1990s, but, in addition, the majority of implementations of linkage statistics in commonly used software do not suffer from this "bias" toward the null hypothesis in the presence of uninformative families. Furthermore, the use of highly informative markers in a multipoint analysis will result in very few families being fully uninformative for IBD sharing.

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References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30:97–101

Cordell HJ (2004) Bias toward the null hypothesis in model-free linkage analysis is highly dependent on the test statistic used. Am J Hum Genet 74:1294–1302

Cottingham RW, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A (2000) Allegro, a new computer program for multipoint linkage analysis. Nat Genet 25:12–13

Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3–19

Hinds D, Risch N (1996) The ASPEX package: affected sib-pair exclusion mapping. Available at: http://aspex.sourceforge.net/. Accessed August 2, 2004

Holmans P (1993) Asymptotic properties of affected–sib-pair linkage analysis. Am J Hum Genet 52:362–374

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454

Ott J (1989) Computer-simulation methods in human linkage analysis. Proc Natl Acad Sci USA 86:4175–4178

Risch N (1990) Linkage strategies for genetically complex traits. III. The effect of marker polymorphism on analysis of affected relative pairs. Am J Hum Genet 46:242–253

Schork NJ, Greenwood TA (2004) Inherent bias toward the null hypothesis in conventional multipoint nonparametric linkage analysis. Am J Hum Genet 74:306–316

T.Cuenco K, Szatkiewicz JP, Feingold E (2003) Recent advances in human quantitative-trait–locus mapping: comparison of methods for selected sibling pairs. Am J Hum Genet 73:863–873

Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. Am J Hum Genet 47: A204

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Conventional Multipoint Nonparametric Linkage Analysis Is Not Necessarily Inherently Biased

To the Editor:

Schork and Greenwood (2004) recently reported that there is an inherent bias toward the null hypothesis in conventional multipoint linkage analysis in which expected values are used for allele sharing between relatives when, in fact, there is no information on their identity-by-descent (IBD) sharing status. The implications of Schork and Greenwood's results are serious, because they suggest that the power of detection of disease genes or QTLs is compromised. Here, we show that their results are based on a comparison of test statistics that have different variance (and, therefore, have different distribution) and so should not be compared directly and that the usual way in which inference is made from multipoint nonparametric linkage is, in fact, correct. In addition, we demonstrate that, for linkage analysis of quantitative traits, the effect of mixing informative and uninformative sib pairs on the test statistic is very small and very unlikely to be of practical importance.

Schork and Greenwood (2004) use the analogy of a coin-tossing experiment to make their main point, and we use the same experiment to contest their conclusion. Suppose a coin is tossed 100 times to test the hypothesis that it is fair (i.e., that it gives a 1:1 ratio of heads to tails). The outcome of the experiment is observed in only 50 tosses, and, of those 50 tosses, 40 are heads. The estimate of the probability of heads ($\hat{p}$) from the observation that 40 of 50 observed tosses are heads is thus 0.80. If we assign the expected values (under the null

hypothesis) for the 50 unobserved tosses (25 tails and 25 heads), the estimate of the probability of heads is 0.65. Schork and Greenwood use the fact that the estimate of 0.65 is <0.80 to make their point that there is an inherent bias toward the null hypothesis when unobserved outcomes are assigned an expected value under the null hypothesis. However, to draw statistical inference from this experiment, we need to compare the observed statistic (in this case, the estimate of the probability of heads) with the variance of that statistic under the null hypothesis (H_0), to create a test statistic. In the case of $\hat{p} = 0.80$, the variance under the null hypothesis is $0.5(1 - 0.5)/50 = 0.005$. If, without loss of generality, we create a test statistic (T) that is the deviation of the estimate from its expectation, divided by the SE of the estimate—that is, $T = (\hat{p} - 0.50)/\sigma(\hat{p}|H_0)$—we obtain the T value $(0.80 - 0.50)/\sqrt{0.005} = 3\sqrt{2}\,(= 4.24)$. The variance of the estimate of 0.65 is $[(50)(0.50)(1 - 0.50) + 0]/100^2 = 0.00125$, and, in this case, the test statistic is $(0.65 - 0.50)/\sqrt{0.00125} = 3\sqrt{2}$. Hence, the two test statistics are identical, and the inference from both experiments is the same, if the correct variance of the sufficient statistic is used. Despite the lower estimate of the probability of heads for the case in which unobserved outcomes were assigned the expected value, the test statistic is the same, because the variance of the estimate is lower. This should not be surprising, because all we have done in the second case is add a constant to a random variable and scale it by another constant.

With linkage analysis, the same analogy holds. Schork and Greenwood (2004) base their conclusions on presented statistics (likelihood-ratio scores) and implicitly assume that the distribution of these test statistics under the null hypothesis is the same for all comparisons, when it is not. For collections of small families, computer programs such as GeneHunter (Kruglyak et al. 1996) and Merlin (Abecasis et al. 2002) calculate the correct variance of the sharing statistic, conditional on all observed marker information; therefore, the correct test statistic and P value are computed (see also Cordell [2004]). For large complex pedigrees, the exact variance of the sharing statistic cannot be computed, and it has been pointed out elsewhere that to assume fully informative markers when there is missing information can reduce power of detection (Kong and Cox 1997). Kong and Cox (1997) present a modification of the test statistic, taking into account that the precision of the estimation of IBD allele sharing varies between pairs of relatives.

