

Chapter 19

Genetic and Environmental Influences on Sexual Orientation

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Introduction

The primary focus of this chapter is to provide an overview of the evidence to date on the quantitative genetics of sexual orientation, including family and twin studies. The bulk of the available evidence suggests moderate heritability for male sexual orientation. Female sexual orientation has been studied much less extensively, but current studies are consistent with a genetic contribution for women as well (Kirk, Bailey, Dunne, & Martin, 2000; Pattatucci & Hamer, 1995). Familial aggregation has been reported in several family studies of both male and female homosexuality (Dawood & Bailey, 2000), although the genetic and environmental influences on this familial clustering have not been clearly defined by the largest twin studies published thus far, which have produced contradictory results. Recent molecular genetic studies will also be reviewed, including the two main strategies that have been used to date – linkage and association analysis. We will also discuss the implications of recent advances in molecular genetic studies.

Prevalence and Distribution of Sexual Orientation

Sexual orientation describes what is erotically attractive to an individual and is usually consistent with sexual identity which refers to an individual's labeling of self as heterosexual, homosexual, or bisexual; both are typically not consolidated until adolescence or later.

Three large surveys of sexual behavior from UK (Johnson, Wadsworth, Wellings, Bradshaw, & Field, 1992), France (ACFS Investigators, 1992), and the USA (Billy, Tanfer,

Grady, & Klepinger, 1993; Laumann, Gagnon, Michael, & Michaels, 1994) have provided current estimates of the frequency of male and female adult homosexual behavior. These estimates vary with the stringency of the respective definitions. For men, the least stringent definition examined in the studies, any homosexual experience ever, yielded an estimate of 4.1, 6.1, and 7.1% for the three samples, respectively. A much more stringent criterion, same-sex activity during the year preceding the survey, yielded rates of 1.1, 1.1, and 2.7% for the three samples, respectively. The criterion closest to that of self-identification as “gay” or “bisexual” was employed only in the American study and applied to 2.4% of men. In general, the rates for female homosexuality appear to be about half that for males; for example, female same-sex activity during the year preceding the survey yielded a rate of 1.3% in the American survey. In addition, male and female sexual orientation appear to be distributed differently in the general population. For men, sexual orientation appears to be bimodally distributed, with most men clustered at the heterosexual end of a continuum, many fewer at the homosexual end, and hardly any in the bisexual middle. For women, however, it tapers gradually from exclusively heterosexual to exclusively homosexual (Bailey, Dunne, & Martin, 2000). Whereas older studies tended to define sexual orientation behaviorally, most current researchers, ourselves included, define sexual orientation psychologically. Because sexual attraction and fantasy are less likely than behavior to be constrained by societal pressures, psychological sexual orientation is thought to be a more stable and fundamental trait. A psychological definition of sexual orientation tends to produce lower prevalence figures compared to a behavioral one and is used in most of the studies demonstrating familiarity and heritability.

Behavior Genetic Studies

As currently practiced, behavior genetic research proceeds in three main stages.

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Family studies are initially conducted to determine whether a trait or characteristic (often called a phenotype) runs in families by comparing rates in families of probands (i.e., individuals possessing the trait) vs. families of controls (that typically represent the base rate in the general population). If there is a heritable genetic contribution to a trait, one expects to find familial aggregation. However, the mere demonstration of clustering of a trait in families does not prove a genetic influence, because some traits run in families for environmental reasons, biological or otherwise.

Twin studies are conducted in order to separate genetic from familial environmental effects by comparing the similarity of monozygotic (MZ, also called identical) and same-sex dizygotic (DZ, also called fraternal) twins who have been reared together. MZ twins share all their genes in common whereas DZ twins share 50% of their segregating genes on average, just like any other pair of full siblings. Since both twins share the same pregnancy, it is assumed that they experience essentially the same prenatal environment. Because both twins have also been reared together, postnatal environmental similarity is assumed to be approximately equal. Thus, if MZ twins are more similar (i.e., concordant) for a trait than DZ twins, this is assumed to reflect their greater genetic similarity and is evidence that genetic factors influence the phenotype. Twin studies allow one to estimate heritability, defined as the proportion of the variance in expression of the trait due to all genetic influence(s) combined. Results from twin studies support a significant environmental contribution to most behavioral traits since MZ twins are not 100% concordant.

