transporter by matching the specificity of the class I molecules and the transporters that serve them.

REFERENCES AND NOTES

1. A. Townsend and H. Bodmer, *Ann. Rev. Immunol.* **7**, 601 (1989); M.-J. Bijlmakers and H. L. Ploegh, *Curr. Opin. Immunol.* **5**, 21 (1993); A. L. Goldberg and K. L. Rock, *Nature* **357**, 375 (1992).
2. H.-G. Rammensee, K. Falk, O. Rotzschke, *Ann. Rev. Immunol.* **11**, 213 (1993).
3. P. Parham, *Nature* **348**, 674 (1990); T. Spies *et al.*, *ibid.*, p. 744; J. Trowsdale *et al.*, *ibid.*, p. 741; E. V. Deverson *et al.*, *ibid.*, p. 738; J. J. Monaco *et al.*, *Science* **250**, 1723 (1990).
4. J. C. Shepherd *et al.*, *Cell* **74**, 577 (1993).
5. J. J. Neefjes, F. Momburg, G. Hammerling, *Science* **261**, 769 (1993).
6. T. N. M. Schumacher *et al.*, *J. Exp. Med.*, in press.
7. H. R. Gaskins *et al.*, *Science* **256**, 1826 (1992); M. Colonna *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3932 (1992).
8. S. J. Powis *et al.*, *Nature* **357**, 211 (1992).
9. A. M. Livingstone *et al.*, *J. Exp. Med.* **170**, 777 (1989); S. Powis *et al.*, *ibid.* **173**, 913 (1991).
10. A. M. Livingstone *et al.*, *Immunogenetics* **34**, 157 (1991).
11. After testing peptides of different sequence and lengths, we identified TYQRTRALI as a candidate for translocation assays in rat microsomes. The substitution Q to N was considered conservative. Microsomes were prepared from rat liver as described (*4, 20*), but were finally resuspended in buffer I (50 mM tetraethylammonium, 1.5 mM magnesium acetate, 1 mM EDTA, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride). Synthetic peptides (*6*) and peptide libraries (*16*) were synthesized and dissolved as described. Peptides were labeled by chloramine T–catalyzed iodination to a specific activity of 10 Ci/mmol. Competition assays were done as described (*4, 6*) with the following modifications: Incubation was done for 15 min at 37°C, and 0.1 to 0.4 μM of peptide (depending on the batch peptide) was used; iodinated TYNRTRALI was used as reporter substrate unless indicated otherwise. Assays were stopped by addition of 1 ml of ice-cold buffer NaI + 10 mM EDTA. Microsomes were sedimented (14,000*g* for 20 min at 4°C), and microsome-associated radioactivity was determined. Endoglycosidase H digestion and electrophoretic analysis were done as described (*13*). Percent uptake was calculated as follows:

$$\frac{[\text{CPM}(+\text{ATP};+\text{competitor})]-[\text{CPM}(-\text{ATP})]}{[\text{CPM}(+\text{ATP})]-[\text{CPM}(-\text{ATP})]}$$

