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Genetics and Male Sexual Orientation

Sexual orientation is a complex trait that is probably shaped by many different factors, including multiple genes, biological, environmental, and sociocultural influences. In a 1993 report, my group (1) provided initial evidence that a locus at the q28 region of the X chromosome was involved in male sexual orientation in some, but not all, individuals. The Xq28 hypothesis was based on both family pedigree analysis, which revealed that gay men had more homosexual male relatives through maternal than through paternal lineages, and linkage analysis of gay male siblings, which found significantly increased sharing of Xq28 DNA markers. Recently, George Rice *et al.* (2) challenged the Xq28 hypothesis on the basis of a new linkage study in Canada and have questioned the degree to which genes contribute to sexual orientation. It appears to us that (i) the family pedigree data from the Canadian study (2) actually support the Xq28 hypothesis; (ii) three other available Xq28 DNA studies did find linkage; and (iii) the heritability of sexual orientation is supported by substantial evidence independent of the X-chromosome linkage data.

The original impetus for the Xq28 hypothesis was the finding that gay male probands had more gay male relatives through maternal than through paternal lineages (1); this is the expected pattern for a trait that is influenced by gene on the X chromosome, which males inherit only from their mothers. According to a poster presented at the International Academy of Sex Research meeting in 1995 (3, 4), the Canadian group found a similar pattern in their own family data. Specifically, they interviewed the probands from 182 families that were ascertained on the basis of having two or more gay brothers and found that 13.4% (35/261) of their maternal uncles were gay as compared to 6.9% (24/364) of their paternal uncles. This is a significant difference in favor of maternal transmission (chi-square value = 7.1, $p = 0.008$), as predicted by the Xq28 hypothesis. The difference could not be attributed to reporting bias because the same families showed a slight excess of lesbian aunts on the paternal side of the family (3, 4). This important family pedigree data was not included in the report by Rice *et al.* (2), which describes only the genotyping results for a subset of 48 families.

DNA linkage analysis provides a more direct test of the Xq28 hypothesis. If male sexual orientation is influenced by a gene or genes at Xq28, then gay brothers should share more than 50% of their alleles at this

region, whereas their heterosexual brothers should share less than 50% of their alleles. By contrast, if there is no such gene, then both types of brothers should display 50% allele sharing.

To date, there have been four X-chromosome linkage studies of male sexual orientation (Fig. 1). Hamer *et al.* (1) analyzed 40 pairs of gay brothers and found that they shared 82% of their alleles in the Xq28 region; this was greater than the 50% allele sharing that would be expected by chance ($p = 0.00001$). In a follow-up study, Hu *et al.* (5) analyzed an independent series of 32 genetically informative pairs of gay brothers and found 67% allele sharing ($p = 0.04$). Hu *et al.* also found that the heterosexual brothers of Xq28-concordant gay sib-pairs had only a 22% likelihood of carrying the same Xq28 allele; this independent test of linkage was statistically significant ($p < 0.05$), giving an overall significance level of $p = 0.004$ for their study. In 1998, the independent research group of Sanders *et al.* (6) reported the results of an X chromosome linkage analysis of 54 pairs of gay brothers. They found 66% Xq28 allele sharing ($p = 0.04$). The results of the study by Sanders *et al.* were indistinguishable from those in the study by Hu *et al.*, both in terms of the degree of allele sharing (66% versus 67%) and the precise

chromosomal location of maximum sharing (locus DXS1108). By contrast, Rice *et al.* (2) studied 52 pairs of gay brothers and found no evidence for linkage to Xq28; they reported approximately 46% allele sharing, a nonsignificant result.

Given the modest sample sizes of these studies, the most accurate estimate of Xq28 linkage can be obtained by combining the data from each of the four datasets. This meta-analysis gives an estimated level of allele sharing of 64% ($p = 0.0001$) (Fig. 1). Basically, the same result is obtained if one discards the highest and lowest reported allele sharing values and uses only the Hu *et al.* (5) and Sanders *et al.* (6) data, which gives a figure for estimated allele sharing of 66% ($p = 0.001$). A 64% allele sharing level corresponds to a λ_s value of 1.4, where λ_s is the ratio for homosexual orientation in the brothers of a gay index subject, as compared with the population frequency, that is attributable to a gene in this region. This modest level of influence is typical for the effect of a single locus on a complex behavioral trait such as sexual orientation.

