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The Transmission/Disequilibrium Test Detects Cosegregation and Linkage

To the Editor:

In a recent paper (Spielman et al. 1993), we described a test of linkage between a disease and a marker locus, to be used when there is a population association between the two. We named this test the "transmission/disequilibrium test" (TDT). Because the TDT is family based, it distinguishes between association due to linkage (with linkage disequilibrium) and association that may arise in the absence of linkage, such as that due solely to artifacts such as population stratification. We showed that, subject to statistical fluctuation, rejecting the null hypothesis specified by the TDT implies a recombination fraction (θ) of $<\frac{1}{2}$, i.e., linkage between marker and disease. However, Hodge (1993, p. 368) has claimed that our test and similar tests (Parsian et al. 1991) "do *not* detect cosegregation . . . [and therefore] are not linkage tests, at least not in the usual sense of the term." Similar statements appear several times in her paper (see, e.g., sec. 4 of her Discussion; p. 379). Hodge also claims that the Parsian et al. test (and by implication the TDT also) "actually detects the same phenomenon as a population association test does, and it does not test for linkage" (p. 376). We argue here that Hodge's assertions rest on unusual definitions of "linkage" and "cosegregation."

Hodge thus makes claims about what the TDT does and what it does not do. We dispute both these claims. With regard to what the TDT *does:* It is well known that association between disease and marker can arise from linkage (with linkage disequilibrium) or, in the absence of linkage, as a consequence of population stratification, for example. Discrimination between these possibilities is achieved by the TDT; in the latter case, the TDT gives no evidence for linkage. Hodge does not dispute this point. She says, "These tests detect exactly what population-based association tests detect (except that they are not subject to problems of population stratification or improper controls, as the population-based tests are)" (Hodge 1993, p. 380). But what appears here as a parenthetical qualification is the raison d'être of the TDT. Thus it is wrong to say without qualification, as she does, that the TDT "actually detects the same phenomenon as a population association test does" (p. 376).

With regard to what the TDT does *not* do: Two aspects of Hodge's claims deserve clarification. First, despite her reference to "the usual sense of the term [linkage]," Hodge's conclusion that the TDT and Parsian tests "are not linkage tests" is based on an unusual definition of "linkage." The ordinary usage (that of Mendel; or see Ott 1991, p. 6) is the following: If θ is the recombination fraction between two traits or loci, then $\theta = \frac{1}{2}$ for unlinked loci, and $\theta < \frac{1}{2}$ for linked loci; this is the customary way to *define* linkage. A linkage test is a test of the hypothesis $\theta = \frac{1}{2}$, and conventionally, when we reject the hypothesis $\theta = \frac{1}{2}$, we infer linkage. Hodge argues, however, that even when we find $\theta < \frac{1}{2}$, we cannot always infer linkage, since according to her there are some cases with $\theta < \frac{1}{2}$ that we should not designate as "linkage." (Specifically, she argues that for her example, even though $\theta = 0$, the term "linkage" should not be used.)

Hodge's conclusion rests entirely on her unusual use of the term "linkage." In view of the normal purpose of a linkage test, we prefer to use "linkage" *whenever* we reject the hypothesis $\theta = \frac{1}{2}$ (i.e., whenever we find $\theta < \frac{1}{2}$), and this is the sense of "linkage" tested for by our procedure. Hodge acknowledges that she herself uses our test to rule out the possibility of association occurring despite $\theta = \frac{1}{2}$, i.e., "to determine whether the population association is real or artifactual" (Hodge 1993, p. 376). For her computer-generated data with $\theta = 0$, she uses the TDT and rejects the null hypothesis with a χ^2 of 9.38 and 1 df. The null hypothesis of the TDT is $\theta = \frac{1}{2}$. Although this null hypothesis is not stated by Hodge, it must be what she is testing. We conclude that she does not view our test procedure as invalid and that the argument concerns only the use of the word "linkage" to describe certain situations. (E.g., she argues that her model with $\theta = 0$ and the disease

allele not "necessary," i.e., her S model, is not a case of linkage.)

Second, we consider the claim that our test, and that of Parsian et al., does not detect cosegregation and is therefore not a test of linkage in the usual sense of the term. From her use of "cosegregation" in this context, we understand Hodge to restrict her use of the term to families in which multiple meiotic products from one parent are observed in affected offspring, i.e., in which an informative parent has two or more affected children (multiplex sibship). This is the situation in studies of affected sib pairs. In such cases, or in classical linkage studies that use lod methods, it is not necessary to have population association; the meiotic products from one parent can reveal cosegregation directly, in the form of departure from independent assortment. An informative parent is either in coupling or in repulsion phase, and therefore *within the parent* there is maximum possible departure from equilibrium, so to speak. If there is linkage, the two phases will not be equally frequent among affected offspring.

But the restriction imposed by Hodge is arbitrary. Cosegregation between marker and disease can be observed even if there is *only one* meiotic product (one affected offspring, a simplex sibship) per informative parent. Since now there will be only one segregation per parent, the "meiotic products" are assembled from different families. However, the detection of linkage in this case requires population association between marker and disease, i.e., linkage disequilibrium as well as linkage. This requirement implies that coupling and repulsion phases are not equal in the population, a situation analogous to, but much less extreme than, that in the informative parent in a multiplex family. *However, the cosegregation of marker and disease is equivalent in the two cases.* In one case, multiple meioses are observed from one parent; in the other, they are accumulated from multiple parents.

For many "complex" diseases, the affected child is the only affected family member. In this regard, the fact that our test and that of Parsian et al. do not require multiple affected offspring is a considerable virtue, not a drawback, since it allows us to use data from these families.

We conclude that (*a*) since both our test and that of Parsian et al. accept or reject the null hypothesis $\theta = \frac{1}{2}$, they are tests of linkage in the classical sense of "linkage," contrary to claims of Hodge (1993); (*b*) both classical linkage analysis, based on multiple (affected) offspring, and the TDT (with one affected offspring per parent) test for cosegregation of marker and disease, but in different ways; and (*c*) therefore Hodge's argument that certain cases where $\theta < \frac{1}{2}$ (specifically $\theta = 0$) should not be termed "linkage" reflects an idiosyncratic definition. It is incorrect to conclude that the TDT does not detect linkage.

RICHARD S. SPIELMAN,* RALPH E. MCGINNIS,* AND WARREN J. EWENS†
**Department of Genetics, University of Pennsylvania School of Medicine, and †Department of Biology, University of Pennsylvania, Philadelphia*

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Reply to Suarez and Hampe and Spielman et al.: Cosegregation, Association, and Linkage

To the Editor:

The letter writers have raised a number of thoughtful points, and I welcome the opportunity to discuss them further. The critical issues, it seems to me, concern the distinction between "association" and "cosegregation," as I will try to make clear. My response will be in seven parts.

1. One point made by Suarez and Hampe (1994), after their instructive history of association studies, concerns cosegregation. They maintain that the family association tests of Parsian et al. (1991) and Spielman et al. (1993) are tests of cosegregation "in the same spirit" as affected-sib-pair linkage tests are, whereas I had ar-