twofold increased risk nearly 85% of the time. This is clearly unacceptable in a positional cloning project. While the precise numbers change, the general conclusion applies to all types of meiotic mapping data.

The simple argument presented above underscores the difficulty of finely mapping genes underlying complex traits. This situation is in contrast to that of a rare simple Mendelian trait, for which the gene *always* lies in the region of maximal sharing delimited by the closest flanking recombinants. Complex traits are different because a single recombinant cannot be trusted to rule out a region as the gene's location—the observed lack of allele sharing may instead reflect the fact that an affected individual happens not to carry the susceptibility gene.

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References

Kruglyak L, Lander ES (1995) High-resolution genetic mapping of complex traits. Am J Hum Genet 57:1212–1223

Risch N (1990*a*) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222–228

——— (1990*b*) Linkage strategies for genetically complex traits. II. The power of affected relative pairs. Am J Hum Genet 46:229–241

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Likelihood Ratio Tests for Linkage and Linkage Disequilibrium: Asymptotic Distribution and Power

To the Editor:

Terwilliger (1995) proposes an interesting likelihood ratio test for linkage disequilibrium that appears conservative under the null hypothesis and powerful when one of several alleles is positively associated with the disease. In a model where p_j is the population frequency of marker allele j, p_D is the population frequency of the disease allele, and λ is a parameter specifying the magnitude of the linkage disequilibrium, he defines the log-likelihood of the data conditional on allele i being positively associated with the disease to be $\ln[L_i(\lambda)] = \Sigma_j [X_j \ln(q_j) + Y_j \ln(r_j)]$, where the observed counts of marker allele j on disease and control chromosomes are X_j and Y_j and where the predicted allele frequencies are $q_j = p_j + \lambda(1 - p_j)$ and $r_j = p_j - \lambda(1 - p_j)p_D/(1 - p_D)$ when $j \neq i$ (i.e., the associated allele); and $q_j = p_j - \lambda p_j$ and $r_j = p_j + \lambda p_j p_D/(1 - p_D)$ when $j \neq i$ (i.e., the nonassociated alleles). (Incidentally, the likelihood function in eq. [1] in the paper should be a product rather than a sum, although the correct formula was used in the computer program that implemented the test.) He then defines the overall likelihood to be a weighted sum of the conditional likelihoods over all marker alleles; that is, $L(\lambda) = \Sigma_i p_i L_i(\lambda)$. A likelihood ratio statistic is then

$$\Lambda = 2\{\mathrm{Max}_\lambda[\ln[L(\lambda)]]/\{\ln[L(\lambda = 0)]\} . \qquad (1)$$

This statistic assumes that allele frequencies are known (as in standard linkage analysis); when allele frequencies are uncertain, a similar likelihood ratio statistic can be defined by maximizing the numerator likelihood with respect to λ and allele frequencies jointly and maximizing the denominator likelihood with respect to allele frequencies only. In either case Λ is assumed to be asymptotically distributed as a 50:50 mixture of 0 and χ_1^2 under the null hypothesis (H_0, $\lambda = 0$). The reasoning given for the 50% point mass at 0 is that the test is "one-sided" (that is, H_0, $\lambda = 0$, is tested against H_1, $\lambda > 0$). Thus, the numerator likelihood will maximize at $\lambda = 0$ (giving $\Lambda = 0$) whenever the unrestricted maximum falls in the inadmissible region $\lambda < 0$, and this occurs with probability 0.5 under H_0.

Using this null distribution for Λ, however, Terwilliger found that the test tended to be conservative. This finding suggests that this distribution is incorrect and that the standard argument for a "one-sided" test does not apply to Λ. To simplify matters in order to gain insight into the apparent "anomalous" behavior of Λ, it is helpful to consider a particular situation under which Λ has some properties similar to LOD scores for phase-unknown sibships. The situation is when both the disease and marker loci are biallelic with known allele frequencies $p_1 = p_2 = p_D = \frac{1}{2}$. In this special case, given the observed data (X_1, X_2, Y_1, Y_2), the overall likelihood can be written as

$$L = (\tfrac{1}{2})\theta^R(1 - \theta)^{N-R} + (\tfrac{1}{2})\theta^{N-R}(1 - \theta)^R , \qquad (2)$$

where $R = X_1 + Y_2$, $N = X_1 + X_2 + Y_1 + Y_2$, and $\theta = (1 - \lambda)/2$. This likelihood function is identical in form to that of a phase-unknown sibship in which there are R gametes of one type and $N - R$ gametes of the other, and where θ is the recombination fraction. In both cases H_0 corresponds to $\theta = \frac{1}{2}$, so that R is a binomial random variable with parameters $(N, \frac{1}{2})$. It is clear that R and $N - R$ are interchangeable without affecting the value of L, so we can set $R \leq N - R$ and define $K = N - 2R$. Thus,

