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Is Schizophrenia Linked to Chromosome 1q?

Levinson *et al*. (*1*) reported the results of a meta-analysis of families showing no major schizophrenia locus on chromosome 1q. These results, based on a multicenter study of affected sibling pairs (ASPs), are in striking contrast to findings of several recent papers reporting susceptibility loci on 1q in extended families. Significant linkage (LOD = 6.5) at 1q21-22 was detected in Canadian families (*2*) and replicated in European origin families (*3, 4*). At 1q42, Blackwood *et al*. (*5*) obtained a LOD of 7.1 in a single Scottish family, while Ekelund *et al*. (*6*) obtained a LOD of 3.2 in Finnish pedigrees. How can these apparently conflicting results be reconciled? We suggest that locus heterogeneity adequately explains the failure of an ASP study with any reasonable sample size to replicate results from large extended families, and we have strong reservations about the limited interpretation of the results in (*1*).

We considered the effect of heterogeneity in two ways. First, we evaluated the power of the ASP mean test under heterogeneity. The number of sib pairs required to detect linkage is inversely proportional to the square of the proportion of linked families (*7*). Fig. 1 shows the effect of heterogeneity on the power to detect linkage given the effect of an allele segregating in the linked families, which increases risk to sibs by a given factor. Three effect sizes—small (factor 1.35), moderate (factor 3), and large (factor 7)—were considered. As shown, a sample of less than 1000 ASPs, as studied in (*1*), has little power to replicate linkage of schizophrenia to a locus that contributes to risk of illness in less than 20% of families. Note that Levinson *et al.* used the relative risk to siblings of affected individuals across the whole sample [λ_{sibs} in (*1*)] to determine power. Our interest is in showing how large a part heterogeneity plays in determining power. In the case of breast cancer, for example, the BRCA1 and BRCA2 genes have a large effect on risk (10- to 20-fold) in mutation carriers (*8*) but, because they are very rare in most populations, they are not readily detectable in large heterogeneous samples.

We also considered the power of nuclear families using SLINK software (*9*). Sixty families (each with 6 individuals in the sibship, equivalent to 15 ASPs) were simulated under a partially penetrant model and analyzed allowing for heterogeneity (*10*). The power to detect a LOD of 3 decreased rapidly; power for 75%, 50%, and 33% of families with mutations segregating at the gene of interest was 80%, 40%, and 5%, respectively.

In concluding that there is no locus of major effect on chromosome 1q, Levinson *et al*. have not appropriately considered locus heterogeneity. The logistic regression used in (*1*) ignores within-sample heterogeneity. Parametric linkage analysis incorporating heterogeneity is used but only with a recessive model. To ensure good power one must also fit a dominant model (*11*).

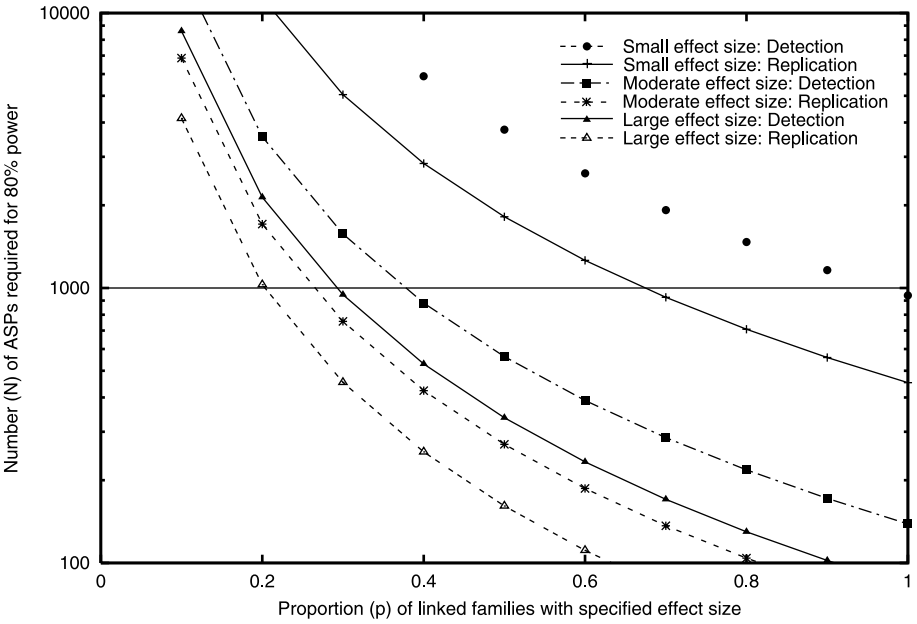

Fig. 1. Power of the ASP mean test at different heterogeneity levels. The power to detect linkage (LOD = 3) and replicaton of linkage (LOD = 1.2) were determined for three effect sizes: small (factor 1.35), moderate (factor 3), and large (factor 7).