Values are means of duplicates for which the standard deviation is less than 15% of the mean.
12. H. Schägger and G. Von Jägow, *Anal. Biochem.* **166**, 368 (1987).
13. M.-J. Bijlmakers *et al.*, *Eur. J. Immunol.* **23**, 1305 (1993).
14. T. Fukumoto, W. R. McMaster, A. F. Williams, *ibid.* **12**, 237 (1982).
15. TYQRTRALX competitor peptides were used instead of TYNRTRTALX, to avoid competition for glycosylation.
16. T. N. M. Schumacher *et al.*, *Eur. J. Immunol.* **22**, 1405 (1992).
17. Analysis by NEPHGE was done as described (*21*) with the following modifications: Ampholytes were pH 3.5 to 10 (2%) and pH 5 to 7 (0.4%); samples were loaded at the anodal side of the gel and run for 2.5 hours at 400 V. The gel was frozen and exposed to Kodak X-AR5 film. Trifluoroacetic acid (TFA) extracts of microsomal pellets (2 × 1% TFA) were analyzed by HPLC as described (*22*) with a reversed-phase C18 column [3 min constant at 5%, increase in 50 min from 5% to >55% (v/v) acetonitrile in 0.1% TFA]. Fractions of 0.5 min were collected, and radioactivity was determined. The identity of the peak in the translocated material (fractions 22 to 24) has not been established. The height of this peak was found to vary between experiments, and the material is not obviously present in input peptides.
18. T. S. Jardetzky *et al.*, *Nature* **353**, 326 (1991); H.-C. Guo *et al.*, *ibid.* **360**, 364 (1992).
19. J. C. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3777 (1993).
20. P. Walter and G. Blobel, *Methods Enzymol.* **96**, 84 (1983).
21. J. J. Neefjes *et al.*, *Hum. Immunol.* **16**, 169 (1986).
22. T. J. Tsomides and H. N. Eisen, *Anal. Biochem.* **210**, 129 (1993).
23. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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TECHNICAL COMMENTS

Male Sexual Orientation and Genetic Evidence

Dean H. Hamer *et al.* report a paired sibling (sib-pair) analysis linking male sexual orientation with a region of chromosome Xq28 (*1*). Much of the discussion of this finding has focused on its social and political ramifications. In contrast, our goal is to discuss the scientific evidence and to highlight inconsistencies that suggest that this finding should be interpreted cautiously.

While interpretation of genetic linkage results for Mendelian traits (such as genetic markers) by lod score statistics has a substantial history, including empirical verification, this is not so for complex, or non-Mendelian traits. There are pitfalls in applying standards derived for Mendelian traits to complex ones (*2*). Interpretation of linkage results for such a trait must, of necessity, occur in the context of prior evidence about the genetic basis for that trait. Family, twin, and adoption studies, the basic paradigms of genetic epidemiology, provide important evidence about the magnitude and nature of a possible genetic contribution to a trait. Furthermore, quantifying the magnitude of a genetic effect, through family and adoption studies, plays a direct role in the interpretation of linkage results. This is because there is a simple correspondence between the familiality of a trait that results from a gene and the expected linkage evidence (*3*). Specifically, inheritance of alleles identical by descent (ibd) at an autosomal contributory locus (or closely linked marker) for an affected (or trait-concordant) sib pair is directly related to the ratio (λ_s) of recurrence for siblings over population prevalence (*3*)

$$z_0 = 0.25/\lambda_s$$

where z_0 is the probability that the sib-pair share no alleles ibd at the trait locus, and λ_s is the sibling recurrence ratio that results from that locus. For an X-linked locus and brother pairs, the equivalent formula is

$$z_0 = 0.50/\lambda_s$$

Hence, interpretation of ibd data for trait-concordant brother pairs depends on prior evidence about plausible values of λ_s for such a locus.

Hamer *et al.* (*1*) and two other recent studies (*4, 5*) suggest the existence of an increased frequency of homosexual orientation among the brothers of gay probands; however, the one study (*5*) that included monozygous (MZ) and dizygous (DZ) cotwins, as well as adopted (unrelated) and biological brothers of homosexual probands, provided inconsistent genetic evidence. While the MZ cotwins showed a higher rate than DZ cotwins of homosexual orientation (29/56 or 52%, as opposed to 12/54 or 22%, respectively), which suggested a genetic contribution, the biological brothers and adoptive brothers showed approximately the same rates (13/142 or 9.2% and 6/57 or 10.5%, respectively). This latter observation suggests that there is no genetic component, but rather an environmental component shared in families. Hence, one's interpretation of the results of this study (*5*) depends on whether one places greater credibility on the data from twins or on the data from adopted and biological siblings. The fact that the study population was obtained through advertisement may have also led to undetermined biases.