Linkage analysis of quantitative traits to map QTL is typically a two-stage procedure with several well-known approximations. In the first stage, IBD probabilities are calculated (or IBD proportions are estimated) and, in the second stage, a regression or variance analysis is performed using the phenotypes and IBD proportions. One implicit assumption of these methods is that the proportion of alleles shared IBD between a pair of individuals is known without error. This is most easily seen in those least-squares regression methods in which the estimated proportions of alleles shared IBD ($\hat{\pi}$) are the x variables, because, in regression analysis, the x values are taken as "fixed." If marker informativeness varies between families (or between pairs of individuals within a family), then this variation is not taken into account in these analyses, and one would not expect these QTL-mapping methods to be invariant with respect to uninformative pairs. The approximation in the use of the expected proportion of alleles shared IBD, instead of the full distribution, has been tested (e.g., by Gessler and Xu [1996]). Gessler and Xu (1996) explicitly make the distinction between the "distribution approach" and the "expectation approach" and conclude that there is little difference between them, in terms of power. Cordell (2004) performed simulations to investigate the "bias" in the test statistic for a number of regression and variance-components QTL-mapping methods. As acknowledged by the author, the simulation parameters used were rather extreme, because there was no sibling resemblance other than that due to a single diallelic QTL, and this QTL explained >90% of the phenotypic variance. Cordell (2004) showed the mean difference in the test statistic when uninformative pairs were left out or were kept in the analysis and showed the SD of that difference for a range of test statistics. However, the scale of the test statistic varies between methods, and the mean and SD of the difference in test statistics do not necessarily show how important these results are in practice. We have performed additional simulations, using both Cordell's parameters and a less extreme set of parameters, and have expressed the mean and SD of the difference in test statistics when uninformative pairs are left out or are kept in, as a function of the average test statistic and the SD of the test statistic. Results are shown in table 1 for the Haseman-Elston LOD (HE-LOD) and variance-components LOD (VC-LOD) methods (see Cordell [2004] for details). Clearly, when put in perspective, the effect on the test statistic either of keeping uninformative pairs in the analysis or of removing them is very small. For example, even in the extreme case of a QTL heritability of 98% and 50% uninformative pairs, the average difference in test statistics is only 4% (HE-LOD) and <1% (VC-LOD) of the average test statistic, and the SD of the difference in test statistics when uninformative pairs are kept in or left out is only 6% (HE-LOD) and 2% (VC-LOD) of the SD of the test statistic. As Cordell (2004) pointed out, the slight increase in the HE-LOD test statistic when uninformative pairs are removed is the result of a decrease in the residual variance in the regression analysis. The decrease in the VC-LOD when uninformative pairs are removed (too small to show in table 1 but reported by Cordell

Table 1

Results from Simulations to Show the Relative Impact of Uninformative Pairs on QTL Analysis for Different Values of Polygenic and QTL Heritability and Different Percentages of Uninformative Markers

Heritability		Uninformative Markers		HE-LOD[b]			VC-LOD[b]		
Polygenic	QTL	(%)	No. of Pairs	$\delta T/E(T)$	$\sigma(\delta)/E(T)$	$\sigma(\delta)/\sigma(T)$	$\delta T/E(T)$	$\sigma(\delta)/E(T)$	$\sigma(\delta)/\sigma(T)$
0	.90	50	1,000[a]	4	7	6	0	1	2
0	.98	50	1,000[a]	4	11	9	0	2	2
.5	.10	10	10,000	0	2	1	0	1	0
.5	.10	20	10,000	0	2	1	0	1	0
.5	.10	30	10,000	0	3	1	0	1	0
.5	.10	40	10,000	0	4	1	0	1	0
.5	.10	50	10,000	0	5	1	0	2	0
.5	.10	60	10,000	0	6	2	0	2	0
.5	.10	70	10,000	0	8	2	0	2	0
.5	.10	80	10,000	0	11	2	0	4	1
.5	.10	90	10,000	0	16	3	0	8	1

[a] The data in the first two rows correspond to the simulated scenario (2) of Cordell (2004) and are based on 10,000 replicates. For all other data, a normally distributed additive QTL was simulated, and results are averages from 1,000 replicates.

[b] δT is the average difference between the test statistic achieved when uninformative pairs are removed from the analysis and the one achieved when they are kept in the analysis. $\sigma(\delta)$ is the SD of the difference between the test statistic achieved when uninformative pairs are removed from the analysis and the one achieved when they are kept in the analysis. $E(T)$ is the average test statistic achieved when uninformative markers are removed from the analysis. $\sigma(T)$ is the SD of the test statistic when uninformative markers are removed from the analysis. All ratios are expressed as percentages.

[2004]) is very small because, presumably, the phenotypes of the uninformative pairs provide information on the estimation of the sibling variance and average covariance, and this information is used in the maximum-likelihood analysis. Hence, removal of uninformative pairs may indirectly decrease information on linkage.

We conclude that commonly used nonparametric allele-sharing methods, as implemented in major statistical-genetics computer programs, do not suffer from an inherent bias toward the null hypothesis when expected values of IBD sharing are used in the absence of observed IBD sharing and that QTL-mapping methods are not invariant but are robust to mixtures of informative and uninformative pairs.

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References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30:97–101

Cordell HJ (2004) Bias toward the null hypothesis in model-free linkage analysis is highly dependent on the test statistic used. Am J Hum Genet 74:1294–1302

Gessler DG, Xu S (1996) Using the expectation or the distribution of the identity by descent for mapping quantitative trait loci under the random model. Am J Hum Genet 59: 1382–1390

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Schork NJ, Greenwood TA (2004) Inherent bias toward the null hypothesis in conventional multipoint nonparametric linkage analysis. Am J Hum Genet 74:306–316

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"Bias toward the Null" Means Reduced Power

To the Editor:

In a recent article published in the *Journal*, Schork and Greenwood (2004) discuss the effects of uncertainty in inferred identity-by-decent (IBD) sharing on nonparametric linkage analysis. Tests based on inferred IBD