Adoption studies are a further method used to separate the effects of genes from the environment. Adoption produces family members who share family environment but are not genetically related, and vice versa. Thus, this method allows one to estimate the contribution of family environment to family resemblance. Studies exist from all three research designs regarding both male and female sexual orientation:

Family Studies

The first contemporary family-genetic study of sexual orientation was conducted by Pillard and Weinrich (1986). They recruited homosexual and heterosexual male probands (index subjects) using newspaper advertisements that did not mention the nature of the study. Probands were interviewed about their own sexuality as well as their siblings' sexual orientations. The researchers obtained permission to contact, and successfully contacted, the large majority of probands' siblings in order to verify proband reports. Results suggested that homosexual probands were quite accurate at assessing their siblings' sexual orientations (provided that they expressed a high degree of confidence, which they were typically able to do). Most importantly, gay male probands had an excess of gay brothers (22%) compared to heterosexual male probands' brothers (4%).

Subsequent studies have used similar methodologies, with one exception. A study with a very different methodology (Bailey et al., 1999) recruited gay and bisexual men from consecutive admissions at an HIV outpatient center. (In most studies, both gay and bisexual men have been included as "homosexual" probands.) The most important aspect of such an ascertainment strategy is that it is more systematic than advertising for volunteers, and it may be less subject to self-selection biases. All available studies have focused on the rate of homosexuality in siblings rather than other first-degree relatives (e.g., parents or offspring), due to the decreased reproduction of homosexual individuals.

Both male and female homosexuality appear to run in families. Table 19.1 contains the results of recent family studies. The rate of homosexuality among brothers of homosexual males has been around 9%. These results have exceeded those for heterosexual controls as well as the prevalence estimates from recent large-scale epidemiological surveys, suggesting that male homosexuality is familial. Homosexual women also appear to have more homosexual sisters than do

Table 19.1 Rates of homosexuality for nontwin siblings in recent studies

Study	Criterion	Brothers		Sisters	
		Homosexual	Heterosexual	Homosexual	Heterosexual
Male probands					
Pillard & Weinrich, 1986	Kinsey 2–6	0.22	0.04	0.08	0.09
Bailey et al., 1991	Subject's rating	0.10	0.00	0.02	0.00
Bailey & Pillard, 1991	Subject's rating	0.09	NA	0.06	NA
Bailey & Bell, 1993	Subject's rating	0.09	0.04	0.03	0.01
Bailey et al., 1999	Subject's rating	0.09	NA	0.04	NA
Female probands					
Pillard, 1990	Kinsey 2–6	0.13	0.00	0.25	0.11
Bailey & Benishay, 1993	Subject's rating	0.07	0.01	0.12	0.02
Bailey et al., 1993	Subject's rating	0.05	NA	0.14	NA
Bailey & Bell, 1993	Subject's rating	0.12	0.00	0.06	0.01

Table entries are proportions. NA entries indicate that studies did not assess the respective rate.

heterosexual controls, though the familiarity estimates have varied more widely for women. Table 19.1 also contains information concerning the cofamiliarity of male and female homosexuality. There is a trend for gay male probands to have more gay brothers than lesbian sisters and for the opposite pattern to be seen for lesbian probands, suggesting that at least some of the familial factors influencing male homosexuality differ from those influencing female homosexuality. However, the largest study to date (Bailey & Bell, 1993) did not find this pattern. Thus, the degree of cofamiliarity (reflecting common familial influences) of male and female homosexuality remains inconclusive.

Twin Studies

The most common methodology used by contemporary human behavior geneticists to disentangle genetic and environmental determinants compares the similarity of monozygotic and dizygotic (DZ) twins who have been reared together. Because both kinds of twins have been reared together, environmental similarity is assumed to be equal (more about this assumption later). Thus, if MZ twins are more similar than DZ twins, this reflects their greater genetic similarity and is evidence that genetic factors influence the phenotype.

The first twin study of (male) homosexuality, by Kallmann (1952), ascertained homosexual twins in the “homosexual underworld” and correctional/mental institutions of New York city. Remarkably, 100% of 37 MZ twin pairs were concordant compared to 15% of 26 DZ pairs. Kallmann’s (1952) study had a number of methodological defects, including its over reliance on (evidently) mentally ill gay men, lack of information on zygosity diagnosis, and especially its anomalously high rate of MZ concordance compared to other studies (Rosenthal, 1970). Still, it is remarkable that despite its promising results, nearly 40 years passed before another large twin study of male homosexuality was attempted.

