CHAPTER EIGHT

The Biological Basis of Human Sexual Orientation: Is There a Role for Epigenetics?

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Abstract

Sexual orientation is one of the largest sex differences in humans. The vast majority of the population is heterosexual, that is, they are attracted to members of the opposite sex. However, a small but significant proportion of people are bisexual or homosexual and experience attraction to members of the same sex. The origins of the phenomenon have long been the subject of scientific study. In this chapter, we will review the evidence that sexual orientation has biological underpinnings and consider the involvement of epigenetic mechanisms. We will first discuss studies that show that sexual orientation has a genetic component. These studies show that sexual orientation is more concordant in monozygotic twins than in dizygotic ones and that male sexual orientation is linked to several regions of the genome. We will then highlight findings that suggest a link between sexual orientation and epigenetic mechanisms. In particular, we will consider the case of women with congenital adrenal hyperplasia (CAH). These women were exposed to high levels of testosterone in utero and have much higher rates of nonheterosexual orientation compared to non-CAH women. Studies in animal models strongly suggest that the long-term effects of hormonal exposure (such as those experienced by CAH women) are mediated by epigenetic mechanisms. We conclude by describing a hypothetical framework that unifies genetic and epigenetic explanations of sexual orientation and the continued challenges facing sexual orientation research.

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1. INTRODUCTION

Sexual orientation is one of the most pronounced sex differences in the animal kingdom. With few exceptions, the overwhelming majority of people are heterosexual: most males desire females as sexual partners and vice versa. Knowledge about whether someone has a sexual preference for males or females is one of the most reliable behavioral predictors of that individual's biological sex, perhaps second only to gender identity (the sense of being male or female). Although heterosexuality is the norm, a small but significant proportion of individuals (2–6%) report having predominantly homosexual attractions (Diamond, 1993). The distribution of men and women between the two extremes of sexual orientation (completely heterosexual vs. completely homosexual) shows some interesting differences. Men are bimodally distributed, with most men being mainly attracted to just one sex (Hamer, Hu, Magnuson, Hu, & Pattatucci, 1993;Vrangalova & Savin-Williams, 2012). On the other hand, fewer women report that they are exclusively attracted to the same sex, but more of them report attraction to both sexes compared to men (Hu et al., 1995;Vrangalova & Savin-Williams, 2012).

The search for the biological basis of sexual orientation is not a recent enterprise. For instance, in the midnineteenth century, it was generally accepted that innate intellectual inferiority explained why certain classes of people were socially disadvantaged (Terry, 1995, pp. 129–169). The brains and bodies of these groups, which included the poor, women, persons of color, and homosexuals, were presumed to reflect their lower status. Richard von Krafft-Ebing, a notable Viennese sexologist, was among those who believed that the homosexual behavior was a result of defective development (Krafft-Ebing, 1965). By the late nineteenth and early twentieth centuries, the discourse had changed somewhat. The bodies of homosexuals were still seen as distinct, but they were now characterized as a third sex (Hirschfeld, 1958). In this framework, homosexuals were seen as inverts, that is, gay men were thought to have some innately feminine tendencies, while lesbian women were more inclined to express masculine traits. Although homosexuals are no longer considered a distinct sex, the inversion paradigm continues to influence the way research on homosexuality is presented, particularly in terms of neurological correlates (Berglund, Lindstrom, & Savic, 2006; LeVay, 1991; Rice, Friberg, & Gavrilets, 2012; Savic, Berglund, & Lindström, 2005).

