

Israel Lowy, M.D., Ph.D.

Bristol-Myers Squibb
Lawrenceville, NJ

Deborah C. Molrine, M.D., M.P.H.
Donna M. Ambrosino, M.D.

MassBiologics
Boston, MA
deborah.molrine@umassmed.edu

Since publication of their article, Dr. Lowy reports having equity in Bristol-Myers Squibb. No further potential conflict of interest relevant to this letter was reported.

1. Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* disease. *Lancet* 2001;357:189-93.
2. Babcock GJ, Broering TJ, Hernandez HJ, et al. Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamsters. *Infect Immun* 2006;74:6339-47.
3. Westerman LE, McClure HM, Jiang B, Almond JW, Glass RI. Serum IgG mediates mucosal immunity against rotavirus infection. *Proc Natl Acad Sci U S A* 2005;102:7268-73.
4. Santosham M, Reid R, Ambrosino DM, et al. Prevention of *Haemophilus influenzae* type b infections in high-risk infants treated with bacterial polysaccharide immune globulin. *N Engl J Med* 1987;317:923-9.

Genomewide Association Study of Leprosy

TO THE EDITOR: Zhang and colleagues (Dec. 31 issue)¹ report that genes in the nucleotide-binding oligomerization domain containing 2 (NOD2)-mediated signaling pathway are associated with susceptibility to infection with *Mycobacterium leprae* in China. India has the world's greatest leprosy disease burden. We therefore genotyped the single-nucleotide polymorphisms (SNPs) that were implicated by Zhang and colleagues in two Indian case-control cohorts (492 patients in New Delhi and 382 in Kolkata).^{2,3} We also genotyped 273 cases and 221 controls from Mali, West Africa.⁴ We observed associations between disease and SNPs at *C13orf31* (the gene encoding chromosome 13 open reading frame 31) (rs3764147, $P=6.1\times 10^{-8}$) and *CCDC122* (the gene encoding coiled-coil domain containing 122) (rs9533634, $P=1.1\times 10^{-5}$) (Table 1); both genes were of unknown function. We did not, however, observe associations between disease and the other four non-major histocompatibility complex (MHC) genes related to the NOD2 pathway (*NOD2*, *RIPK2* [the gene encoding receptor-interacting serine-threonine kinase 2], *TNFSF15* [the gene encoding tumor necrosis factor (ligand) superfamily member 15], and *LRRK2* [the gene encoding leucine-rich repeat ki-

nase 2]) (Table 1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org), despite reasonable power to detect the effect sizes observed by Zhang et al.¹ An analysis of 27 additional SNPs at *NOD2* in the New Delhi cohort provided support for the absence of a consistent association at this locus (Table 2 in the Supplementary Appendix). These results indicate heterogeneity among populations and suggest that future functional studies should focus on populations in which the relevant genetic association may occur. Nevertheless, a robust association of the Crohn's disease chromosome 13q14.11 locus⁵ containing *C13orf31* and *CCDC122* with leprosy in China, India, and Mali provides support for a molecular link between mycobacterial infection and Crohn's disease.

Sunny H. Wong, M.B., Ch.B.
Adrian V.S. Hill, D.M., D.Phil.
Fredrik O. Vannberg, D.Phil.

Wellcome Trust Centre for Human Genetics
Oxford, United Kingdom
vannberg@well.ox.ac.uk

for the India-Africa-United Kingdom Leprosy Genetics Consortium

Table 1. Associations with Leprosy for Replicated Single-Nucleotide Polymorphisms on Chromosome 13q14.11, According to Case-Control Population.*

SNP	Gene	Original Study odds ratio (95% CI)	Present Study odds ratio (95% CI)	New Delhi	Kolkata	Mali	Combined Populations
rs3764147	<i>C13orf31</i>	1.68 (1.57–1.80)	1.59 (1.34–1.89)	2.7×10^{-3}	6.4×10^{-2}	1.1×10^{-5}	6.1×10^{-8}
rs9533634	<i>CCDC122</i>	0.76 (0.70–0.82)	0.70 (0.59–0.82)	1.5×10^{-3}	3.6×10^{-1}	1.1×10^{-5}	1.1×10^{-5}

* *C13orf31* denotes the gene encoding chromosome 13 open reading frame 31, *CCDC122* the gene encoding coiled-coil domain containing 122, CI confidence interval, and SNP single-nucleotide polymorphism.

No potential conflict of interest relevant to this letter was reported.

