Am. J. Hum. Genet. 54:715–718, 1994

Replication of Genetic Linkage by Follow-up of Previously Studied Pedigrees

Elliot S. Gershon and Lynn R. Goldin

Clinical Neurogenetics Branch, National Institute of Mental Health, Bethesda

Summary

Independent replication of linkage in previously studied pedigrees is desirable when genetic heterogeneity is suspected or when the illness is very rare. When the likelihood of the new data in this type of replication study is computed as conditional on the previously reported linkage results, it can be considered independent. We describe a simulation method using the SLINK program in which the initial data are fixed and newly genotyped individuals are simulated under $\theta = .01$ and $\theta = .50$. These give appropriate lod score criteria for rejection and acceptance of linkage in the follow-up study, which take into account the original marker genotypes in the data. An estimate of the power to detect linkage in the follow-up data is also generated.

Introduction

How can linkage be replicated when it is initially found in a complex disease? In general, replication requires a new experiment with new data. Because of possible genetic heterogeneity, it has appeared prudent to develop new information on linkage in previously positive pedigrees. A linkage finding can be "replicated" in the same pedigrees for which significant results were originally found, either when additional marker data make more meioses informative or when new case onsets or new relatives are studied. It is self-evident that repeat performance of the same experiment in the same individuals, even with different methods, may uncover errors in the original experiment, but it is not a replication. In assessing the significance of a replication, inclusion in a new analysis of both new and previously adduced information can bias the replication, if it is not correctly accounted for.

In three notable instances, genetic linkages to psychiatric disorders have been tested on additional information from the pedigrees in which linkage was originally reported (Kelsoe et al. 1989; Baron et al. 1993; Gurling and Sharma, in press). In each instance, the new information was not supportive of linkage. The investigators in two of the reports noted that, in part of the original pedigrees, lod scores remained positive, although not greater than 3, which implies that linkage was not clearly rejected (Kelsoe et al. 1989; Baron et al. 1993). In the present paper, we consider what constitute appropriate tests of linkage in follow-up studies of this kind.

When attempting to replicate linkage by using additional information from a pedigree or a series of pedigrees, ascertainment becomes an issue. In the standard lod score analysis of linkage in pedigrees, ascertainment is generally disregarded, for the following reasons described by Elston: "as a function of θ (recombination fraction) and of Φ (phase of marker and trait), maximizing the lod score is equivalent to maximizing the likelihood of the marker data conditional on the trait data. This conditional likelihood does not depend on the mode of ascertainment, provided that, conditional on the trait data, the marker data and the mode of ascertainment are independent" (Elston 1989, p. 487). When a pedigree is followed up precisely because of the previous positive finding, however, the marker data are not independent of the ascertainment. The follow-up marker/clinical data are conditional on the original clinical phenotype and marker data.

It is incorrect to add new data on genotypes and

Received March 4, 1993; accepted for publication December 15, 1993.

Address for correspondence and reprints: Elliot S. Gershon, M.D., CNG/NIMH, 10/3N218, 9000 Rockville Pike, Bethesda, MD 20892.

diagnoses to the initial data and then report a new total lod score as if it were a separate result, because of the bias in favor of linkage and because of the general principle of statistics whereby increasing the number of statistical tests on the same data increases the type I error (see Chotai 1984). In some instances, one can derive a conditional lod score for an enlarged pedigree. It is demonstrated elsewhere, for a case of linkage analysis when there is ascertainment of nuclear families through a proband with a particular phenotype *and* marker genotype, that the ascertainment probability is the same as the unconditional likelihood of the particular genotype/phenotype set chosen for an acceptable nuclear family (Gershon and Matthysse 1977). The same reasoning holds for a nuclear family ascertained through an affected sib-pair with particular marker genotypes and in some other instances.

The conditional likelihood of (the genotype/phenotype distribution of) such a pedigree, X, ascertained through (the genotype/phenotype distribution of) a portion of it, Y, can be expressed in terms of unconditional likelihoods as $L(X,\theta)/L(Y,\theta)$, where L is the unconditional likelihood function under a given genetic model. The lod score can then be expressed as $(\log_{10} L[X,\theta] - \log_{10} L[Y,\theta]) - (\log_{10} L[X,.5] - \log_{10} L[Y,.5])$. With rearrangement, this becomes the difference between two lod scores, $\text{lod}(X,\theta) - \text{lod}(Y,\theta)$.

On follow-up data in a family with appropriate structure, to calculate a lod score that is not inflated by the previous positive lod score, we can use the same reasoning. Consider a pedigree in which A is the distribution in the original pedigree of phenotypes and markers and B is the distribution in the follow-up data. The lod score–testing replication, conditional on the original data, is the difference between lod scores, i.e., $\text{lod}(A \text{ and } B,\theta) - \text{lod}(A,\theta)$.

This is a test of replication that is not biased by the previous result. One could choose the value of θ on which the lod score maximized in the initial report, or one could maximize the value of the expression as a function of θ. This example is chosen to emphasize the point that it is the increment or decrement in lod score that is of interest when a previously linked pedigree is followed up with new data and not whether the total lod score remains above a certain value.