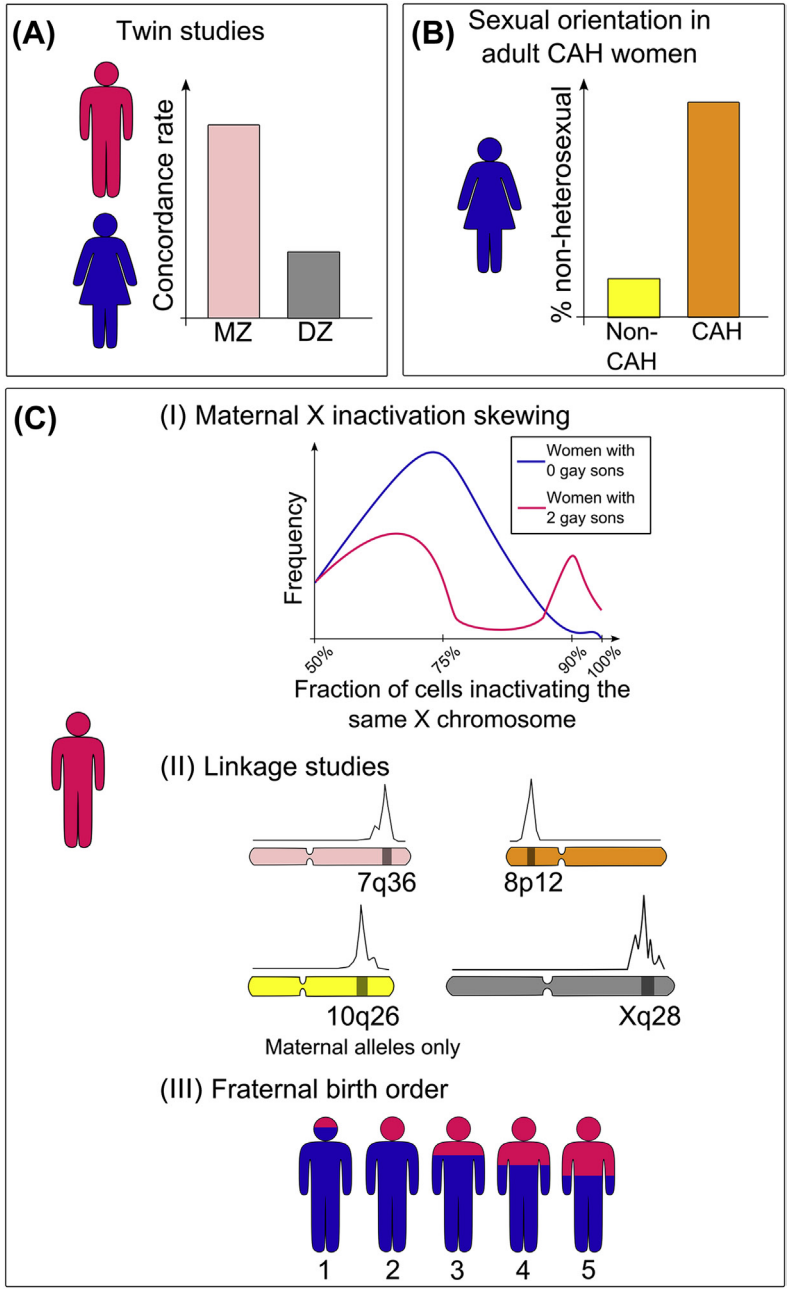
In this chapter, we will consider the role of epigenetics in human sexual orientation (Figure 8.1). First, we will discuss the role of genetics in influencing this trait and review significant findings from 1994 to 2014. Second, we will highlight findings suggesting a link between epigenetics and sexual orientation, with a particular focus on female sexual orientation and prenatal hormone exposure. Third, we will consider data from animal models about potential epigenetic mechanisms that could underlie long-term or organizational effects of prenatal hormones.

2. THE GENETICS OF SEXUAL ORIENTATION

The first clues that sexual orientation (particularly in men) was strongly influenced by genetics came from family and twin studies. Gay men have a higher number of homosexual relatives in comparison to heterosexual men (Bailey & Pillard, 1991; Pillard & Weinrich, 1986). Similarly, in the families of nonheterosexual women, there is evidence of clustering of this trait (Pattatucci & Hamer, 1995). Twin studies have also indicated a significant role for genetics. Although the exact concordance rates in monozygotic (MZ) twins differs between studies, they are uniformly higher than concordance rates in dizygotic (DZ) twins or nontwin siblings, and all suggest that sexual orientation is a highly heritable trait (Bailey, Dunne, & Martin, 2000; Bailey & Pillard, 1991; Kendler, Thornton, Gilman, & Kessler, 2000; Kirk, Bailey, Dunne, & Martin, 2000).

There have been few molecular genetic studies in this area with the majority done by Dean Hamer's group at the NIH. They reported the first linkage of male homosexuality to a specific genetic location in 1993 (Hamer et al., 1993). They first noticed that male homosexuality appeared to be maternally loaded (i.e., gay male probands had more gay male relatives on their maternal side), which led them to focus on the X chromosome. They found that male sexual orientation was linked to a region near the end of this chromosome called Xq28, which is large, complex, and gene dense (Figure 8.1(C)). In two out of three subsequent studies (one from Hamer's group and two from independent teams, this finding was replicated (Hamer, 1999; Hu et al., 1995; Rice, Anderson, Risch, & Ebers, 1999). A meta-analysis across all four studies revealed that Xq28 allele sharing was significantly elevated among gay brothers (Hamer, 1999). Of note is that female sexual orientation does not appear to be linked to Xq28 (Hu et al., 1995).