1. Zhang F-R, Huang W, Chen S-M, et al. Genomewide association study of leprosy. *N Engl J Med* 2009;361:2609-18.
2. Malhotra D, Darvishi K, Sood S, et al. IL-10 promoter single nucleotide polymorphisms are significantly associated with resistance to leprosy. *Hum Genet* 2005;118:295-300.
3. Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis* 1999;179:187-91.
4. Meisner SJ, Mucklow S, Warner G, Sow SO, Lienhardt C, Hill AV. Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy per se in West Africans. *Am J Trop Med Hyg* 2001;65:733-5.
5. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;40:955-62.

TO THE EDITOR: In a genomewide association study, Zhang and colleagues identified the NOD2 pathway associated with susceptibility to leprosy. Their findings were consistent with those of studies showing the role of NOD2 in the recognition of mycobacteria.¹ However, NOD2 interacts with toll-like receptors (TLRs) during recognition of mycobacteria, and polymorphisms in either TLR2² or TLR4³ have been shown to influence susceptibility to leprosy. Therefore, it may seem surprising that no signal of association was detected between TLRs and leprosy in the study by Zhang et al. An explanation of this apparent discrepancy is that haplotypes in TLR genes vary greatly among populations in different geographic locations.⁴ For example, TLR4 polymorphisms that influence the susceptibility to infections are virtually absent in East Asia, in contrast to other populations.⁴ We therefore suggest that genomewide association studies involving African or European populations would identify a different and possibly larger number of genes influencing susceptibility to leprosy.

Mihai G. Netea, M.D., Ph.D.

Bart Jan Kullberg, M.D., Ph.D.

Jos W.M. van der Meer, M.D., Ph.D.

Radboud University Nijmegen Medical Center
Nijmegen, the Netherlands
m.netea@aig.umcn.nl

No potential conflict of interest relevant to this letter was reported.

1. Ferwerda G, Girardin SE, Kullberg BJ, et al. NOD2 and Toll-like receptors are nonredundant recognition systems of Mycobacterium tuberculosis. *PLoS Pathog* 2005;1:279-85.
2. Bochud PY, Hawn TR, Aderem A. Cutting edge: a Toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. *J Immunol* 2003;170:3451-4.
3. Bochud PY, Sinsimer D, Aderem A, et al. Polymorphisms in Toll-like receptor 4 (TLR4) are associated with protection against leprosy. *Eur J Clin Microbiol Infect Dis* 2009;28:1055-65.

4. Ferwerda B, McCall MB, Alonso S, et al. TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans. *Proc Natl Acad Sci U S A* 2007;104:16645-50.

TO THE EDITOR: A similar proinflammatory immune response has been observed in paucibacillary leprosy and in the contained form of Crohn's disease.¹ In contrast, such an immune response has not been detected in the more aggressive, multibacillary form of leprosy and in the more aggressive form of Crohn's disease.¹ These findings are now mirrored in an intriguing commonality between human genetic defects in multibacillary leprosy and Crohn's disease reported on by Zhang et al. The accompanying editorial² posits that a subgroup "of Crohn's disease cases may have a mycobacterial cause." A candidate culpable organism³ causes Johne's disease in ruminants, an affliction evocative of Crohn's disease. Interspecies comparisons⁴ may be illuminating. Cattle with the NOD2 defect are three times as likely to contract Johne's disease as cattle with wild-type NOD2 in the same herd.⁵

Robert J. Greenstein, M.D.

Sheldon T. Brown, M.D.

James J. Peters VA Medical Center
Bronx, NY
bgaxis@aol.com

Dr. Greenstein reports submitting patent applications based on the inhibition of mycobacteria including the *M. avium* subspecies *paratuberculosis* by medications used to treat Crohn's disease. No other potential conflict of interest relevant to this letter was reported.

1. Gilberts EC, Greenstein AJ, Katsel P, Harpaz N, Greenstein RJ. Molecular evidence for two forms of Crohn disease. *Proc Natl Acad Sci U S A* 1994;91:12721-4.
2. Schurr E, Gros P. A common genetic fingerprint in leprosy and Crohn's disease? *N Engl J Med* 2009;361:2666-8.
3. Greenstein RJ, Collins MT. Emerging pathogens: is Mycobacterium avium subspecies paratuberculosis zoonotic? *Lancet* 2004;364:396-7.
4. Greenstein RJ. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. *Lancet Infect Dis* 2003;3:507-14.
5. Pinedo PJ, Buergelt CD, Donovan GA, et al. Association between CARD15/NOD2 gene polymorphisms and paratuberculosis infection in cattle. *Vet Microbiol* 2009;134:346-52.