Several additional twin studies have been conducted in the past two decades, and their results are given in Table 19.2. These studies have been generally consistent in detecting moderate to large heritabilities for both male and female sexual orientation. However, there have been methodological limitations, in particular, most of the large twin studies of sexual orientation recruited probands via advertisements in gay or lesbian publications. Such sampling is likely to result in volunteer bias that affects twin concordances and heritability analyses, though in most scenarios this would be more likely to lead to a false negative study. The largest twin study of sexual orientation to date (Bailey et al., 2000) recruited twins systematically from a twin registry and reported lower

Table 19.2 Concordance rates for twin studies of homosexuality

Study	MZ concordance	DZ concordance
Male studies		
Kallmann, 1952	1.00	0.15
Heston & Shields, 1968	0.60	0.14
Bailey & Pillard, 1991	0.52	0.22
Buhrich, Bailey, & Martin, 1991	0.47	0.00
Bailey et al., 2000	0.20	0.00
Female studies		
Bailey et al., 1993	0.48	0.16
Bailey et al., 2000	0.24	0.15
Combined male and female		
King & McDonald, 1992	0.25	0.12
Whitam, Diamond, & Martin, 1993	0.66	0.30
Kendler et al., 2000	0.32	0.13

twin concordances for homosexuality than in prior studies, although their findings were also consistent with moderate to large heritabilities for male and female sexual orientation. A further analysis of these data using multivariate structural equation modeling estimated heritability of the latent variable of male homosexuality around 30% and for female homosexuality around 50% (Kirk et al., 2000).

In the discussion below, we focus on some of the largest twin studies of male and female sexual orientation (Bailey et al., 2000; Bailey & Benishay, 1993; Bailey & Pillard, 1991; Kendler, Thornton, Gilman, & Kessler, 2000) conducted to date.

In Bailey and Pillard (1991) as well as in Bailey et al. (1993), homosexual probands were recruited via advertisements in gay or lesbian publications (e.g., “Do you have a twin or an adoptive brother?”). Two kinds of probands were recruited: probands with twins or probands with adoptive brothers or sisters (for the male and female studies, respectively). Adoptive siblings were raised with the probands but are genetically unrelated to them. Probands were interviewed, especially concerning the sexual orientations of their twins. Probands’ twins were also contacted when possible and confirmed that probands were quite accurate in assessing their twins’ sexual orientations.

In the male study (Bailey & Pillard, 1991), 52% of the MZ cotwins were also gay or bisexual, compared to 22% of the DZ cotwins and 11% of the adoptive brothers. In the female study (Bailey et al., 1993), 48% of the MZ cotwins were also lesbian or bisexual, compared to 16% of the DZ cotwins and 6% of adoptive sisters. Thus, for both men and women, the rates conformed to a partially genetic model, with highest concordance in the most genetically similar (MZ) group and lowest concordance in the least similar (adoptive) group.

In order to calculate heritability from this study, several assumptions were made. First, although sexual orientation was measured on a dichotomous scale (i.e., heterosexual vs.

homosexual), the underlying causal structure was dimensional. That is, genetic influences are polygenic (i.e., numerous genes each with small effect), and environmental influences are similarly multifactorial. This corresponds to a multifactorial threshold model (Reich, Cloninger, & Guze, 1975). Second, a population base rate was assumed for homosexual orientation. The possibilities considered ranged from 4 to 10% for males and from 2 to 10% for females. Third, since ascertainment methods are frequently viewed as leading to concordance-dependent bias, different degrees of such bias were assumed, from none at all to the case in which probands from concordant pairs were three times more likely to be ascertained than probands from discordant pairs. For both men and women, heritability estimates ranged from approximately 0.30 to approximately 0.70.

Kendler et al. (2000) recruited 756 twin and nontwin sibling pairs from a US national probability sample and found that 32% of MZ twins were concordant for homosexual orientation vs. 13% of DZ same-sex twins. The concordance rates for MZ twins were lower than those reported in previous studies which recruited via advertisements in gay publications, suggesting that twin pairs concordant for sexual orientation may be more likely to respond to such advertisements than are twin pairs discordant for sexual orientation. It is also worth noting that unlike previous studies in this area, this study assessed sexual orientation by a single item on a self-report questionnaire.

Bailey et al. (2000) have conducted the largest twin study to date. They recruited male and female twin pairs systematically from the Australian Twin Registry and assessed their sexual orientation as well as two related traits: childhood gender nonconformity and continuous gender identity. Twin concordances for homosexual orientation were lower than in prior studies with 20% of male MZ twins concordant vs. 0% of DZ twins, and 24% of female MZ twins vs. 10.5% of DZ twins. Univariate analyses showed that familial factors were important for all traits, but were less successful in distinguishing genetic from shared environmental influences. Multivariate analyses suggested that the causal architecture differed between men and women and, for women, provided significant evidence for the importance of genetic factors to the traits' covariation.

Adoption Studies

A few of the family and twin studies listed in Tables 19.1 and 19.2 have included adoptive siblings who were reared in the same household as the homosexual probands in their samples. Hence, rates of homosexuality have been estimated for genetically unrelated adoptive siblings. Consistent with a genetic hypothesis, for both sexes the proportion of homo-

sexuals (and bisexuals) was significantly greater for MZ cotwins than for either DZ same-sex cotwins or adoptive siblings.