To date, two follow-ups to this group of studies have been published. Mustanski et al. (2005) performed the first genomewide scan for markers


(A)
Twin studies
Concordance rate
MZ
DZ
(B)
Sexual orientation in adult CAH women
% non-heterosexual
Non-CAH
CAH
(C)
(I) Maternal X inactivation skewing
Women with 0 gay sons
Women with 2 gay sons
Frequency
50%
75%
90%
100%
Fraction of cells inactivating the same X chromosome
(II) Linkage studies
7q36
8p12
10q26
Xq28
Maternal alleles only
(III) Fraternal birth order
1
2
3
4
5

associated with male sexual orientation. Their sample group included subjects who were part of the earlier studies, and when they limited their analysis to just these individuals, they also found linkage to Xq28. When all subjects were considered, the highest linkage scores were seen at chromosomes 7 (7q36) and 8 (8p12). Interestingly, this study also observed linkage at chromosome 10 (10q26) that resulted from excess sharing of maternal alleles only. The relevance of this particular finding to the potential involvement of epigenetic mechanisms will be discussed later. In summary, this study reinforced the view that genetics plays a major role in male sexual orientation and that at least one type of male homosexuality may be inherited maternally. A genomewide linkage scan from 2010 also used homosexual brother pairs but was unable to identify any significantly linked regions (Ramagopalan, Dyment, Handunnetthi, Rice, & Ebers, 2010). However, the number of brother pairs in this study was much smaller than in Mustanski et al.

At the 2012 Annual Meeting of the American Society of Human Genetics, the results of two new genetic linkage/association studies were presented although neither has been published at press time. The first study was a large-scale linkage study on 410 independent pairs of homosexual brothers from Alan Sanders, which largely agrees with the results of Mustanski et al. (Sanders et al., 2012). In this study, the strongest linkage peak was seen on chromosome 8 and overlaps with the peak seen in the 2005 study. The second strongest linkage peak was at Xq28. This study is currently in review (Sanders, personal communication). The second study was carried out by the personal genomics company 23andme. Although this was the largest and best-powered genomewide association study (GWAS) on sexual orientation, it did not find any genetic markers that were significantly associated with sexual orientation (Drabant et al., 2012). This is likely due to the

Figure 8.1 ***Sexual orientation has biological underpinnings.*** The accumulated evidence strongly suggests that sexual orientation has biological origins. (A) Twin studies on both male and female twins have found that the concordance rate for homosexuality is significantly higher in MZ twins than in DZ twins. (B) The proportion of adult women with CAH who identify as nonheterosexual is many times higher than the proportion in non-CAH women. (C) Multiple lines of evidence indicate the involvement of genetic and epigenetic factors in male sexual orientation. (I) The frequency of extreme skewing in X chromosome inactivation is significantly higher among women with two gay sons than among women with no gay sons. (II) Male sexual orientation has been linked to several regions in the human genome. (III) Each male pregnancy increases the chance that the following male child will be gay by 33% relative to the baseline population rate. CAH, congenital adrenal hyperplasia; MZ, monozygotic; DZ, dizygotic. (See the color plate.)

differences in the types of subjects collected—Sanders et al. used brother pairs whereas Drabant et al. cast a much wider net. We will expand on this point in the conclusion.

Taken together, these studies show that genetics plays a role in sexual orientation, at least for men. This is not entirely surprising. Since sexual reproduction is essential to species propagation, placing sexual orientation under genetic control would ensure tight regulation of this behavior. Nevertheless, there seems to be an additional layer of molecular control, which is likely to involve epigenetic mechanisms.

3. EPIGENETICS AND SEXUAL ORIENTATION IN HUMANS

Direct evidence of epigenetic mechanisms in human sexual orientation is sparse. There are several lines of evidence that indicate an involvement of these mechanisms but a direct link is yet to be demonstrated. In this section, we will review the relevant data and highlight a recent hypothesis that has gained prominence about how epigenetics may help explain the occurrence of homosexuality. This will lead us into a discussion about the role of prenatal hormones in female sexual orientation and potential epigenetic mechanisms that may account for this long-term effect of prenatal hormone exposure.

