Letter to the Editor

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Reply to Hodge et al.

To the Editor:

We thank Hodge et al. (2001 [in this issue])for the chance to discuss an important question: how should we analyze linkage data when we suspect that our families may be segregating more than one disease-susceptibility gene and that disease in some of them may be nonhereditary (i.e., due to nonhereditary factors or to chance)? In our original article (Whittemore and Halpern 2001), we argued against the admixture model of Smith (1963), which is often used to address this problem. This model assumes that all families are hereditary and that a fraction (α) of them segregate deleterious alleles of a gene in a region of interest, while the remaining families segregate alleles of other genes. Testing for linkage at a marker in the region involves specifying the frequencies and penetrances of genotypes of the gene of interest and then maximizing Smith's admixture likelihood with respect to both the fraction α and the recombination fraction (θ) between marker and trait locus. The problem is that, when the likelihood-ratio statistic from this model (i.e., the HLOD) is large, the maximum-likelihood estimate of α is often reported, with its implication that 100α% of disease X is due to a gene in the linked region.

In our original article, we showed that defining, interpreting, and estimating the parameter α is fraught with fundamental logical problems and major statistical pitfalls. We argued that α is not meaningful except under strong and unrealistic assumptions about the data. Moreover, even in the unlikely event that the data meet all these assumptions, estimates of α are quite sensitive to misspecification of the unknown phenocopy rate. Finally, even if the data meet all the necessary assumptions and the investigator specifies the phenocopy rate correctly, the estimates of α that are produced by standard linkage programs are calculated incorrectly and therefore are biased in the presence of phenocopies. We showed how to fix the last problem by correcting the software estimates, but, nevertheless, we recommended against using the HLOD, even as a tool for detection of linkage.

In their letter, Hodge et al. agree with us about the difficulties with α, but they take issue with our recommendation. They cite simulation studies, which suggest (*a*) only slight power loss for the HLOD test compared with the test based on the correct model and (*b*) superior power for the HLOD test compared with NPL (i.e., nonparametric linkage) tests. They also note the need for additional power comparisons between HLOD tests and NPL tests.

We agree with Hodge et al. that the relative power of HLOD and NPL tests needs more work. We also agree that, in some situations, the HLOD test may have greater power than does an NPL test. But the published evidence that they cite does not convince us that such power advantage holds more generally, when the data arise from mechanisms that differ from the rather special models used to generate the simulated data. For example, the models used in several of the papers cited by Hodge et al. assume that all cases of the disease are hereditary, which limits the generalizability of their findings. Furthermore, in the analysis of the simulated data, the correct penetrances of the relevant genotypes are sometimes assumed to be known, which is unrealistically favorable to the HLOD test.

Any power comparison among tests must begin by equating their performance under the null hypothesis—that is, when there is no gene to detect. However, the distribution of the HLOD test statistic under this null hypothesis is complex. Faraway (1993) studied it in the simple, idealized case when the outcome (recombinant vs. nonrecombinant gamete) is known for all informative meioses in all families. Even in this simple case, he found the distribution to be complicated, and he suggested using an approximation to it. In practice, the recombinant statuses of all meioses are seldom known, and probability distributions must be assigned to them. It is not clear whether Faraway's results extend to this situation.

Moreover, Faraway did not evaluate agreement between his approximate distribution and the true distribution in the extreme tails of the latter. Lander and Kruglyak (1995) have argued that pointwise linkage-test statistics must achieve a nominal significance level of $\sim 10^{-5}$, in order to provide an overall significance level of .05 in a genomewide scan. We know little about the performance of the HLOD test statistic (*a*) in the extreme

tails of its null distribution and (*b*) when the recombinant statuses of informative meioses must be inferred. In contrast, the null distribution of the NPL test proposed by Kong and Cox (1997) has been shown to conform well to the theoretical distribution on which its *P* values are based, even in its extreme tails (Nicolae et al. 1998). This issue is important, because even a small inflation of the pointwise type I–error rate could yield an overall false-positive rate that unacceptably exceeds the nominal 5% level.

In conclusion, we thank Hodge et al. for supporting our warnings that estimates of α can be misleading. And we agree with them on the need for further research on the relative power of HLOD and NPL tests to detect linkage. This research should examine test sizes in the tails of the null distributions. The models used to generate the simulated data should include nonhereditary disease, at least two disease-causing genes whose variants have different penetrances, and genes whose variants are common enough so that some families segregate more than one of them. The models used to analyze the simulated data should not be based on the correct values of either the phenocopy rate, the penetrances, or the deleterious-allele frequencies. Meanwhile, whatever may be the possible virtues of the HLOD test, we believe that its use for detection of linkage presents unresolved difficulties.

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