Molecular Genetic Studies

Once a solid foundation of support for significant genetic influence on a trait has been built by means of behavior genetics (family, twin, and adoption studies), as has been the case for sexual orientation especially with males, molecular genetic studies are the next logical step. The two primary varieties of these studies are linkage and association designs. Linkage analysis exploits the key biological phenomenon during generation of sperm and eggs of meiotic recombination, or crossing over, during which both the maternally and paternally derived chromosomes lie in close proximity and undergo exchange of genetic material between the homologous chromosomes, e.g., between a paternally derived chromosome and its maternally derived counterpart. The chance of crossing over between two loci (locations on a chromosome) is referred to as the recombination fraction. Genes and other genetic markers (DNA sequence variations known as polymorphisms) that are close together are less likely to be separated by this process than are those that are farther apart. Therefore, they are usually inherited together by the progeny cells and are genetically linked. Due largely to the complexity of the genetic contributions to male sexual orientation and uncertainty regarding key parameters (mode of inheritance, number of relevant genes, etc.), the type of linkage analyses preferred are nonparametric allele-sharing methods and, more specifically, the affected sibling pair (ASP) method. ASP designs measure the frequency with which a genetic marker allele (or variant) is inherited from a particular parent (referred to as IBD, meaning identical by descent) in a pair of siblings both manifesting the trait. The presence of a trait-influencing gene is revealed when the IBD allele sharing between affected siblings exceeds the expected 50%.

Association studies are based on linkage disequilibrium (LD). This means that a gene variant influencing a trait was initially associated with specific alleles of nearby polymorphic loci. As generations (and the meioses that produce sperms and eggs) pass, the trait-influencing gene and marker allele may remain statistically associated because their proximity reduces the number of recombinations or crossing over that occurs between them. An advantage of association tests is that the chromosomal region examined is usually much smaller than the region examined by testing for linkage in families. Association is often more powerful than linkage in that a valid association may be detected in a sample when linkage is not detectable, even when the gene is playing only a modest role. Most association studies in the past were the

population-based type where the allele frequencies of a group of unrelated cases were compared against those of a group of unrelated controls, and this is the only type of association study done with male sexual orientation. A potential pitfall of population-based case-control studies is that some populations, although they appear homogeneous to superficial examination, are in reality composed of different ancestral human groups, each one potentially with a different allele distribution at the studied loci. If one or more such groups is represented in a largely different proportion in one of the samples of an association dataset (i.e., either in the controls or in the cases), false negative or false positive association findings may easily arise due to methodological artifact.

Linkage Studies

The findings to date from behavior and molecular genetic studies predict that the genetics of male homosexuality will not be simple, and this prediction is consistent with the results of linkage research thus far. Several linkage analyses of male homosexuality to the X chromosome have previously been reported (Hamer, Hu, Magnuson, Hu, & Pattatucci, 1993; Hu et al., 1995; Rice, Anderson, Risch, & Ebers, 1999; Sanders et al., 1998). These studies have been largely based on the assumption that oligogenic (a “few” genes contribute) transmission was most likely, and therefore relied on the ASP method of linkage analysis. See Table 19.3 for a comparison and contrast of the samples examined. While the Xq28 chromosomal region in Hamer et al.’s (1993) study showed a significant linkage signal, with supporting data in a second dataset from the same group in

a follow-up study (Hu et al., 1995), it is still indeterminate (as in many complex traits) whether this finding represents a true positive. An independent group (Sanders et al., 1998) found inconclusive evidence of linkage to Xq28 in 1998, and a third group (Rice et al., 1999) found no support for linkage to Xq28 in 1999. Combining all four linkage studies, with respective affected sibling pair (ASP) sample sizes of 40 (1993), 33 (1995), 54 (1998), and 52 (1999), yields a “multiple scan probability” (MSP) of 0.00003 which is a suggestive p-value when considering all of the chromosomes, i.e., the entire genome (Sanders & Dawood, 2003). The replication MSP (excluding the original positive report from 1993) of 0.07 is at the level of a “trend” and thus not quite statistically significant. This pattern of results is one that has been predicted for complex traits with oligogenic inheritance on the basis of simulation studies: stochastic variation in the degree of co-segregation of any one locus with a trait, which produces variation in the magnitude of linkage findings across samples. Of course, the sample size of the individual linkage studies should be considered a major factor.

In the discussion below, we discuss the first major linkage study of male sexual orientation (Hamer et al., 1993) in greater detail, including a review of the main criticisms of this finding.