Why did Rice *et al.* not find the Xq28 linkage that was observed in the three preceding studies? One possible explanation is derived from the fact that only 48 (26%) of the 182 families originally interviewed by Rice *et al.* were actually studied at the level of their DNA (2). According to data presented in 1995 (3), it appears that the genotyped families were not a representative subset of the starting population because they displayed an excess of paternal rather than ma-

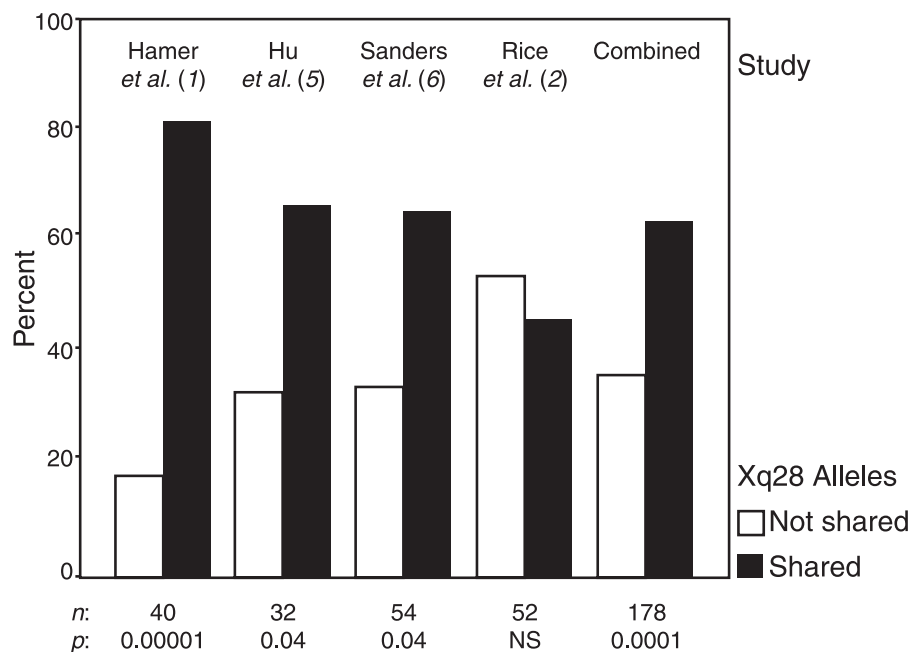


Fig. 1. Comparison of four studies of linkage between alleles on the X chromosome and male homosexuality. Meta-analysis (combined data, last set of columns) shows overall estimated allele sharing of 64% ($p = 0.0001$). Sample size, n . Statistical significance, p .

ternal gay relatives—exactly the opposite pattern found in the total dataset. One would not logically expect such a nonrepresentative subset of the families to display any X-chromosome linkage. It would be interesting in the future to examine genotypes for the remainder of the Canadian families, especially those with maternal loading.

A second possible explanation for why Rice *et al.* did not observe linkage is the modest statistical power of their sample. A population of 52 sib-pairs has 65% power to detect 64% allele sharing at the 0.05 level of significance. Thus there was a 35% chance that Rice *et al.* would not detect linkage simply by chance.

A third consideration is the lack of defined criteria for homosexuality in the study by Rice *et al.* Hamer *et al.* (1) and Hu *et al.* (5) assessed sexual orientation using the 6-point Kinsey scales of sexual attraction, fantasy, self-identification, and behavior. By contrast, Rice *et al.* (2) depended on the investigator's judgment, in some cases based on a single question to the research subject (7). The validity and reliability of this method of phenotype classification are unknown.

A final difference between the linkage studies is that Rice *et al.* (2) did not methodically exclude families inconsistent with the hypothesis of X chromosome linkage. The use of defined inclusion and exclusion criteria to select appropriate families for the study of a putative X-linked locus was a key feature of the studies by Hamer *et al.* (1) and Hu *et al.* (5). As noted by King (8), this is a valuable strategy to detect linkage for a complex trait, such as sexual orientation, for which one particular locus (Xq28) accounts for only a portion of the total variance. Hu *et al.* (5) found no Xq28 linkage in families who did not meet their inclusion criteria. Although Rice *et al.* (2) found that excluding two families that failed to meet such criteria did not change their linkage results, it appears unlikely that they collected sufficient data on their subjects or families to systematically apply this sort of selection.