From these three studies, it is somewhat difficult to obtain an accurate (or consistent) estimate of λ_s. To calculate λ_s an appropriate control group is required. For controls, Pillard and Weinrich (*4*) studied the brothers of heterosexual probands and obtained a rate of homosexual orientation of 2/38 or 5.3% for those directly interviewed, compared with a rate of 15/68 or 22% for the brothers of gay probands. From these data, we obtain a value of λ_s =

0.22/0.053 = 4.1. This value of λ_s includes all genetic and nongenetic sources of familial aggregation.

Bailey and Pillard (5) did not employ a formal reference group. However, the adopted brothers of gay probands may, in fact, be the most appropriate referents because they have no genetic relationship to the proband, only an environmental one. Hence, estimating λ_s from biological and adoptive brothers gives a direct assessment of the *genetic* effect. Contrasting the biological with the adoptive brothers from this study (5), we obtain λ_s = 0.092/0.105 = 0.9. Even if we use the rate from the DZ twins (who are also genetic brothers) instead of the biological brothers, we obtain only a modest value of λ_s = 0.222/0.105 = 2.1.

Hamer *et al.* (*1*) obtain a frequency of homosexual orientation equal to 14/104 or 13.5% for the brothers of gay probands. It is difficult to derive an accurate comparison rate from their study. They used the uncles and male cousins (combined) of lesbian probands (from another study) as referents, reporting a rate of 14/717 or 2.0%. However, a more appropriate reference group would be the brothers of the lesbians, as they are matched in terms of generation and relationship to the brothers of the male probands. The rate for the brothers of the lesbians was reported to be 4.7%, giving a value for λ_s of 0.135/0.047 = 2.9.

The value of λ_s estimated from family studies includes all genetic and shared environmental factors. Unless all recurrence in brothers is a result of one genetic locus, the value of λ_s for any particular contributing gene relevant to a linkage study of that gene will be less than the value of λ_s observed. Only the values of λ_s from the study of Bailey and Pillard (5), which used adopted brothers as referents, exclude shared environmental factors.

Hamer *et al.* (*1*) argue that the results of their family study are consistent with an X-linked locus contributing to male sexual orientation. For the study based on singleton probands (*1*), the recurrence rates for homosexual orientation are 13.5% for siblings, 9/215 or 4.2% for uncles, and 12/243 or 4.9% for cousins. These results are not consistent with any genetic model, for which the rates should increase from population prevalence to cousins (third-degree relative) to uncles (second-degree relative) to brothers (first-degree relative).

Hamer *et al.* (*1*) suggest that their results are consistent with X-linkage because maternal uncles have a higher rate of homosexual orientation than paternal uncles, and cousins related through a maternal aunt have a higher rate than other types of cousins. However, neither of these differences is statistically significant. Further, small sample sizes make these data compatible with a range of possible genetic and environmental hypotheses.

The hallmark characteristic of an X-linked trait is no male-to-male transmission. Because few homosexual men tend to have children (8), a study of male homosexual orientation will reveal few opportunities for male-to-male transmission, giving the appearance of X-linkage. In this context, examining the rate of homosexual orientation in the fathers of homosexual men [for example 0/76 (*1*)] is not meaningful. In the study by Hamer *et al.*, there were only six sons of homosexual males, clearly an inadequate number for a meaningful test. Hamer *et al.* also present four pedigrees as being consistent with X-linkage [figure 3 in (*1*)]. Only one homosexual male in these four pedigrees has a child (a daughter). In the context of trait-associated lack of male reproduction, such pedigrees would be relatively easy to obtain. Thus, the family data presented in (*1*) provide no consistent support for X-linkage or for the subsequent linkage results.

Hamer *et al.* (*1*) present 40 homosexual brother pairs, of whom 33 are concordant for Xq28 markers and seven are discordant. Hence, the proportion of brother pairs sharing no alleles is 7/40 = 0.175. From the formula given above, this would correspond to a sibling recurrence ratio resulting from this locus of λ_s = 0.50/0.175 = 2.9.