Hamer et al.’s (1993) report consisted of two major analyses: a pedigree study and a linkage study. First, they examined family pedigrees in a “randomly ascertained” sample of homosexual probands. As reported in Table 19.1, the probands had a high rate of gay brothers, 13.5%. Furthermore, their pedigrees showed an excess of gay uncles and male first cousins on the maternal side compared to the paternal side, though the difference was not significant. This excess was more pronounced in a subsequent analysis of an

Table 19.3 Sample characteristics of linkage male homosexuality samples

Study	Subject sources	ASPs	DNA	Tools	Inclusion	Exclusion
Hamer et al., 1993	Local clinics, local homophile organizations, homophile publications	40	Proband, homosexual brothers, parents, other siblings	Interview, Kinsey scale, family history	2 (exactly) homosexual brothers	Maximum of one lesbian per family, no male to male transmission
Hu et al., 1995	Local clinics, local homophile organizations, homophile publications	33	Proband, homosexual brothers, parents, other siblings	Interview, Kinsey scale, family history	2 (exactly) homosexual brothers	Maximum of two lesbians per family, no male to male transmission, no bisexuals
Sanders et al., 1998	Homophile organizations, homophile media	54	Proband, homosexual brothers, parents	Kinsey scale, family history	2 (or more) homosexual brothers	No known evidence of male to male transmission
Rice et al., 1999	Homophile media	52	Probands, homosexual brothers	Interview, family history	2 (or more) homosexual brothers	None stated

Number of ASPs are calculated by the $n - 1$ method for independent ASPs where n is the number of homosexual brothers per sibship. DNA refers to the family members from whom blood was sought for genetic analyses. Tools refer to the clinical methods used to assess the trait of sexual orientation. Major inclusion and exclusion criteria for families are listed.

additional sample of 38 families with two gay brothers. If there are genes for male homosexuality, then these families should be especially rich with them. The probands' maternal uncles and cousins (through maternal aunts; no gay cousins were sons of maternal uncles) had rates of male homosexuality of 10.3 and 12.9%, respectively, compared to rates of 1.5 and 3.1% for paternal uncles and cousins. This pattern of results is precisely what one would expect if an X-linked gene influenced male sexual orientation. In X-linked inheritance, males with the trait inherited the gene from their mothers, and hence have more maternal than paternal relatives with the trait.

Because of the suggestion of X-linkage, Hamer et al. (1993) then searched the X chromosome using linkage analysis. Specifically, they looked at the pairs of gay brothers without evidence of paternal transmission (e.g., they excluded a few cases in which the father may have been gay; the pairs analyzed in the linkage study included all eligible pairs analyzed in both pedigree studies). They examined 22 genetic markers distributed across the X chromosome in order to see if brothers concordant for homosexuality were also concordant for the markers. For chromosomal region Xq28 at the tip of the long arm of the X chromosome, 33 of 40 pairs of gay brothers shared all the markers. This was statistically different from the expected rate (20 of 40), suggesting that a gene influencing male sexual orientation lies within that chromosomal region.

Some skepticism has derived from concerns about Hamer et al.'s (1993) study. Risch, Squires-Wheeler, and Keats (1993) raised three main issues. First, they suggested that the pedigree finding, that gay men had an excess of gay maternal relatives, could be due to bias. They speculated that people may know more about their mothers' side of the family (presumably because mothers are more socially oriented, on average). Second, they argued that even if the finding of an increased rate of gay maternal relatives were true, it could be due to fertility patterns. Even if a gene for male homosexuality were autosomal (i.e., not X-linked) gay men are unlikely to have inherited it from their fathers, because men with the gene tend to be gay and gay men tend not to have children. Third, Risch et al. argued that Hamer et al. overestimated an important parameter, l , that reflects the increased prevalence of a trait in first-degree relatives compared to the background, or general population, rate. This parameter, which affects probability estimates, has not yet been precisely estimated in a large and careful study. Risch et al. chose values from available studies to yield the lowest plausible value of l , which would have rendered the linkage analysis statistically nonsignificant. In our view, the concerns raised by Risch et al. are worth the attention of future research, but are not fatal flaws in Hamer et al.'s study.

Other reasons for skepticism have less to do with Hamer et al.'s study than more general concerns about linkage

analysis of genetically complex traits. By genetically complex traits, we mean those whose transmission patterns do not fit classic Mendelian patterns such as autosomal dominant or recessive, or X-linked dominant or recessive. All evidence suggests that male sexual orientation is inherited, if at all, in a complex manner. Linkage analysis has provided important breakthroughs for Mendelian traits, but it has also provided some false leads, especially for genetically complex behavioral traits (Risch & Merikangas, 1993). Indeed, to date not a single molecular finding concerning behavior has been widely accepted as valid by the scientific community, and several highly publicized findings have failed to replicate. This is in part because the number of studies examining any one trait has been relatively small. But it could also reflect the likely possibility that genes underlying behavior variation are typically of small effect and thus difficult to detect. Thus, it is especially important that linkage findings be replicated.

