Mapping the Effects of Genetic Susceptibility and *Mycobacterium avium* subsp. *paratuberculosis* Infection on Crohn's Disease: Strong but Independent

We read with interest the report of Sechi et al. (1) on the associations among Crohn's disease (CD), *Mycobacterium avium* subsp. *paratuberculosis*, and carriage of mutant alleles of the NOD2/CARD15 gene.

We believe that the study by Sechi et al. was well conceived, is critical to understanding the etiology of Crohn's disease, and should stimulate additional interest in the field. Unfortunately, their analysis contains substantial inaccuracies, fails to address their study hypothesis directly, and does not correctly characterize the outcome of their study. Our intention is to correct these problems and show how this study can provide useful direction for further study.

There are very substantial errors in the odds ratios (ORs) presented by the authors, the corrections of which are prerequisite to understanding the study findings. The authors report three ORs. The OR for the bivariate association between CD and *M. avium* subsp. *paratuberculosis* infection is not 4.94, but rather 8.04 with a 95% confidence interval (CI) of 2.73 to 23.65 and a *P* of <0.001. The authors report an OR of 4.07 for the bivariate association between CD and the carriage of at least one mutant NOD2/CARD15 allele. We can confirm this OR and confidence interval. And finally, the authors report an OR of 1.7 for the association between CD and *M. avium* subsp. *paratuberculosis* among those carrying at least one of the mutant alleles. This OR should be 7.00 (95% CI, 1.01 to 48.31; *P*, 0.048). In addition, a multivariate logistic regression model including both *M. avium* subsp. *paratuberculosis* status and mutant allele carriage as well as an interaction term (i.e., the *M. avium* subsp. *paratuberculosis* variable multiplied by the mutant allele variable) finds strong independent effects of *M. avium* subsp. *paratuberculosis* exposure on CD (OR, 6.94; 95% CI, 2.29 to 21.07; *P*, 0.001) and mutant allele carriage on CD (OR, 3.16; 95% CI, 0.99 to 10.06; *P*, 0.052), but the interaction term was not significant (OR, 1.01; *P*, 0.992).

There are critical implications that flow from these corrected odds ratios, implications that go to the heart of the issues addressed in the article. We quote from the article: "It is possible that genetically identifiable subpopulations may have different tendencies to develop CD when exposed to the same infectious agent. The following case study suggests an exploitation of these considerations in a group of Mediterranean patients with CD."

This clearly indicates that the authors' hypothesis is that there exists some interactive relationship, whether positive or negative, between *M. avium* subsp. *paratuberculosis* infection and mutant allele carriage in the development of CD.

If this hypothesis is correct, then a couple of features should be evident in the data. First, the relationship between *M. avium* subsp. *paratuberculosis* and CD should be quite different for the mutant and nonmutant allele carriage groups and should be different from that indicated by the overall odds ratio. On the contrary, according to our calculations, the odds ratios for *M. avium* subsp. *paratuberculosis* in both groups are identical (7.0 and 6.9, respectively), and these odds ratios are not significantly different from the overall odds ratio of 8.0 noted above. This is in direct contradiction to the authors' findings. In other words, regardless of whether you carry the mutant allele or not, *M. avium* subsp. *paratuberculosis* infection has the same tendency to produce CD. Second, if the authors' study hypothesis is true, a logistic regression model should show a statistically significant interaction term. That is, in addition to the independent effects of *M. avium* subsp. *paratuberculosis* and mutant allele carriage on the development of CD, there should be an "extra" effect of having both *M. avium* subsp. *paratuberculosis* infection and the mutant allele. Our calculations indicate that the interaction term is not even close to significant (OR, 1.01; *P*, 0.992). Thus, with these two pieces of evidence, we refute the authors' study hypothesis.

What does this study find, and what kinds of hypotheses would be consistent with these data? First, this study confirms that carriage of mutant alleles of the NOD2/CARD15 gene and *M. avium* subsp. *paratuberculosis* infection are independently associated with CD. Given the sometimes contradictory nature of findings in this field, this is not insignificant. Furthermore, the associations are much stronger than originally reported by the authors. The second point of interest is that these data are consistent with two quite different causal models or hypotheses which this study cannot distinguish because of its retrospective nature but which future research should endeavor to elucidate.

The first hypothesis is the independent, or "direct effects," model. In this model, *M. avium* subsp. *paratuberculosis* infection and mutant allele carriage cause CD but have no synergistic effect and are not themselves causally connected. Our logistic regression model supports this with independent odds ratios of 6.9 and 3.2 for *M. avium* subsp. *paratuberculosis* infection and mutant allele carriage, respectively.

The second hypothesis, which you might call the "sequential" model, is that mutant allele carriage, through some unknown mechanism, results in a higher likelihood of *M. avium* subsp. *paratuberculosis* infection, which in turn causes CD. There is support in the data for this model as well. Namely, we have found an odds ratio of 2.9 (*P* = 0.037) for the association between *M. avium* subsp. *paratuberculosis* infection and mutant allele carriage. The fact that some Crohn's disease patients did not have detectable *M. avium* subsp. *paratuberculosis* infections does not necessarily refute this possibility because a previous infection may have cleared up or been otherwise undetectable at the time of the study.

We would like to commend the authors for their significant research and hope that our analysis will make their findings more helpful to research teams interested in conducting larger studies of the effects of *M. avium* subsp. *paratuberculosis* infection and genetic susceptibility on Crohn's disease.

REFERENCE

1. **Sechi, L. A., M. Gazouli, J. Ikonomopoulos, J. C. Lukas, A. M. Scanu, N. Ahmed, G. Fadda, and S. Zanetti.** 2005. *Mycobacterium avium* subsp.

paratuberculosis, genetic susceptibility to Crohn's disease, and Sardinians: the way ahead. J. Clin. Microbiol. **43:**5275–5277.

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Ed. Note: The authors of the published article declined to respond.