Though the results in initial genome scans are likely to be overestimates of effect size, the effects found in the studies reporting linkage to chromosome 1q21-22 and 1q42 are unlikely to be small in magnitude. Such effects will account for a sizable proportion of the variance in liability in particular families. The distribution of risk to schizophrenia can be well described by a model that incorporates genes of major effect and substantial locus heterogeneity. Under heterogeneity, ASP studies will require extremely large samples. Linkage analyses with large families and identification of cytogenetic variants associated with schizophrenia are appropriate strategies when heterogeneity is expected.

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Levinson *et al.* (*1*) reported no evidence of linkage of schizophrenia to chromosome 1q using a combined sample of 779 small nuclear families (average size of 5 individuals) from diverse ethnic backgrounds. Their principal objective was to determine if they could replicate our highly significant linkage (LOD = 6.5, or a likelihood ratio 3 million to 1 in favor of linkage) of schizophrenia to chromosome 1q21-22 (*2*). Notably, we used a different study design, ascertaining 22 larger Canadian pedigrees (average size of 14 individuals) of similar ethnicity, with schizophrenia segregating in a unilineal dominant-like pattern over multiple generations (*2*). This raises the question of whether their failure to replicate linkage says more about the relative merits of the two study designs than it does about the genetics of schizophrenia.

Optimal ascertainment and study design are essential to increase power for gene mapping of complex disorders like schizophrenia, where genetic heterogeneity complicates determination of linkage (*3*). Sampling multigenerational families with similar segregation patterns or from population isolates can help reduce genetic heterogeneity, thus increasing power to detect linkage, as illustrated in hereditary deafness (*4*). Larger families may also provide additional power from unaffected subjects when likelihood methods using all pedigree information are used (*2*). Of course, optimal study design depends on the genetics of the disease being investigated, with some diseases or loci being more amenable to mapping based on smaller families (*5*). Notably, all four schizophrenia genome scans reporting LOD scores >4 have involved larger multigenerational pedigrees, population isolates, or both (*2, 6–8*).

It is not surprising that the Levinson *et al*. study failed to find significant linkage to chromosome 1q, and that subgrouping by ethnicity or number of affected individuals per nuclear family, or using different genetic models or analysis methods, could not overcome the limits imposed by the initial design. It is probable that less than 50% of the families in their combined sample are linked to any particular locus. Combining data sets may actually reduce power to detect linkage because genetic heterogeneity increases. Power may be further lowered by subgrouping, particularly when each family is relatively uninformative for linkage on its own (*9*). Replication may be more difficult than initial linkage detection in complex disorders, even in studies with similar designs. However, Gurling *et al*. (*10*), using 13 multigenerational pedigrees with unilineal segregation of schizophrenia (average size of 14 individuals), did find suggestive linkage of schizophrenia to 1q22-23.

Population-wide effects of the underlying loci may be small despite strong linkage signals in selected samples. But Levinson *et al*. appear to have confused the primary goal of linkage studies—localizing susceptibility genes—with estimating a locus-specific effect size at the population level. Contrary to their report, our study did not predict a population-wide "genetic effect" from the results. Levinson *et al*. [note 23 in (*1*)] inappropriately predicted population-wide relative risk to siblings (λ_{sibs}) from our linkage results (*2*), and then claimed this was an "over-estimate." Linkage studies have identified dozens of loci and genes for hereditary hearing loss, almost all of which are rare (*4*). These genes have provided important information about the pathogenesis of hereditary deafness, but each would have a small locus-specific effect size. This is likely to be the case with many schizophrenia susceptibility loci, including the 1q21-22 locus.

Levinson *et al*. (*1*) concluded that they could not determine whether or not our LOD of 6.5 is a "false-positive" result. But their study was not suited to address such a question because, for complex disorders, follow-up in an independent sample in order to distinguish true- from false-positive initial findings makes neither statistical nor biological sense (*11*). Although no definite conclusions can be drawn until the chromosome 1 gene has been identified, given the strength of evidence for linkage in our small sample of larger, genetically more informative individual families, the failure of Levinson *et al*. to detect linkage to 1q suggests a failure of their study design for this locus. Indeed, the six genome scans using their samples (except for a subgroup of the JHU sample) have failed to find significant linkage of any chromosomal region to schizophrenia.