For most pedigree data, however, it may not be possible to partition the likelihoods into new versus old meioses. Another approach would be to determine the significance of the new result by simulation. We will demonstrate this approach by using a fictitious pedigree (see fig. 1). It should be noted that this structure was chosen for convenience of simulation only.

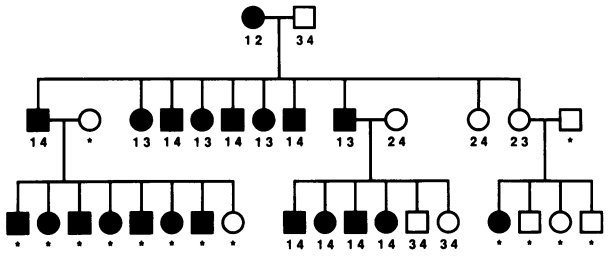

Figure 1 Pedigree with 33 individuals. Blackened symbols indicate affected individuals. Marker genotypes with alleles 1–4 are *fixed* as shown for 19 individuals and are simulated for the remainder (indicated with an asterisk [*]). The maximum lod score for the pedigree with the 19 individuals genotyped and clinical information on all 33 is 3.39 ($\theta = 0$). When there is no clinical information on the third generation, the lod score is 4.06 ($\theta = 0$).

Methods

A simulation example in one pedigree (fig. 1) is offered in support of the assertion that the lod score of a followed-up pedigree cannot be evaluated as if it were an independent result. For simplicity, the new information in the pedigree was designed not to change the apparent phase and informativeness of the original data. That is, the ancestral couple and the persons marrying into the pedigree do not have changed diagnoses (on follow-up), so that the original information, which implies a coupling phase between illness and allele 1 of the marker locus, is unchanged in the follow-up analysis.

The initial part of this pedigree has 19 persons genotyped as shown in figure 1. The marker genotypes were chosen purposely so that linkage would be present. Under a model of dominant illness inheritance with reduced penetrance (frequency of disease allele = .01; penetrances of the three genotypes at the disease locus are .8, .8, and .01), the maximum lod score is 3.39 at $\theta = 0$. Assuming the same model of inheritance, we used the program SLINK (Ott 1989; Weeks et al. 1990*b*) in order to simulate marker genotypes (four equally frequent alleles) for an additional 14 persons in the two sibships indicated in figure 1, which can be considered the follow-up study. The additional individuals were simulated under linkage at $\theta = .01$ (1,000 replicates) and under *no* linkage at $\theta = .5$ (10,000 replicates). For each simulation, we determined the distribution of lod scores and the average lod score.

Table 1

Distribution of Lod Scores for Complete Pedigree (fig. 1), Simulated under Linkage and Nonlinkage

Lod Score (X)	Percentage of Replicates with Lod Score > X for θ = [a]	
	.01	.50
.5	100	100
1.0	100	97.1
1.5	99.9	77.2
2.0	99.7	48.2
2.5	97.8	20.0
3.0	95.6	9.5
3.5	92.6	5.0
4.0	83.9	2.6
4.5	76.6	1.0
5.0	61.2	.53
5.5	59.1	.44
6.0	0	0

[a] For θ = .01, 1,000 replicates were simulated; for θ = .50, 10,000 replicates were simulated.

Results

The distributions of lod scores under linkage and nonlinkage are shown in table 1. Under the condition of linkage, the average lod score increased to approximately 5.0; whereas under the nonlinkage condition, the average lod score of the enlarged pedigree decreased to 2.07. These changes reflect the additional linkage information in the pedigree. However, under *no* linkage, the lod score was $\geq$3.0 in 9.5% of the simulations. Clearly, if a lod score of 3.0 is the criterion for linkage, then this is an unacceptable type I error rate. To have a 1% type I error in this pedigree, the criterion for replication of linkage would have to be set at 4.5. With this criterion, the power to detect linkage with the information in this followed-up pedigree is 76.6%, under the simulated parameter value θ = .01. One can also use these data to determine a criterion for rejecting linkage. For example, if linkage were rejected when the lod score was <2.0, then a true linkage (at θ = .01) would be rejected <1% of the time.

This simulation demonstrates how the significance level of a lod score can be adjusted in a follow-up study that contains many of the same individuals. This is analogous to having a criterion that is based on the increment of the lod score from the new data, since the initial data were a given for the simulation. In addition, the procedure gives one an estimate of the power of the follow-up replication study to detect linkage, if it is true.

In actuality, it may be difficult to break down data into old versus new informative meioses. If new markers have been genotyped, each marker may be informative for a slightly different subset of the data. When a new system is used at the same locus or when a haplotype is introduced, it may be necessary to go through each individual and determine where there is new information and where there is not, prior to simulation. Another approach would be to carry out the simulations by using multipoint marker data.

