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The common *NOD2* risk variants in African Americans with Crohn's disease are due exclusively to recent Caucasian admixture

Oloruntosin Adeyanju¹, David Okou¹, Clifton Huang^{1,4}, Archana Kumar¹, Cary Sauer^{1,4}, Courtney Galloway¹, Mahadev Prasad¹, Jon Waters¹, David Cutler², Michael Zwick², Tanvi Dhere³, and Subra Kugathasan^{1,4}

¹Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA

²Department of Human Genetics, Emory University School of Medicine, Atlanta, GA

³Division of Gastroenterology and Hepatology, Department of Medicine, Emory University School of Medicine, Atlanta, GA

⁴Children's Health Care of Atlanta, Atlanta, GA

Abstract

Background—Crohn's disease (CD) is highly heritable. *NOD2* has emerged as the main susceptibility gene among individuals of European ancestry; however, *NOD2* does not appear to contribute to CD susceptibility among many non-European populations. Today's African American (AA) population represents an admixture of West African (80%) and European (20%) ancestry. Since genotype-based tools are becoming increasingly available for CD, it is important that we validate the risk variants in different populations, such as admixed AAs.

Methods—We analyzed the *NOD2* variants among admixed AAs (n=321, 240 with CD and 111 healthy controls) and non-admixed West Africans (n=40) by genotyping for 4 known disease-causing *NOD* variants. We extracted the publically available 1000 Genomes data on *NOD2* variants from 500 subjects of West African origin. Association with disease was evaluated by logistic regression.

Results—An association with CD was found for the classical SNP 1007fs (2.6% CD, 0% HC, p=0.012); there was no association when the genotypic and allelic frequencies of the risk alleles were compared for SNPs R702W and G908R. No known *NOD2* risk alleles were seen in either the West African cohort or in subjects of African ancestry from the 1000 Genomes project.

Conclusions—The *NOD2* gene is a risk for CD in AAs, although the allele frequencies and the attributable risk are much lower compared to Caucasians. The risk alleles are not seen in the West African population suggesting the risk for CD contributed by *NOD2* among AAs is due exclusively to recent European admixture

Author Keywords

African Americans; Crohn's disease; *NOD2*

Introduction

Crohn's disease (CD) is a highly heritable disorder, with studies showing about 50% concordance among monozygotic twins, and siblings of patients with CD having a much higher risk of developing CD compared with the general population.(1) In the past decade, candidate gene studies and genome-wide association studies have uncovered well over 50 susceptibility loci for CD.(2) Among the confirmed genes, the association between *NOD2* and CD susceptibility has been established beyond a doubt in European populations; homozygous or compound heterozygous carriers of the 3 classical polymorphisms (1000fs, R702W, G908R) have a 17-fold higher risk of developing disease, and heterozygous carriers have a 2.4-fold greater risk.(3) Nonetheless, it is clear that CD affects all races, and that disease prevalence in other races, including African Americans (AAs), approaches that of Europeans. African Americans are a recently admixed population, with an estimated European admixture of around 20%, with the other 80% coming from an ancestral African population (4–7). It is known that the majority of the African ancestral genome found in AAs originated in West Africa. Today, little is known about the risk conferred by *NOD2* polymorphisms among AAs with CD. As genetic-based testing begins to impact diagnostic and treatment decisions, the importance of this information for all CD patients will continue to grow, and with it the need to understand the applicability of genetic findings from all patient populations.

In total, 4 disease-associated SNPs, all resulting in nonsynonymous amino acid changes, have been reported in the European (8) and Ashkenazi Jewish (9) populations; these are 1000fs, R702W, G908R, IVS8+158 (or JW1). We also included R790Q, a variant unique to the African ancestral population (10) for our analysis. Our study aimed to define the association between already known CD-associated *NOD2* risk variants in the AA population. We hypothesized that any association between known *NOD2* variants in AAs with CD could be attributable to recent European admixture.