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Response: Macgregor *et al*. and Bassett *et al.* suggest that linkage findings on chromosome 1q can be replicated in studies of extended pedigrees using parametric heterogeneity lod score analyses, and that our study could not succeed because we used smaller pedigrees and nonparametric statistical methods. We agree on a central point—that there may well be schizophrenia susceptibility genes on chromosome 1q, given the significant findings of four recent studies *(1–4)*. We expect numerous susceptibility genes to be definitively identified in the years ahead, most likely in regions such as 1q, which have produced evidence for linkage in several samples. However, we do not agree that classical locus heterogeneity can explain a substantial proportion of schizophrenia cases, that the structure and ethnicity of our pedigrees explain the difference in results, or that nonparametric methods are inappropriate methods to test for linkage when heterogeneity is present.

We hypothesized (*5*) that linkage to schizophrenia could be identified in one or more regions of chromosome 1q, and that a large pedigree sample could help to localize the findings, as has appeared to be the case in some but not all of our previous studies (*6–15*). Our subsequent multicenter findings did not disprove linkage on 1q, although false positive results could not be ruled out. Our results suggested that if there are susceptibility genes on 1q, their population-wide effects are likely to be small, and that the large linkage scores observed on 1q21-22 could reflect an upward bias due to maximization of linkage statistics across the genome in small samples (*16*). The magnitude of gene effects

within individual subjects could be much larger than the populationwide estimates, but these estimates predict power to detect linkage.

The comments above assert that locus heterogeneity can explain the divergent results on 1q. Heterogeneity is likely in the context of multigenic inheritance, i.e., susceptibility alleles of varying population frequencies in the same gene and in different genes, with additive or epistatic interactions conferring risk of disease. However, both comments argue for classical locus heterogeneity, in which the same phenotype occurs as the result of distinct major locus effects in different families. This model cannot be reconciled with the high risk to MZ twins and low risk to siblings of probands (*17*). Further, if classical locus heterogeneity explained a modest fraction of schizophrenia cases, finding single pedigrees that are sufficiently large and densely-affected to map each locus should be relatively straightforward. Bassett *et al*. point out that this has occurred for many deafness syndromes, but most of these show classical Mendelian inheritance. Very large, dense schizophrenia pedigrees have rarely been found. However, we agree that if a rare genetic cause of schizophrenia could be identified in even one family, this could be of enormous benefit to our understanding of schizophrenia pathophysiology more generally.

The Brzustowicz *et al.* study (*1, 12*; see comment by Bassett *et al*.) illustrates the problem with the classical heterogeneity hypothesis. Their pedigrees, recruited because they appeared to be segregating a dominant disease, included one family with 15 affected cases, and 21 others with an average of 3 affected cases (similar to many of our pedigrees). Contrary to expectation, significant linkage was not reported in the largest family. To explain why their maximum linkage result on chromosome 13q was observed under a recessive model assuming linkage in 75% of families (similar to 1q), Brzustowicz *et al*. suggested (*12*) that common recessive alleles could produce pedigree patterns that appear to be autosomal dominant. But such common alleles should produce many families with two or three affected cases, and one would expect to detect linkage in our Irish and Welsh samples (*5*). Similarly, how would Macgregor *et al*. explain that the strongest support for their 1q42 finding came from a large sample of ASPs from the general Finnish population (*4*)? The most parsimonious explanation would be that population-wide genetic effects of susceptibility genes on chromosome 1q are relatively weak and that stochastic variation in the proportion of families showing evidence for linkage in each sample (especially smaller ones) accounts for the wide variation in linkage results (*16, 18*). This does not preclude the possibility of mapping a gene in a small sample with an atypically high proportion of families in which the gene is segregating—but statistical support for such an association is likely to come from a larger sample.

Both Macgregor *et al.* and Bassett *et al*. suggest that our study design and analysis reduced the power to detect to linkage, but our designs were more diverse than the comments indicate. The Bonn, Cardiff, and Chicago projects recruited primarily ASPs, while the others sought the densest available pedigrees but did not exclude ASPs. Of the 1905 genotyped affected cases, 1210 were from sibships with two ill siblings, and 688 were additional ill siblings, parents, aunts/uncles, grandparents or cousins of probands. Nonparametric analyses were employed because they do not depend on estimates of transmission parameters. Logistic regression analysis was used to determine whether differences among samples significantly affected results. Allele sharing in sibling pairs is the most straightforward dependent variable for this analysis (which is not designed to test for interfamily heterogeneity).