$$L = \theta^R(1 - \theta)^R[\theta^K + (1 - \theta)^K]/2 . \qquad (3)$$

The likelihood ratio is therefore

$$LR = \{\theta^R(1-\theta)^R[\theta^K + (1-\theta)^K]/2\}/(1/2)^{R+K}, \quad (4)$$

so that

$$\begin{aligned} 2\ln(LR) = 2\{&(R + K - 1)\ln 2 \\ &+ R[\ln\theta + \ln(1-\theta)] \\ &+ \ln[\theta^K + (1-\theta)^K]\}. \end{aligned} \quad (5)$$

The likelihood ratio test statistic (Λ) is the maximum of this function with respect to θ, over the admissible range $0 \leq \theta \leq 1/2$. Omitting some mathematical details, the first and second derivatives of $2\ln(LR)$ with respect to θ evaluated at $\theta = 1/2$ are

$$\{d[2\ln(LR)]/d\theta\}_{\theta=1/2} = 0 \quad (6)$$

and

$$\{d^2[2\ln(LR)]/d\theta^2\}_{\theta=1/2} = 4K(K-1) - 8R. \quad (7)$$

It follows that $2\ln(LR)$ is either a maximum or minimum at $\theta = 1/2$, depending on whether $4K(K-1) - 8R$ is negative or positive. A maximum at $\theta = 1/2$ would imply $\Lambda = 0$, since in this case there is no other stationary point in the likelihood function. The probability that $\Lambda = 0$ is therefore equal to the probability that $2R > K(K-1)$, which implies $(K^2/N) < 1$. Under H_0, as N increases, R approaches a normal distribution with mean $N/2$ and variance $N/4$, so that $K^2/N = (N-2R)^2/N$ becomes approximately χ^2_1. The probability that $\Lambda = 0$ is therefore equal to the probability that $\chi^2_1 < 1$, which is ~ 0.68. Λ is therefore not asymptotically distributed as a 50:50 mixture of 0 and χ^2_1, but a mixture of 0 with probability .68 and some other distribution with probability .32. The nonzero part of the distribution, which can be determined exactly for any given value of N, applies when $(K^2/N) > 1$, that is, $R < (N - \sqrt{N})/2$. (It is not χ^2, because the log-likelihood function is 0 at both the maximum and H_0 [$\theta = 1/2$], which violates one of the regularity conditions assumed by standard asymptotic theory.) However, since both Λ and K^2/N increase monotonically with decreasing R for $R < (N - \sqrt{N})/2$, they are perfectly correlated in rank and are equivalent in the sense that if the true distribution of each statistic is known, the two tests will produce identical P values on the same data, provided that Λ is positive. Since the asymptotic distribution of K^2/N is known to be χ^2_1, it provides a more convenient test than Λ. Similarly, it may be possible to construct a test that is almost equivalent to Terwilliger's test for linkage disequilibrium but with the advantage of a simpler asymptotic distribution.

The fact that LOD scores for phase-unknown sibships maximize at $\theta = 1/2$ with probability $\sim .68$ under nonlinkage may be surprising to some. Because the likelihood is symmetrical about $\theta = 1/2$ for phase-unknown sibships (i.e., the LOD scores at $\theta = 1/2 - t$ and $\theta = 1/2 + t$ are identical), it is easy to be misled into thinking that if the sibship size is large, the LOD score function will almost never maximize at exactly $\theta = 1/2$, so that the maximum LOD score is almost never 0. For example, Risch (1989) states that for a phase-unknown sibship the LOD score (scaled by a factor of 2ln10) is distributed asymptotically as χ^2_1. It is interesting that if there are J phase-unknown sibships and the sibshipwise LOD scores are summed to give an overall LOD score function, then the probability that the maximum total LOD score is 0 is $P[2\,\Sigma_j\, R_j > \Sigma_j\, K_j(K_j - 1)]$, which is asymptotically $P(\chi^2_J < J)$. As J becomes large, this probability tends to .5, so that the maximum total LOD score is 0 with probability .5. This result suggests that LOD scores (scaled by a factor of 2ln10) based on a large number of families can be referred to one-sided χ^2 test, regardless of whether the constituent families are phase known or phase unknown.

This mathematical demonstration applies to Terwilliger's test in only one extremely simple situation. It is not clear how the null distribution of Λ behaves under other more general conditions. However, Terwilliger's simulations show that as the number of alleles increases, the test becomes even more conservative, so that the deviation of the null distribution from the assumed distribution is not confined to a few isolated situations. The test would become more powerful if the correct null distribution (obtained, for example, by Monte Carlo methods) were used instead of a conservative null distribution.