What about pedigrees in which follow-up reveals that the previous genotype data must be revised? One solution is for the same simulation to be used, but the revised data would be included with the new data, and the part of the original data that is not revised would become the whole set of original data. When trait phenotypes must be revised, simulation of marker genotypes, of course, will not assess the effect of the change. When the change occurs in an ancestral or marrying-in person, we would consider this a correction of the original data, as opposed to a replication study. The procedure of Hodge and Greenberg (1992), in which lod scores of a pedigree are compared with a particular person considered affected versus unaffected, is useful in identifying persons whose affection status is crucial to the linkage result. These persons are most important to study on follow-up.

In cases where descendants, who were at risk and were followed up, have become ill, one can revise the initial data to omit their prior genotype information and include their new affection status. In a disease with variable penetrance, this should not greatly change the initial conclusions. The simulation of genotypes in the followed-up pedigree would then proceed as discussed above, and the replication results can be rationally assessed.

Discussion

In the type of follow-up study that we have described, one needs to determine a lod score criterion in order to have an appropriate type I error. This is similar to the use of simulation to "correct" a lod score to account for multiple data analyses (Weeks et al. 1990*a*). One would like the overall type I error of the study to be small. However, in complex diseases, the initial type I error rate of the lod score may be inflated because of multiple analysis models, disease classification models, and the number of markers typed (Clerget-Darpoux et

al. 1990). Nonetheless, we can use the simulation method to determine the type I error of a replication study with fixed initial genotypes. One can argue that, since the replication is a test of only *one* hypothesis, the signficance level does not need to be as stringent as when a large number of unlinked markers are initially screened. A *P*-value of .001, or even .01, would be acceptable, as compared with the lod score ≥ 3 criterion, whose asymptotic *P*-value equivalent is .0001, when a fixed-sample χ^2 test is assumed (Ott 1991). Some recent follow-ups of previously studied pedigrees in psychiatry had *reductions* in lod scores of >2 lod units, as reported by the authors in each study (Baron et al. 1987, 1993; Kelsoe et al. 1989; Gurling et al., in press), which would support a conclusion that replication had failed.

There will be some circumstances where linkage replication results may be ambiguous, but other data will serve to confirm the original finding. Finding an association or a candidate gene mutation within the linkage region, e.g., would bypass the issue of replication of linkage. Characterizing the mutation, after all, is the goal of linkage studies in disease.

The increasing density and informativeness of the human genetic linkage map will have an influence on what constitutes a suitable initial report of linkage and also on what constitutes replication. At the present time, linkage should not be claimed until a maximal amount of genetic marker information has been extracted from the pedigrees. Normally, this will require several closely linked, highly polymorphic markers. Inspection of the data, so that there are not parents homozygous at all markers and so that there are not genetic marker map inconsistencies, would also be needed in the initial publication.

In follow-up studies, as elsewhere, replication is a term that should be distinguished from repetition. Replication should apply to new data only; where the new data are conditional on previous data, this should be accounted for in the analysis. In the design of a follow-up replication experiment for genetic linkage, care must be taken that the sample size (of new data) in the replication is adequate to retest the hypothesis of the original experiment. Interpretation of follow-up replication experiments should be done cautiously. The simulation procedure described here, using the SLINK program, provides an empirical basis for assessing the statistical power and significance of follow-up replication results. It is incorrect to invoke the current lod score of an entire pedigree or series of pedigrees as being consistent with linkage, on the basis of a lod score criterion that is appropriate for a pedigree asscertained without knowledge of genotypes. In several actual instances described here, the earlier data in the same pedigrees clearly supported linkage, and the later data clearly do not.

References

Baron M, Freimer NF, Risch N, Lerer B, Alexander JR, Straub RE, Asokan S, et al (1993) Diminished support for linkage between manic depressive illness and X-chromosome markers in three Israeli pedigrees. Nature Genet 3:49–55

Baron M, Risch N, Hamburger R, Mandel B, Kushner S, Newman M, Drumer D, et al (1987) Genetic linkage between X-chromosome markers and bipolar affective illness. Nature 326:289–292

Chotai J (1984) On the lod score method in linkage analysis. Ann Hum Genet 48:359–378

Clerget-Darpoux F, Babron M-C, Bonaiti-Pellie C (1990) Assessing the effect of multiple linkage tests in complex diseases. Genet Epidemiol 7:245–254

Elston RC (1989) Man bites dog? the validity of maximizing lod scores to determine mode of inheritance. Am J Med Genet 34:487–488

Gershon ES, Matthysse S (1977) X-linkage: ascertainment through doubly ill probands. J Psychiatr Res 13:161–168

Gurling HMD, Sharma T. Genetic linkage analysis and clinical approaches to the resolution of heterogeneity in the schizophrenias. In: Gershon ES, Cloninger CR (eds) Genetic approaches to mental disorders. American Psychiatric Press, Washington DC (in press)

Hodge SE, Greenberg DA (1992) Sensitivity of lod scores to changes in diagnostic status. Am J Hum Genet 50:1053–1066

Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, et al (1989) Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. Nature 342:238–243

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

——— (1991) Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore

Weeks DE, Lehner T, Squires-Wheeler E, Kaufmann C, Ott J (1990*a*) Measuring the inflation of the lod score due to its maximization over model parameters in human linkage analysis. Genet Epidemiol 7:237–244

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