Materials and Methods

Subjects

The study population included self-identified unrelated individuals: AAs with CD (n=210), healthy AA controls (n=111). In addition, we included 40 non-admixed West Africans recently migrated from Ghana, Nigeria, Cameroon and Ivory Coast who were living in the USA at the time of recruitment and enrolled as healthy controls. It is known that the founder population of African ancestry in AAs originated primarily from the slave trade in West Africa including the nations described above. All the AAs and West Africans reported in this study represent an independent cohort not studied before in any IBD genetic studies. We extracted *NOD2* data from the 1000 Genomes diversity project from 500 individuals of West African origin. In addition, we genotyped 220 Caucasian subjects with CD and 210 Caucasian control subjects for comparison. The study protocol was approved by an Institutional Review Board, and informed consent was obtained from each participant or from the legal guardians of minors.

Genotyping

Samples were plated for genotyping and blinded to the laboratory. Five SNPs (1000fs, R702W, G908R, JW1, and R790Q) were genotyped using Applied Biosystem's TaqMan 5' exonuclease assays, with alleles determined on an Applied Biosystems 7900HT Fast Real-Time PCR System analyzer.

Analysis

Association analyses for each SNP were performed on confirmed CD cases compared with matched healthy controls using the PLINK software. Results were stratified by ethnicity and compared to 1000 Genomes data.

Results

We genotyped 5 previously published SNPs that map across the *NOD2* locus. The SNPs R702W, G908R, and 1007fs known to be associated in Caucasians were included, as was the SNP JW1, which is found in Caucasians mainly of Jewish ethnicity. The SNP R790Q was also genotyped, as this SNP is found only in African ancestral populations, and not typically found in Caucasians of European origin. Allelic association reached statistical significance only for 1007fs (2.6% CD, 0% HC, $p=0.012$). There was no association when the genotypic and allelic frequencies of the risk alleles were compared for SNPs R702W (5.47% CD, 5.08% HC, $p=0.83$), G908R (1.19% CD, 0.42% HC, $p=0.322$), R790Q (3.8% CD, 3.4% HC, $p=0.78$), and JW1 (7.8% CD, 4.3% HC, $p=0.07$). All SNPs examined are either uncommon or non-existent in the West African population studied (Table 1), as well as in the 500 individuals from the 1000 Genomes database. The complete lack of *NOD2* variants in the West African population, coupled with the low allele frequency of *NOD2* variants among AAs, point to the risk for CD from known *NOD2* variants in African Americans being the result of European admixture.

Discussion

All studies in Caucasians of European origin show that *NOD2* mutations are more frequent among CD cases than controls. A recent meta-analysis of 29 studies found an overall *NOD2* carrier risk of 3.2 among Caucasians with CD;(11) however, numerous publications from Asia, North Africa, and the Middle East reveal that known disease-causing *NOD2* mutations are rare or absent among non-Caucasians. The 3 common disease-causing *NOD2* mutations associated with CD are assumed to have arisen in European ancestral populations or by admixture.(12, 13) In this study, first we confirmed independently that *NOD2* is a risk for AAs, and secondly, we show that these *NOD2* variants are not present among a non-admixed West African population.

The clinical implications of having *NOD2* mutations in CD are now well known. Numerous studies point to an association with ileal disease, and *NOD2* is a risk factor for internal penetrating/stricturing disease in CD. It remains to be seen whether *NOD2* variants have prognostic utility in admixed individuals, such as AAs, who constitute about 15% of the US population and in whom each chromosome is likely to be a mosaic of blocks of DNA from different ancestral populations. Recent studies estimate European admixture in AAs to be around 20%, with the other 80% arising from ancestral African genome.(4–7) Since genotype-based tools are becoming increasingly available for CD, it is crucial that we accurately understand risk variants in the admixed AA population.

Very little has been published on common *NOD2* mutations in the African ancestral population. R702W and G908R mutations were not seen in 118 individuals of Yoruba descent, and Leu1007fsinsC was absent in 24 individuals with African ethnicity in the SNP500CANCER project. Recent study genotyping *NOD2* mutations in 7 sub-Saharan populations (including 25 Yorubans) likewise found no *NOD2* mutations(14). In this paper, we show that the common CD-associated *NOD2* variants are indeed absent in non-admixed West African individuals. Our findings are further supported by the 1000 Genomes project data confirming the absence of these 3 SNPs among at least 500 African populations.

We conclude that *NOD2* is a risk for African Americans with CD, but their allele frequencies are much lower: about 20–25% of the frequencies seen in Caucasians of European origin. Our data strongly suggest that the risk conferred by *NOD2* in AAs is the exclusive result of Caucasian admixture. Deep sequencing will explore whether other SNPs within the *NOD2* loci could contribute to disease susceptibility in African ancestral populations.