It is interesting to note that in spite of the use of a conservative null distribution, Terwilliger's test appeared to be more powerful than a conventional Pearson χ^2 test when applied to simulated data. One reason for this may be that the test was specifically designed to detect a single common marker allele positively associated with the disease, and the test data were simulated under this condition. The standard Pearson χ^2 test does not particularly favor the detection of a single common positively associated allele, and so it is expected to be less powerful than the new test in this situation, although it may be superior under a different situation—for example, if there were more than one allele positively associated with the disease. When we investigated the power of several other test statistics for association on a real data set relating to fragile X, we found that tests that aim to detect association with a single allele were inferior to tests that take account of differences between observed and expected frequencies of all alleles (Sham and Curtis 1995). It is a general principle that tests that deliberately set out to detect evidence for a particular alternative hypothesis will be more powerful, if the par-

ticular alternative hypothesis is close to reality, than tests that are more general or "model free." Which tests will be more appropriate for the detection of linkage disequilibrium will depend on the nature of linkage disequilibrium that tends to occur in real situations, and this is a topic that merits further investigation.

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References

Risch N (1989) Linkage detection tests under heterogeneity. Genet Epidemiol 6:473–480

Sham PC, Curtis D (1995) Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 59:97–105

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. Am J Hum Genet 56: 777–787

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Reply to Sham et al.

To The Editor:

Many solutions to the problem of why my proposed statistic (Terwilliger 1995) for detection of linkage disequilibrium is overly conservative have been proposed. Some have gone so far as to suggest that the obvious typographical error in my equation (1), which Sham et al. [1996, in this issue] point out should have been written as

$$L_i = \prod_{j=1}^{m} q_j^{X_{ij}} r_j^{Y_{ij}},$$

was actually an endemic mathematical error in my model. Let me assure you that the simulations and the software have all been performed using the correct form of equation (1), as shown above; the error in the manuscript is purely a typographical one. This matter has nothing to do with the overconservative distribution observed. Sham et al. (1996) rationalize that the observed increased point mass at zero is analogous to the already well-studied situation in phase-unknown linkage analysis (e.g., Nordheim et al. 1984). I partially explained the increased point mass at $\alpha = 0$ for the multipoint statistic by arguing that "the admissible proportion of the total parameter space becomes smaller and smaller" with an increase in the number of markers (Terwilliger 1995, p. 784). It has been further brought to my attention (M. Knapp, personal communication) that the maximization procedure I employed in some cases does not fully maximize the likelihood over allele frequencies and λ, leading to a decrease in the value of the statistic in some situations. It is likely that all of these factors play some role in why the assumption of the $0.5\chi^2_{(1)}$ distribution for this statistic is overly conservative, but to date I know of no concrete answer to what the actual distribution is.

Sham et al. (1996, p. 1093) claim that my approach "assumes that [marker] allele frequencies are known." In point of fact, I have never made this assumption—I always have treated them as nuisance parameters in the analysis. It is trivial to analytically maximize the null hypothesis likelihood over the allele frequencies, but under the alternative hypothesis, it is a very complicated numerical maximization problem. When the number of alleles becomes large, it becomes an extremely computationally intensive task. To make the maximization more tractable, I restricted the admissible parameter space for the allele frequencies when maximizing the likelihood.

The effect is that sometimes a global maximum is not achieved, especially when there are either a number of rare marker alleles, or a rather small data set. This effect contributes to some of the conservativeness of the statistical method I proposed. However, the effect is not large unless the true state of nature in compatible neither with the null hypothesis of no association nor the specified alternative or there are a number of marker alleles that occur very infrequently in the data set at hand. Improved software for the globally maximized likelihood calculations for both case-control linkage disequilibrium analysis and haplotype relative risk analysis is available via anonymous ftp from ftp.well.ox.ac.uk.

The letter by Sham et al. (1996) is largely devoted to a rediscovery of previously characterized properties of phase-unknown likelihoods. A comprehensive theoretical analysis of this situation has been made by Nordheim et al. (1984, p. 785), who examined the "unusual performance of likelihood methods for genetic linkage models with unknown phase" because the "maximum likelihood method for recombination frequency yields estimates of 0.5 for many possible sets of data." The issue has been considered separately by many other investigators as well (e.g., Tai and Chen 1989; Dcerge 1995). In my article (Terwilliger 1995), I drew an analogy to phase-unknown linkage analysis in describing how my likelihoods are computed. However, the analogy is not direct beyond there, because,