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0002-9297/98/6201-0031$02.00

Am. J. Hum. Genet. 62:204–205, 1998

Efficient Strategies for Genome Scanning with Affected Sib Pairs

To the Editor:

Holmans and Craddock (1997) present the results of their investigations into the performance of different approaches designed to reduce the number of genotypings required to detect linkage, using a sample of affected sib pairs and their parents; but their method of evaluation is fundamentally flawed, and hence their results do not provide useful information. Two techniques are applied—sample splitting and grid tightening—to produce a two-stage test. In the first stage a reduced number of subjects and/or markers are genotyped, and only regions reaching a certain LOD-score criterion in stage 1 are followed up, in stage 2, by genotyping of all subjects at all markers. Holmans and Craddock present their results in terms of both the power of the procedure to detect linkage and the number of genotypings required. However, in many cases, increasing the number of subjects genotyped in the first stage actually reduces power. Intuitively, it is clear that the detection of linkage rests on being able to identify regions likely to contain linked markers in the first stage and then to follow them up adequately in the second stage. Yet, Holmans and Craddock's results seem to show that a more thorough search in the first stage leads to a decrease in the probability that linkage will be detected, sometimes to a substantial degree (e.g., from .62 to .52 or from .61 to .48). Furthermore, in 3 of their 18 scenarios they recommend a threshold that is higher for the first stage than for the second. This means that one could find a LOD score >3 in the first stage, which one would have to discard and not follow up, even though, if the same LOD score were to be found in the second stage, it would be taken to imply linkage.

The explanation for these paradoxical findings lies with the test strategy that Holmans and Craddock have used. What they propose as a two-stage test for linkage is to choose in advance a LOD score that must be achieved in stage 2 and then to choose as the stage 1 criterion that LOD score that will produce an overall type I error rate of .05. For example, the stage 2 criterion may be set to 3, and then simulations are performed, with the specified data set and scanning procedure, to discover that LOD score that, if used for the stage 1 criterion, will produce a genomewide probability of .05 for an unlinked locus to get through to stage 2 and produce a LOD of 3. As a test for linkage, this is perhaps valid in a narrow sense, but even intuitively it might be expected to perform badly, since it lacks any intrinsic

appeal. Here, the stage 2 LOD score has no real meaning but just serves to act as a benchmark that must be attained. Values of 3.0, 3.3, and 3.6 are used, but no justification is given for choosing them; nor could there be one. It would be perfectly reasonable to find the power of a procedure to attain a certain LOD score or to attain a statistic having a certain *P* value (type I–error probability). What makes no sense is to aim to attain a certain LOD score in the second stage but then to achieve a specified type I–error probability overall by manipulating the threshold applicable for the first stage. The more natural approach, which I am sure would yield completely different results with regard to power and efficiency, would be to fix the threshold for the first stage (probably at .5–1.0) and, in the second stage, to aim either for some predetermined LOD score or for a LOD producing a certain overall *P* value.

The effects of Holmans and Craddock's approach are clear to see. The stage 1 criterion has to be made high enough so that only a small number of unlinked regions will achieve it and hence go on to produce false-positive results in stage 2. The more subjects and markers that are typed in the first stage, the more likely it is that high LOD scores will be thrown up by chance, and hence the higher the stage 1 criterion must be set. The higher this criterion is, the harder it may be for a truly linked locus to achieve it, and so such loci may be more frequently discarded. Thus, doing more genotyping in the first stage generally leads to a reduction in power, despite involving an increase in the total amount of genotyping required.

The first scenario that Holmans and Craddock present illustrates this clearly. A wide, 20-cM grid is used for stage 1, narrowing to 10 cM in stage 2, and the LOD score to be taken to indicate linkage, after stage 2, is chosen to be 3. When only 100 of the 200 sib pairs are typed, the threshold to move from stage 1 to stage 2 is set to a modest and sensible .89, and the overall power is .62. However, when all 200 pairs are typed, the stage 1 threshold has to be raised to 2.14, so many true linkages are missed, and the power falls to .57. Using 100 pairs together with their parents needs a threshold of 1.57 and yields a power of .52. Finally, initially using all 200 pairs and their parents apparently demands a stage 1 threshold of 3.1 and has a power of only .54. This would mean that, if one got a LOD of 3.05 with the initial 20-cM grid scan, one would not follow up this finding, even though it would count as a positive result if it were to be found in stage 2.

Given that genotyping is becoming ever cheaper and easier, given that linkage can easily be missed in sib-pair samples, and given that performing a genome scan but missing a disease locus is highly undesirable, my own personal view is that the initial scan should probably be fairly thorough, using all available subjects and a relatively narrow marker grid.

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0002-9297/98/6201-0032$02.00

EDITOR'S NOTE.—This letter is a truncated version of the letter submitted by Dr. Curtis.

Am. J. Hum. Genet. 62:205–207, 1998

Reply to Curtis

To the Editor:

Curtis (1998 [in this issue]) has raised some criticisms regarding our paper on efficient strategies for genome screening for linkage (Holmans and Craddock 1997). We reply to them as follows:

Curtis has said that our decision to fix the stage 2 criterion "lacks any intrinsic appeal" and "makes no sense." However, we would like to point out that judgements regarding the significance of a linkage study are generally based on the final LOD score obtained. Therefore, to facilitate comparison between the various strategies, it is desirable that a given stage 2 LOD score should correspond to the same significance level in all the strategies, as far as possible. This can most easily be done by fixing the stage 2 criterion and varying the stage 1 criterion, to obtain the desired type I error probability. In practice, one would not regard such criteria as benchmarks of "significant" versus "nonsignificant" linkage—their purpose is to ensure a fair comparison of the power of the various strategies and as a guide to which LOD scores correspond at *P* value of $\leqslant .05$.

We chose 3.6 as one of our criteria since this was recommended by Lander and Kruglyak (1995) as corresponding to a genomewide *P* value of .05 and is in widespread use. The criterion of 3.0 was chosen as the traditional criterion for significant linkage. The criterion 3.3 was adopted when it became clear that 3.6 was too stringent for the strategies to give a *P* value of .05. It is clear from our results that higher criteria would make