To date, at least three major replication attempts have been reported, including Hu et al. (1995) from Hamer's own research group. Rice et al. (1999) obtained pedigree information from 182 families with at least two gay brothers. They failed to find a significant excess of gay maternal uncles or cousins. In a subset of 41 sibling pairs, they also failed to replicate the finding of linkage to Xq28. Unlike Hamer et al. (1993), however, Rice et al. did not exclude brother pairs with strong evidence of paternal transmission, and it is unclear how many of their subjects would have met Hamer et al.'s (1993) inclusion criteria. Nevertheless, the failure to replicate either of Hamer et al.'s (1993) key findings surely diminishes the probability that they are correct.

In contrast, Hu et al. (1995) from Hamer's lab have reported a successful replication. In this second study, Hu et al. included data from heterosexual brothers as well as gay brothers and found that brothers' similarity for sexual orientation was statistically related to the sharing of Xq28 markers. That is, not only did gay brothers tend to share the markers, but gay-straight pairs tended not to share the markers. The magnitude of the genetic effect was smaller in Hamer's second study, however, and the result was barely statistically significant.

Because of the conflicting replication results, the status of the Xq28 linkage finding is unresolved. When studies are small, replications count more than failures to replicate. Nevertheless, larger studies will be needed to determine whether male sexual orientation is influenced by a gene in Xq28. Currently, the largest linkage study to date of male sexual orientation is underway with DNA samples being collected from a target sample of 1000 families with two or more siblings concordant for homosexuality (Sanders et al., 2005), and researchers in the field eagerly await the results of this large-scale study.

Association Studies

Association studies explore the relation between genetic variation at a specific locus and phenotypic variation. Association studies require that one has a very specific hypothesis, in contrast to linkage studies, which may search the entire genome and examine genetic markers rather than genes. Two association studies have been performed to date for male homosexuality. Macke et al. (1993) used a population-based case-control method to examine DNA sequence variation in the androgen receptor gene, reasoning that some variants may affect sexual differentiation of the brain. This study employed a sample of 197 homosexual males and about 213 unselected (with respect to sexual orientation) male controls with variants of the androgen receptor located on the X chromosome (but not at Xq28) and found no significant differences in the distributions of mutations in homosexual and heterosexual men (i.e., no evidence for association). This gene was selected for examination partly due to its location on the X chromosome since there is some evidence for excess maternal transmission of male homosexuality, which would be consistent with X-linked transmission. However, using linkage analysis, the authors showed that sibling pairs concordant for homosexuality were no more likely than chance to share the same androgen receptor allele.

Another reason the androgen receptor was chosen for examination was not due to its position but rather due to its function, which is to transduce messages from androgens ("male" hormones) to the nucleus of the cell, thus affecting other genes responsive to androgens. In general, many different "candidate" genes may be nominated for examination by means of association testing, but the strength of their candidacy is often in question (relative to any other gene expressed at some point in the brain, which most are). For example, studies in animal models where gene variation may be introduced and the effects systematically examined may propose genes to examine in humans, but there is an unresolved question regarding the validity of extrapolating complex behaviors from species sometimes as different from humans as fruit flies.

Dupree, Mustanski, Bocklandt, Nievergelt, and Hamer (2004) conducted a more recent association study of male sexual orientation to investigate whether differences in the gene encoding the aromatase enzyme influence sexual orientation in men. Aromatase cytochrome P450 (CYP19) is necessary for the conversion of androgens to estrogens and plays an important role in the sexual differentiation of the brain in rodents. This study found no differences between heterosexual and homosexual men in the expression of aromatase mRNA by microarray analysis, suggesting that variation in this gene is not likely to be a major factor in the development of individual differences in male sexual orientation (Dupree et al., 2004).

Additional Molecular Genetic Studies

Two recent studies (Bocklandt, Horvath, Vilain, & Hamer, 2006; Mustanski et al., 2005) have provided additional evidence supporting a heritable component to male and female sexual orientation, and we discuss both studies in further detail below.

Mustanski et al. (2005) conducted the first full-genome scan of sexual orientation in men by genotyping 456 individuals from 146 families with two or more gay brothers with 403 microsatellite markers at 10 cM intervals. They failed to replicate linkage to Xq28 in the full sample; however, they reported three new regions which approached the criteria for near significance (7q36) and for suggestive linkage (8p12 and 10q26). These chromosomal regions may be used in future replication studies with new samples and denser linkage maps.