THE AUTHORS REPLY: Using three case-control samples from India and West Africa, Wong et al. undertook a follow-up study of our recently identified leprosy susceptibility SNPs and confirmed the strong associations of *C13orf31* and *CCDC122*, highlighting the commonality of leprosy susceptibility loci among diverse populations. Although they did not detect the associations in the other

non-MHC susceptibility loci, the use of combined small and mixed patient cohorts, with limited analysis of the SNPs most strongly implicated in our analysis, may have resulted in some missed associations, especially if the risk alleles were tagged by different SNPs in populations of differing ancestries, the genetic effects of risk alleles were more moderate in populations other than Chinese populations, or both.

Netea and colleagues express surprise that our study did not highlight TLR genes and further imply that our findings may be relevant only for East Asian populations. We searched our genome-wide association study data for any evidence of TLR associations and found none. We agree with these correspondents that genetic variation due to differences in ancestral origin may explain this result,¹ but we think a more likely explanation is that the NOD2 pathway is more influential than the TLR pathway in determining disease outcome. The implication of variants of *C13orf31* and *CCDC122* in Indian and West African populations shows that genetic risk factors for leprosy are shared by different populations.

The observation by Greenstein and Brown provides further support for the pathogenicity com-

mon to leprosy and Crohn's disease. Although we observed an overlap of the most strongly implicated SNPs in susceptibility to both leprosy and Crohn's disease, a joint analysis of these diseases (through a genomewide association study) should be pursued for a comprehensive investigation of common genetic risk factors. Moreover, confirmation of the association between *C13orf31* and *CCDC122* and susceptibility to Crohn's disease and leprosy in diverse populations calls for an investigation of the biologic function of these genes, which will probably illuminate a pathogenic mechanism of both diseases.

Fu-Ren Zhang, M.D., Ph.D.

Shandong Provincial Institute of Dermatology and Venereology
Jinan, China
zhangfuren@hotmail.com

Martin L. Hibberd, Ph.D.

Jian-Jun Liu, Ph.D.

Genome Institute of Singapore
Singapore, Singapore

Since publication of their article, the authors report no further potential conflict of interest.

1. Smirnova I, Mann N, Dols A, et al. Assay of locus-specific genetic load implicates rare Toll-like receptor 4 mutations in meningococcal susceptibility. *Proc Natl Acad Sci U S A* 2003; 100:6075-80.

Barrett's Esophagus

TO THE EDITOR: Sharma (Dec. 24 issue)¹ says that ablation is not recommended for nondysplastic Barrett's esophagus because of an unacceptably high number of patients who would need to be treated (the number needed to treat) to prevent one case of adenocarcinoma. The estimate of 250 as the number needed to treat is artificially high, however. The number needed to treat is 1 divided by the absolute risk reduction. In the meta-analysis by Wani et al., the incidence of adenocarcinoma in patients with untreated nondysplastic Barrett's esophagus was 0.6% per patient-year of follow-up, as compared with 0.16% in patients treated with ablation.² The absolute risk reduction is therefore 0.6%–0.16%, or 0.44% per patient-year of follow-up. The studies in this meta-analysis varied in the length of follow-up, but all follow-ups were multiple years in length. Assuming a follow-up of just 5 years, the number needed to treat would be 45, not 250. Other studies of ablation for nondysplastic Barrett's esophagus also

show a much lower number needed to treat — approximately 23 rather than 250.³

Sharma's estimate of the number needed to treat limits ablation to prevention of adenocarcinoma only; however, ablation also prevents the development of high-grade dysplasia and subsequent progression to cancer.⁴ For the inclusion of the aggregate end points of high-grade dysplasia and cancer, the number needed to treat would be lower still.

Robert A. Ganz, M.D.

Minnesota Gastroenterology
Bloomington, MN
gastrodude@visi.com

Dr. Ganz reports being a coinventor of the Halo system of radiofrequency ablation and having equity in and serving on the board of directors of the manufacturing company, BARRX Medical. No other potential conflict of interest relevant to this letter was reported.

1. Sharma P. Barrett's esophagus. *N Engl J Med* 2009;361: 2548-56.
2. Wani S, Puli SR, Shaheen NJ, et al. Esophageal adenocarci-