The exponentiated lod score does not reflect the probability of the observed data under the null hypothesis, but rather the relative likelihood of the observed data under the null (no linkage) hypothesis as compared with the alternative (linkage) hypothesis. This ratio depends on the specific alternative hypothesis (*2*); for sib-pairs, this is the value λ_s. The lod score of 4.0 obtained by Hamer *et al.* depends on the assumption of a value of λ_s = 2.9 for the X-linked locus (this value was actually estimated from the linkage data). If one assumes a locus with smaller effect, the lod score would be lower. Specifically, the lod score can be given in this case by the formula

$$40 \log 2 + 33 \log (1 - 0.5/\lambda_s) + 7 \log (0.5/\lambda_s)$$

because 33 pairs were concordant and seven discordant. Using a value for λ_s of 2.9 gives a lod score of 4.0. However, using a smaller value of λ_s, for example 1.5, gives a lod score of 2.9. As discussed above, the appropriate value of λ_s to use for this linkage analysis would be of considerable debate, and the prior evidence suggests a value of 2.9 may be unrealistically large. In any event, the statistical significance of this finding depends on this X-linked locus having a major effect (that is, $\lambda_s > 2$).

Also with regard to statistical significance, the lod threshold of 3 was defined for Mendelian traits (such as markers) and has been verified empirically to produce a false positive rate of less than 5% (9). No such empirical studies of lod thresholds for non-Mendelian traits have been conducted; however, based on the handful of reports of linkage for behavioral traits that proved unreplicable, the empirical false positive rate for a lod score of 3 would be far greater than 5%. Under these circumstances, we would argue that the possibility of a type-1 error should be considered.

Hamer *et al.* (*1*) cite several "theoretical benefits" to the sib-pair approach, suggesting that it may be more robust than traditional pedigree approaches. As we have shown above, sib-pair analysis is not assumption free. Lod score analysis with pedigrees has also been shown, both theoretically and empirically, to be robust to such problems as reduced penetrance, phenocopies, and multiple genes (*10*). On the other hand, neither approach is robust enough to withstand violation of the most important assumption—the existence (or magnitude, or both) of a major gene.

There is little disagreement that male homosexual orientation is not a Mendelian trait. In fact, a priori, one would expect the role of a major gene in male homosexual orientation to be limited because of the strong selective pressures against such a gene. It is unlikely that a major gene underlying such a common trait could persist over time without an extraordinary counterbalancing mechanism.

With regard to sexual orientation, we recognize that many of the limitations in data are a reflection of the difficulties in conducting studies with so many political and social ramifications. However, this also means that such studies must be scrutinized carefully and dispassionately. Linkage results identifying major genes for complex traits must be evaluated in the total context of family, twin, and adoption studies for that trait and must be consistent with results obtained from those studies.

Neil Risch
Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, CT 06520–8034
Elizabeth Squires-Wheeler
Department of Medical Genetics, New York State Psychiatric Institute, 722 West 168 Street, New York, NY 10032
Bronya J. B. Keats
Department of Biometry and Genetics, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112–1328

REFERENCES

1. D. H. Hamer, S. Hu, V. L. Magnuson, N. Hu, A. M. L. Pattatucci, *Science* **261**, 321 (1993).
2. N. Risch, *ibid.* **255**, 803 (1992).
3. ———, *Am. J. Hum. Genet.* **46**, 229 (1990).
4. R. C. Pillard and J. D. Weinrich, *Arch. Gen. Psychiatr.* **43**, 808 (1986).
5. J. M. Bailey and R. C. Pillard, *ibid.* **48**, 1089 (1991).
6. J. M. Bailey and D. S. Benishay, *Am. J. Psychiatr.* **150**, 272 (1993).
7. N. Risch, *Am. J. Hum. Genet.* **46**, 222 (1990).
8. A. P. Bell and M. S. Weinberg, *Homosexualities: A Study of Diversity Among Men and Women* (Simon & Schuster, New York, 1978).
9. D. C. Rao, B. J. B. Keats, N. E. Morton, S. Yee, R. Lew, *Am. J. Hum. Genet.* **30**, 516 (1978).
10. F. Clerget-Darpoux, C. Bonaiti-Pellie, J. Hochez, *Biometrics* **42**, 393 (1986); N. Risch, E. Claus, L. Giuffra, *Multipoint Mapping and Linkage Based Upon Affected Pedigree Members: Genetic Analysis Workshop 6* (Liss, New York, 1989), pp. 183–188; N. Risch and L. Giuffra, *Hum. Hered.* **42**, 77 (1992).