Bocklandt et al. (2006) measured X chromosome inactivation in a sample of 97 mothers of homosexual men and 103 age-matched control women without gay sons. They reported that extreme skewing of X-inactivation was significantly higher in mothers of gay men (13%) compared to controls (4%) and increased in mothers with two or more gay sons (23%). These findings support a role for the X chromosome in regulating sexual orientation in some homosexual men (Bocklandt et al., 2006), although it is unclear whether these unusual X-inactivation patterns influence the development of sexual orientation in sons via a direct mechanism such as the fraternal birth order effect (Blanchard, 1997) or whether it is the indirect result of a different mechanism. These results have yet to be replicated.

Methodological Issues

The most common criticism aimed at the studies listed in Table 19.2 concerns the "equal environments assumption" that the trait-relevant environment is no more similar for MZ twins than for DZ twins or adoptive siblings. A frequent objection to human twin studies (e.g., Lewontin, Rose, & Kamin, 1984) is that parents treat MZ twins especially similarly and that this similar treatment, rather than the twins' similar genotype, could explain their similar behavior. Indeed, MZ twins are more likely to have been dressed alike and to have shared the same room as children, among other things. The question is whether such treatment makes them more similar, and the evidence suggests that this is not the case, at least for traits studied so far (Plomin, Defries, & McClearn, 1990). For example, MZ twins whose parents make an effort to treat them alike do not behave more similarly than do MZ twins whose parents make an effort to treat them differently. MZ twins whose parents mistakenly believe

that they are DZ twins are as similar as they should be based on their true zygosity. It is true (and unfortunate) that the equal environments assumption has not been directly studied in the context of sexual orientation, but it is also true that existing evidence does not contradict the equal environments assumption.

A more serious potential problem concerns ascertainment bias. Ideally, one could recruit probands by interviewing every member of a well-defined population of, say, gay men and asking them if they were twins. Psychiatric genetics has been able to ascertain twins systematically by interviewing consecutive psychiatric admissions, but this strategy was obviously unavailable to those studying homosexuality. The problem with ascertaining twins via advertisements is that self-selection factors are likely to distort results. The most likely way in which this would occur is that gay men whose twins are also gay would be more willing to volunteer than gay men with heterosexual twins (e.g., because the latter might fear conflict from their twins). Kendler and Eaves (1989) have called this kind of bias “concordance-dependent ascertainment bias.” This type of bias inflates concordances compared to the population rates, though it does not lead to spurious findings of heritability. Spurious findings could be obtained, however, if concordance-dependent bias was stronger for MZ than for DZ twins. Although there is no evidence that this is so, it cannot presently be excluded. A systematic ascertainment strategy is the most crucial methodological goal for future population genetic studies of sexual orientation.

One final limitation of twin and family studies is worth emphasizing. Even accepting its validity, the evidence reviewed so far is uninformative regarding proximate etiological mechanisms. Genetic evidence does not necessarily support a neuroendocrine explanation, for example. One could envision a host of other genetic pathways to homosexuality. But available studies cannot distinguish among them. Molecular strategies that can identify specific genes for sexual orientation will be much more useful in elaborating the developmental pathways from genes (if they exist) to behavior.

Environmental Influences

The environmental contribution to phenotypic variance is directly comparable to heritability and may be broken down into two subcomponents or parameters: the proportion of variance that is “shared” by family members and the other “nonshared” or “unique”. Shared environmental influences are those that make members of a family similar to each other (such as having the same parents and growing up in the same house), while the rest of the variance is described as everything else that siblings do not share.

The most convincing result of the twin studies to date is that environment is sometimes an important determinant of sexual orientation. If it were not so, MZ twins would always have the same sexual orientations, but about half the time, homosexual probands have heterosexual twins (and this is probably an underestimate). Experiences or developmental antecedents that differed between homosexual and heterosexual cotwins would be promising candidates to illuminate relevant environmental factors. Thus, perhaps the most intriguing possible application of the twin method concerns the study of discordant MZ twins (Reiss, Plomin, & Hetherington, 1991). For example, we found in both our male and female studies (Bailey et al., 2000; Dawood, Pillard, Horvath, Revelle, & Bailey, 2000) that discordant MZ twins and nontwin siblings also reported quite different childhood experiences. On questionnaire measures, the homosexual twins reported more gender-atypical behavior, and often in interviews twins mentioned this as an early indication of an important difference between them. This suggests that in many cases, relevant environmental factors operate during childhood. This would contrast, for example, with factors such as adult sexual experiences that have sometimes been alleged to be important in determining sexual orientation (Dawood et al., 2000).