Acknowledgments

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References

1. Massey DC, Parkes M. Genome-wide association scanning highlights two autophagy genes, ATG16L1 and IRGM, as being significantly associated with Crohn's disease. *Autophagy*. 2007; 3:649–651. [PubMed: 17921695]
2. Lee JC, Parkes M. Genome-wide association studies and Crohn's disease. *Briefings in functional genomics*. 10:71–76. [PubMed: 21436303]
3. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol*. 2008; 8:458–466. [PubMed: 18500230]
4. Tang H, Jorgenson E, Gadde M, et al. Racial admixture and its impact on BMI and blood pressure in African and Mexican Americans. *Human genetics*. 2006; 119:624–633. [PubMed: 16738946]
5. Fernandez JR, Shriver MD, Beasley TM, et al. Association of African genetic admixture with resting metabolic rate and obesity among women. *Obesity research*. 2003; 11:904–911. [PubMed: 12855761]
6. Parra EJ, Kittles RA, Argyropoulos G, et al. Ancestral proportions and admixture dynamics in geographically defined African Americans living in South Carolina. *American journal of physical anthropology*. 2001; 114:18–29. [PubMed: 11150049]
7. Tishkoff SA, Reed FA, Friedlaender FR, et al. The genetic structure and history of Africans and African Americans. *Science*. 2009; 324:1035–1044. [PubMed: 19407144]
8. Lesage S, Zouali H, Cezard JP, et al. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *American journal of human genetics*. 2002; 70:845–857. [PubMed: 11875755]
9. Sugimura K, Taylor KD, Lin YC, et al. A novel NOD2/CARD15 haplotype conferring risk for Crohn disease in Ashkenazi Jews. *American journal of human genetics*. 2003; 72:509–518. [PubMed: 12577202]
10. Bonen DK, Nicolae DL, Moran T, et al. Racial differences in NOD2 variation: characterization of NOD2 in African-Americans with Crohn's disease (abstr). *Gastroenterology*. 2002; 122:A-29.
11. Ng SC, Tsoi KK, Kamm MA, et al. Genetics of inflammatory bowel disease in Asia: Systematic review and meta-analysis. *Inflammatory Bowel Diseases*. 2011
12. Croucher PJ, Mascheretti S, Hampe J, et al. Haplotype structure and association to Crohn's disease of CARD15 mutations in two ethnically divergent populations. *Eur J Hum Genet*. 2003; 11:6–16. [PubMed: 12529700]
13. Zaahl MG, Winter T, Warnich L, et al. Analysis of the three common mutations in the CARD15 gene (R702W, G908R and 1007fs) in South African colored patients with inflammatory bowel disease. *Molecular and cellular probes*. 2005; 19:278–281. [PubMed: 15967635]
14. Gasche C, Nemeth M, Grundtner P, et al. Evolution of Crohn's disease-associated Nod2 mutations. *Immunogenetics*. 2008; 60:115–120. [PubMed: 18253730]

Table 1

NOD2 allele frequencies in AAs compared with West Africans and Caucasians.

Gene	SNP number	African Americans				West Africans		Caucasians	
		Cases (n=210)	Control (n=111)	OR	P	Controls (n=40)	1000 genome (n=500)	Cases n=220	Control n=210
<i>NOD2</i> (C>T, variant T)	rs2066844 R702W (SNP8)	0.054	0.050	1.08	0.83	0.01	0.011	0.07	0.063
<i>NOD2</i> (G>C, variant C)	rs2066845 G908R (SNP12)	0.012	0.004	2.83	0.32	0	0	0.12	0.088
<i>NOD2</i> C_insertion	rs2066847 1000fs (SNP13)	0.026	0	NA	0.01	0	0	0.1	0.02
<i>NOD2</i> carrier (R702W + G908R + 1000fs)		0.09	0.04		0.03			0.3	0.17
<i>NOD2</i> (G>A, variant A)	rs5743279 R790Q	0.038	0.034	1.13	0.78	0.02	0.032	NA	NA
<i>NOD2</i> (C>T, variant T)	RS5743289 JW1 or IVS8+158	0.078	0.042	1.93	0.07	0.02	0.032	0.02	0.006