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Response: Studies of the role of genetics in sexual orientation are still at an early stage of development. The entire modern literature consists of less than a dozen papers, of which ours (*1*) was the only one to examine extended families or DNA markers. Hence we thoroughly agree that further empirical study will be required to corroborate the role of genes in this complex dimension of human behavior. However, we suggest that the analytical approach advocated by Risch *et al.* is less than optimal for the initial analysis of complex traits such as sexual orientation.

The critique by Risch *et al.* concerns primarily the relationship between the data obtained by family studies and those obtained by DNA linkage analysis. Specifically, Risch *et al.* posit an inverse proportionality between λ_s, the ratio of recurrence for siblings versus population prevalence, and z_0, the proportion of sib-pairs that share no alleles at a candidate locus (*2*, *3*). However they do not note that this relationship is true if and only if λ_s and z_0 are measured on the same population. This was not the case in our study. Although the probands for the family study were ascertained without regard to family history, the families for the linkage study were deliberately screened for absence of direct father-to-son transmission and of multiple female homosexuals—characteristics that would not be consistent with sex-limited, X-linked inheritance. This selection, which was clearly described in our research article (*1*), was apparently successful because it enriched for families containing gay maternal uncles and maternal cousins through an aunt.

Because our family and linkage studies were performed on distinct populations, the recalculation by Risch *et al.* of our linkage data is invalid. In fact, all of the recent successes in the genetic mapping of complex traits—such as those concerning mental retardation, breast cancer, and Alzheimer's disease—have depended on linkage analyses performed on selected subpopulations. If these studies had been evaluated with the formula used by Risch *et al.*, they would have been incorrectly judged as insignificant.

Risch *et al.* also state that our pedigree results are "not consistent with any genetic model, for which the rates should increase from population prevalence to cousins (third degree relative) to uncles (second degree relative) to brothers (first degree relative." This statement is incorrect. Risch *et al.* are describing the expected order of rates for an autosomal dominant trait, not for an X-linked trait for which the expected order of rates in cousins and uncles is reversed. The rates for a trait influenced by an X-linked locus are expected to increase from population prevalence to maternal uncles (related to their nephews by 25% of their X-linked genes) to maternal cousins through an aunt (related by 37.5% of their X-linked genes) to brothers (related by 50% of their X-linked genes). This is precisely the order that we observed.

Finally, Risch *et al.* refer to a possible "major gene" that "underlies" male sexual orientation. We did not say that Xq28 "underlies" sexuality, only that is contributes to it in some families. Nor have we said that Xq28 represents a "major" gene, only that its influence is statistically detectable in the selected population that we studied. The sib-pair linkage approach is designed to determine whether or not there is any correlation between a particular chromosomal region and a trait, not to quantify the role of the locus in the population at large.

As noted by Risch *et al.*, the question of the appropriate significance level to apply to a non-Mendelian trait such as sexual orientation is problematic. We reported a lod score of 4.0, which is 10-fold higher than the conventional cutoff level of 3.0, but only replication of our result will tell whether the observed linkage is truly significant. Ultimately, however, the question of whether or not sexual orientation is influenced by genes will depend not on linkage analysis or any other statistical approach, but on the isolation and characterization of the relevant loci. The current evidence suggests that Xq28 will be a useful starting place for this search.

Dean H. Hamer
Stella Hu
Victoria Magnuson
Nan Hu
Angela M. L. Pattatucci
Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

REFERENCES

1. D. H. Hamer, S. Hu, V. L. Magnuson, N. Hu, A. M. L. Pattatucci, *Science* **261**, 321 (1993).
2. J. W. James, *Ann. Hum. Genet.* **35**, 47 (1971).
3. N. Risch, *Am. J. Hum. Genet.* **46**, 229 (1990).

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