It is important to emphasize that “environment” as construed by behavior geneticists differs from “environment” as it is understood by most laypeople. Environment comprises all causes of variation that are not genetic, where genetic is understood in the specific sense encoded in germline DNA. (Even somatic mutations, which are not typically shared by close relatives, are environmental in this sense.) There is a biological environment – random developmental and intrauterine factors, illness, diet, injury, etc. – as well as a psychosocial environment. Distinguishing between these kinds of environmental factors for sexual orientation will require that specific testable theories be offered.

Results of studies in Table 19.2 suggest that the most important environmental factors are those that typically differ even between MZ twins reared in the same family. Thus, for example, cold, distant fathers are unlikely to be important, because it is unlikely that a father would behave in a cold and distant manner toward only one MZ twin. In contrast, and contrary to intuition, biological differences between MZ twins are not uncommon (Torrey, 1994; Turner, 1994). For example, the twin transfusion syndrome, in which twins receive unequal blood supply, can cause substantial differences in MZ twins’ health (and indeed mortality of one twin is common). MZ twins with congenital brain anomalies typically have normal cotwins. Molenaar, Boomsma, and Dolan (1993) have argued that much of apparent within-family environmental variation may be attributable to poorly understood and effectively random developmental processes. There is now considerable speculation, and some

evidence, that epigenetic phenomena including differential DNA methylation might be one such class of random process (Oates et al., 2006).

The Fraternal Birth Order Effect

Several excellent reviews of biological research on human sexual orientation have recently been published (Mustanski, Chivers, & Bailey, 2002; Rahman, 2005) which can provide more comprehensive reviews of neuroendocrine and neurodevelopmental theories of sexual orientation. We will note here, however, that perhaps the most replicated finding in sexual orientation research is the fraternal birth order effect in homosexual men (Blanchard, 1997) whereby homosexual men have a greater number of older brothers than heterosexual men do, in diverse community and population-level samples. The estimated odds of being homosexual increase by approximately 33% with each older brother, and statistical modeling using epidemiological procedures suggest that one in seven homosexual men may owe their sexual orientation to the fraternal birth order effect (Cantor, Blanchard, Paterson, & Bogaert, 2002). While purely genetic factors could not explain this effect, recent evidence suggests that the effect is related to prenatal events (Blanchard, 2004; Bogaert, 2006). Currently, a maternal immune response to male-specific, Y-linked antigens is the most plausible explanation for this effect (Blanchard & Bogaert, 1996) which becomes stronger with each male pregnancy. However, empirical studies supporting this hypothesis have yet to be conducted.

Directions for Future Research

During the past two decades, a growing body of evidence has accumulated suggesting that familial and genetic factors affect both male and female sexual orientation. The genetic evidence is substantially stronger for male than for female sexual orientation, and multiple genes could well contribute significant influences on the development of sexual orientation.

Although ongoing studies investigating genetic sources of variation in sexual orientation will contribute a critical aspect for understanding the origins and development of sexual orientation, perhaps the most interesting topic for future research in this area lies in studying nonshared environmental sources of variation and the epigenetic relationship between environmental and genetic factors.

Plomin (1994) has suggested that longitudinal studies and behavior genetic methodologies will be useful in studying the effects of nonshared environments. The study of discordant monozygotic twins, who are genetically identical,

can be particularly important since nonshared environmental factors are responsible for monozygotic twins discordant for sexual orientation. For example, future molecular genetic studies could examine epigenetic modifications of DNA between twins using a genomewide screen of differentially methylated regions to identify potential discrete differences between homosexual and heterosexual MZ twins.

Discordant MZ twins could also be used to examine specific environmental influences on the development of sexual orientation. For example, there is strong evidence from both retrospective and prospective studies (Bailey & Zucker, 1995) supporting an association between childhood gender nonconformity and adult sexual orientation. Though significantly larger for males, the effect sizes reported for both sexes are among the largest ever reported in the realm of sex-dimorphic behaviors. Male and female identical twins discordant for sexual orientation might well differ in other gender-related traits, such as childhood gender nonconformity which is also significantly heritable for both men and women (Bailey et al., 2000). If so, it suggests that the non-shared or unique environmental influences that lead to different sexual orientations may also contribute to the development of other gender-related traits, including childhood gender nonconformity.

At this stage, few conclusions can be drawn with certainty regarding genetic and environmental determinants of sexual orientation. Important methodological research innovations hold the most potential for furthering our knowledge on the origins and development of human sexual orientation. Future research should also attempt to integrate different biological approaches in order to provide valuable information about the specific pathways by which genes exert their influence on sexual orientation and its correlates.

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