As stated by Rice *et al.* (2), there is substantial evidence from family and twin studies, such as those reported by Pillard, Bailey and colleagues (9), that sexual orientation is genetically influenced. Rice *et al.* also argued, however, that "there would be strong selective pressure against such a gene." Linkage analysis of a single locus on a limited number of families can show no such thing. Moreover, there are many plausible evolutionary scenarios whereby a gene that predisposed some individuals toward homosexuality could survive or even increase in the population (10).

In summary, a meta-analysis of all available DNA linkage data continues to support a modest but significant role of the Xq28 re-

gion in male sexual orientation. Although there is a 0.01% chance that the observed link represents a "false positive," there is a greater than 10% chance that the conclusions in the report by Rice *et al.* represent a "false negative," resulting from their use of a small, apparently nonrepresentative subset of families for genotyping. Moreover, their family pedigree data appear to actually support X-chromosome linkage. The search for sexual orientation genes will ultimately depend on the identification of functionally relevant polymorphisms in molecularly defined genes, rather than on the purely statistical evidence afforded by linkage analysis. In the meanwhile, the genetic analysis of sexual orientation, like any complex trait, should pay careful attention to all available family and molecular data.

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12 May 1999; accepted 7 July 1999.

Response: In their initial X chromosome linkage study, Hamer *et al.* (1) stated, "As with all linkage studies, replication and confirmation of our results are essential." We agree. To be convincing, such confirmation needs to be obtained by groups using similar methods but working independently of the initial investigators. Hamer presents a "meta-analysis" which appears statistically significant, but does not address the issue of nonreplicability by including his own data in the analysis. Considering only the two studies performed by independent investigators, ours (2) and the unpublished data of Sanders *et al.* (3), we obtain Xq28 allele sharing of 60/106 = 56.6%, which is not statistically greater than the null hypothesis value of 50% sharing ($p > 0.05$). In fact, the two independent replication studies combined deviate significantly from the results from Hamer's group (1, 4) (chi-square = 6.53, $p < .02$). Thus, the con-

clusion remains that the original studies of Hamer and colleagues are not replicated.

As stated in our report (2), our goal was to replicate the linkage at Xq28. We recruited sibships containing at least two gay brothers. Index subjects were only casually asked about second- and third-degree relatives (uncles and male cousins). The rate of maternal uncles reported as gay (35/261 = 13.4%) was higher than that for paternal uncles (24/346 = 6.9%; $p < 0.01$). However, the total number of paternal uncles is even more significantly increased (maternal uncles 346 versus 261, $p < 0.001$). A comparison of maternal to paternal relatives presupposes that the likelihood of ascertainment of this trait is the same for both, but this has not been established. Family history studies of more easily identified traits are known to be unreliable on distant relatives (5), and we place little confidence in such studies, our own included (2). Hamer considers our pedigree data to be important and appears to accept these findings unequivocally. Not all persons in the pedigree study by Hamer *et al.* were interviewed directly (1). Furthermore, a recent larger family history study (6) did not find an excess of maternal gay uncles or gay cousins through maternal aunts, inconsistent with an X-linkage hypothesis, and not replicating the original pedigree study by Hamer *et al.* (1).

Of the original 182 families ascertained by us, 48 were genotyped because these families were willing to provide blood for DNA studies. These families did not deviate from the larger group in any significant way regarding family history. The subset of genotyped individuals was not different from the total sample in its constitution of paternal gay relatives from the total population from which it was sampled. In particular, only two of our 48 genotyped families had gay fathers. Therefore, the genotyped families were not "paternally" loaded (*vide infra*).

Hamer questions the power of our sample to detect linkage. If Xq28 allele sharing in the gay brother pairs is actually 67%, as he states, our power to detect sharing at the 5% significance level, assuming 50 independent pairs, is 82%, a level which would be considered adequate under most statistical standards. The sharing in our sample, the largest published to date, was nowhere near the 67% figure. We can reject the hypothesis of 67% sharing with $p < 0.002$ (exact binomial probability).

The individuals in our linkage study were all self-identified as gay. On the basis of a more detailed questioning regarding sexual attractions, fantasies, and behaviors on a subset of these individuals, all subjects had Kinsey scores (7) of at least 5. We agree with Hamer that such individuals are unlikely to be incorrectly classified. "It seems unlikely, given the stigma of homosexuality, that a

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heterosexual would masquerade as gay" (8). Thus, our study population was unlikely to include heterosexual males, misclassified as being gay. Hamer criticizes our categorization of directly interviewed subjects, but appears not to find fault with the casual family history data which he states supports his conclusion.

