
NOD2 genotype			0.38			
WW	120 (93.0%)	48 (88.9%)				
MW	9 (7.0%)	6 (11.1%)				
MM	0	0				

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	ASCA$^+$ (n=129)	**ASCA$^-$ (n=54)**	**p**	***NOD2*$^+$ (n=15)**	***NOD2*$^-$ (n=168)**	**p**
Immunomodulators	85 (65.9%)	30 (55.6%)	0.19	7 (46.7%)	108 (64.3%)	0.18
Infliximab	53 (42.1%)	24 (44.4%)	0.77	3 (20.0%)	74 (44.9%)	0.06
Surgery	80 (63.0%)	25 (47.2%)	0.05	8 (53.3%)	97 (58.8%)	0.68

Abbreviations: SD, standard deviation IBD, inflammatory bowel disease GI, gastrointestinal ASCA, antibodies to *Saccharomyces cerevisiae* NS, not significant

Table 3

Adjusted Odds Ratios for any Ileal (isolated ileal or ileocolonic) Disease Versus Isolated Colonic Disease

	OR	95% CI	p-value
ASCA (/25 U)	1.18	1.04-1.34	0.012
Age at diagnosis (/10 years)	0.79	0.60-1.04	0.09
Family history of CD	1.90	0.58-6.19	0.29
Smoking	0.95	0.59-1.53	0.84
Disease duration (/10 years)	1.00	0.66-1.49	0.98

Abbreviations: OR, odds ratio CI, confidence interval ASCA, antibodies to *Saccharomyces cerevisiae* CD, Crohn's disease

TABLE 4

Adjusted Odds Ratios for Complicated CD Behavior

	OR	95% CI	p-value
ASCA (/25 U)	1.13	1.01-1.27	0.04
Age at diagnosis (/10 yrs)	1.08	0.81-1.43	0.62
Family history of CD	0.63	0.22-1.77	0.38
Smoking	1.14	0.70-1.87	0.59
Ileal involvement	3.97	1.84-8.54	<0.001
Disease duration (/10 yrs)	1.75	1.11-2.75	0.02

Abbreviations: OR, odds ratio CI, confidence interval ASCA, antibodies to *Saccharomyces cerevisiae* CD, Crohn's disease

Table 5

Hazard Ratios for Having Surgery

	HR	95% CI	p
ASCA (/25 U)	1.11	1.02-1.22	0.02
Age at diagnosis (10 yrs)	0.96	0.75-1.23	0.76
Family history of CD	2.39	1.11-5.14	0.03
Smoking	1.50	1.14-1.99	0.004
Ileal involvement	1.40	0.75-2.62	0.30
Disease duration (/10 yrs)	1.11	0.78-1.57	0.57

Abbreviations: HR, hazard ratio CI, confidence interval ASCA, antibodies to *Saccharomyces cerevisiae* CD, Crohn's disease