Regarding affected sibling pair analysis, it is well-known that as the proportion of linked families drops below 30 to 40% in a classical heterogeneity model (*19, 20*), all methods of linkage analysis become rapidly less powerful, as Macgregor *et al*. elegantly describe. Parametric and nonparametric analyses have similar power when heterogeneity is present, even though the nonparametric methods do not formally model the heterogeneity. For example, in an unpublished simulation study of 770 pedigrees containing 1000 ASPs plus affected parents and offspring in a proportion of families, we studied two dominant transmission models which produced population-wide λ_{sibs} estimates of 1.27 and 1.25 (55.3 or 55% sharing), which were predicted by theoretical locus-specific λ_{sibs} values of either 1.3 in 100% of families, or of 3.5 in 30% of families (heterogeneity). Power to detect linkage ($P = 0.00002$) was 0.84 and 0.87 for heterogeneity lod score (hlod) analysis under the "correct" model, 0.79 and 0.76 for nonparametric linkage (NPL) analysis, and 0.76 and 0.65 for the maximim lod score (MLS). The scores were intercorrelated at 0.92 for hlod and NPL, and 0.87 for hlod and MLS. ASP (MLS) analysis was most powerful under recessive transmission or if the sample consisted only of ASPs. Hlod, NPL, and MLS scores all can detect linkage in the presence of heterogeneity, but not if the genetic effect in the population being studied is too low.

We also stress that statistical significance of linkage data for complex disorders should be interpreted with caution. Simulation-based P values are generally preferred, because theoretical P values are highly dependent on model parameters, marker informativeness, and other factors. The lod score of 6.5 observed by Brzustowicz *et al*. on 1q21-22 was associated with a simulation-based P-value of 0.0002 to 0.00002 (*1*), or approximately 20:1 or lower genome-wide odds for linkage (*21*) (not 3,000,000:1, which represents a pointwise theoretical value). Bassett *et al*. mention four other studies that yielded lod scores greater than 4, an arbitrary threshold. One is a finding on distal chromosome 2q in a small sample of nuclear families from an isolated region of Finland; the lod score went down when the analysis incorporated genealogical connections among the pedigrees (*22*). Another is a finding on chromosome 6q25 in an extended Swedish pedigree, where the lod score varied considerably depending on allele frequency estimates (*23*). The third is a finding in extended Palauan pedigrees, where the haplotype vectors were constructed by Markov chain Monte Carlo methods which are not exact (*24*). A more cautious intepretation would be that each of these five findings probably achieves but does not greatly exceed the threshold for genomewide significance. Most are from small samples which can produce upwardly biased results. While each of these findings is impressive and will hopefully lead to a successful gene cloning effort, the precise level of significance in each case is not clear cut and is probably not critical: given the weak locus-specific effects that are being detected for schizophrenia, observing evidence for linkage in several studies is probably more important than the precise P value.

We believe that progress is best served by multiple approaches. Samples with various sizes and ascertainment strategies have produced important linkage results. Epidemiological data for schizophrenia are consistent with the hypothesis that there are multiple interacting susceptibility loci and that at least some of these loci may be important in many or most populations (*17*). Very large multicenter or prospectively-ascertained linkage samples can help to confirm and localize some of these findings. Negative results from large studies should not be interpreted as excluding any locus, but positive findings should give strong encouragement to efforts to identify the relevant genes in the implicated regions.

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ERRATUM

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TECHNICAL COMMENTS: Response to a Comment on "No major schizophrenia locus detected on chromosome 1q in a large multicenter sample" by D. F. Levinson (20 Dec. 2002, www.sciencemag.org/cgi/content/full/298/5602/2277a). In further discussion after publication, the authors of the Technical Comment (A. S. Bassett *et al.*) and the Response (D. F. Levinson *et al.*) have concluded that there was an error in the Response. The empirical *P* values reported by L. M. Brzustowicz *et al.* [*Science* **288**, 678 (2000)] were incorrectly interpreted in the Response as pointwise (uncorrected) values, but they were actually corrected for multiple testing as described by F. Bonnet-Brilhault *et al.* [*Eur. J. Hum. Genet.* **7**, 247 (1999)] and C. R. Cloninger *et al.* [*Am. J. Med. Genet.* **81**, 275 (1998)]. The genome-wide *P* value for linkage to schizophrenia on proximal 1q in the Canadian sample was 0.0002 to 0.00002, a highly significant result. The Response also noted that significant linkage had not been reported in the largest family in the Brzustowicz *et al.* sample. As a point of clarification, the Z_{max} in this family at D1S1679 was 2.98 under a recessive model of inheritance, considering individuals with schizophrenia or schizoaffective disorder as affected. Single-family lod scores were not presented in the original publication because of space limitations.