A major emphasis is placed by Hamer (1, 8) on "proper selection" of families on the basis of family history, but a coherent rationale for this selection procedure has not been given, to our knowledge. Presumably, the rationale for this selection procedure is based on the notion that only a subset of gay men carry an X-linked gene, and families need to be selected to enrich for such a gene. The best description of their selection process was given by Hamer and Copeland (8, p. 108). "First, the family should have exactly two gay brothers. If there were only one gay man there'd be no enrichment for the gene, and if there were more than two, we ran the risk of selecting rare and unusual genes. Second, there should be at most one lesbian in the family. This is because the family studies showed that male and female homosexuality were not commonly found together and we wanted to use typical (sic) families. Finally, we did not want families with gay fathers and gay sons, because this pattern would not be consistent with X-chromosome linkage." Hamer *et al.* (1) and Hu *et al.* (4) did not exclude gay brother pairs with gay uncles or cousins on the paternal side, nor did we. According to these precise criteria, two of our families would be excluded because the fathers were gay, and two would be excluded because they contained three gay brothers. None of the siblings in our study had gay sons and none had more than one lesbian relative. One sib pair with a gay father shared Xq28 alleles, while the other pair with a gay father did not. One sib trio shared Xq28 alleles among the three sibs, while for the other trio, two were concordant and one discordant. Tallying the observed sharing in the remaining 44 sib pairs who met Hamer's criteria, we obtain 19 of 44 (=43%) chromosomes shared, which is less, but not significantly less, than the entire sample. Hamer argues that we collected insufficient data on our subjects or families to systematically apply this sort of selection. In fact, the opposite seems to be the case. We

have good information on whether the father of the sibships is gay and whether the gay sibling pairs have gay sons and on the number of lesbian mothers or sisters. With the use of identical inclusion/exclusion criteria as employed by Hamer *et al.* (1) and Hu *et al.* (4), we obtained no evidence of excess allele sharing for Xq28 markers.

On the other hand, the logic of these proposed exclusion criteria is not sound. Hamer and colleagues have not put forth a coherent genetic model on which such rules would be based. Presumably, an allele at Xq28 would account for a subset of gay male behavior, and sporadic influences might account for the rest (8). If this were the case, the probability that a gay brother pair shares an X-linked gene would not decrease if the father or any paternal relative is also gay or if there are lesbian relatives in the family. Furthermore, a sibship with three gay brothers would be even more likely to have inherited the causative X-linked allele than a simple sib pair. The exclusion of sibships with more than two gay brothers based on the "risk of selecting rare and unusual genes" is particularly paradoxical, because this is precisely the type of gene Hamer postulates. The logic of excluding families with lesbian relatives contradicts his own statement (1) that lesbians and gay men do not aggregate in families. If male and female homosexuality are genetically independent, the number of lesbian relatives in a family should be irrelevant. Given these considerations, it would be helpful to see the genotyping results of the families excluded from Hamer's initial study. These data have not been reported, to our knowledge.

One difference between our study and that of Hamer *et al.*, not mentioned by Hamer, is that we used controls (brother pairs with multiple sclerosis) to allow blinded scoring of allele sharing. Apparently no such controls (blind or otherwise) were used by Hamer and, in fact, the initial genotyping was performed by Hamer himself (8). Different individuals in the laboratory should perform different steps: phenotypic characterizations and genotypic analyses.

We still contend that an X-linked gay gene could not exist in the population with any sizeable frequency, due to the strong

selection against it. Hamer reports that gay men in his study had one-tenth the number of offspring of their heterosexual brothers. This degree of selection is comparable to or greater than that for many X-linked genetic diseases such as hemophilia, which exist at low frequencies (approximately 1/10,000) in the population as a result of selection pressure.

We agree with Hamer that our results do not exclude the possibility of genetic effects underlying male homosexuality. But with the use of similar methods of family ascertainment, phenotyping, and genotyping, we were unable to confirm evidence for an Xq28-linked locus underlying male homosexuality. Sanders *et al.* (3) came to the same conclusion with their linkage study.

The basis of sexual orientation remains uncertain, but the pathways involved can be expected to be complex. The controversies and methodological difficulties notwithstanding, the study of sexual orientation contains fascinating riddles, and further careful systematic study has the potential to reveal important insights about who we are.

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21 June 1999; accepted 7 July 1999