The first indication that epigenetic mechanisms may be involved in sexual orientation emerged from the twin studies described earlier (Bailey et al., 2000; Bailey & Pillard, 1991; Kendler et al., 2000; Kirk et al., 2000). The concordance rate between MZ twins was always higher than in DZ twins but even the highest observed rate of concordance, 52% (Bailey & Pillard, 1991), was far below what would be expected for a trait that is exclusively genetically influenced and strongly suggests a role for environmental effects in influencing sexual orientation. Many researchers increasingly believe that environmental effects are translated into biological consequences through epigenetic mechanisms (Jirtle & Skinner, 2007).

Due to the sensitive nature of this topic, we would like to clarify that "environmental effects" does not refer to hypotheses about the causal role of dominant mothers and distant fathers or sexual abuse in homosexuality that have long been discredited scientifically but continue to retain their cachet among some circles (Brannock & Chapman, 1990; Siegelman, 1974; Whitam & Zent, 1984). These are highly unlikely to account for the discordance between MZ twins anyway. Nor are we referring to the social

environment (although we cannot definitively exclude it), which is unlikely to significantly shape sexual orientation, particularly in males, since this trait appears to be determined from an early age. Rather, we mean variations between each twin during development, which can include differences of the intrauterine environment. Although the nutrient bath in which both twins develop may be highly similar, there could be differences that could affect epigenetic markers on genes relevant to sexual orientation. We already know that the DNA methylation profile is not identical between MZ twins at the time of birth (Gordon et al., 2012). There is also increasing evidence that discordance among MZ twins in other traits is related to DNA methylation differences (Dempster et al., 2011; Kuratomi et al., 2008). Our group is currently evaluating the hypothesis that discrepancies in DNA methylation are related to discordance in sexual orientation in MZ twin pairs.

There are other clues that the in utero environment may be a player in sexual orientation. The fraternal birth order effect is one of the most replicated and robust findings in sexual orientation research. Each son increases the odds of homosexuality in the next son by 33% relative to the baseline population rate (Blanchard, 1997; Blanchard & Bogaert, 1996; Jones & Blanchard, 1998). Although this may seem like a large increase, the probability of a gay son reaches 50% only after 10 older brothers. The birth-order effect only holds true if all the brothers are from the same mother—if the older brothers are from another mother, there is no effect. The number of older sisters does not have an effect either. The biological mechanism underlying fraternal birth order is still unclear. One hypothesis that has yet to be tested is that a male pregnancy triggers male-specific antigens in the mother, and each successive male child increases this immune response (Blanchard & Bogaert, 1996; Blanchard & Klassen, 1997). Whether this hypothesis or another proves accurate, it is highly probable that epigenetic mechanisms mediate the long-term consequences of the in utero events.

As detailed above, Mustanski et al. (2005) observed a linkage of male homosexuality to 10q26. This chromosomal stretch is of particular interest in the context of epigenetic mechanisms as it is only linked to male sexual orientation when there is an excess sharing of alleles of maternal origin. This finding suggests the involvement of genomic imprinting. In line with this, 10q26 contains a region that is differentially methylated in the germline based on parent-of-origin (Strichman-Almashanu et al., 2002).

Epigenetic mechanisms that specifically affect the X chromosome have also been implicated in sexual orientation. In individuals with two X chromosomes, one copy of the X chromosome is inactivated so that X gene

dosage is equivalent to individuals who only have one X. In theory, the choice of which X chromosome to undergo inactivation is random and happens independently in each cell. Therefore, at the population level, the maternal X should be inactivated in 50% of cells, and the paternal X should be inactivated in the other 50%. In practice, a slight departure from this 1:1 ratio (or skewing) is not uncommon. However, mothers of gay men show extreme skewing of X inactivation (ratios of ≥9:1) at rates far higher than mothers with only heterosexual sons (Bocklandt, Horvath, Vilain, & Hamer, 2006). The rate of extreme skewing seems to be positively correlated with the number of gay sons.

A recent theoretical paper put forth a model about how epigenetic markers could lead to homosexuality and explain its continued existence even though it imposes a significant penalty on fitness or the ability of that organism to reproduce (Rice et al., 2012). This model can be broken down into three core assertions. The first is that sex-specific epigenetic marks (which could take the form of histone modifications, DNA methylation, and/or noncoding RNAs) lead to sex-specific traits. The sex-specific marks that are present in the parents are usually erased during gametogenesis (so that the "correct" sex-specific mark can be placed during embryogenesis). If this erasure fails to occur and carries over to the zygote, development of traits that are discordant with the sex of that individual (like homosexuality) can occur. For instance, if a feminizing epigenetic mark remains in the ovum, and it is fertilized, then the trait under the control of that mark in the offspring might also be feminized. The second core assertion is that sensitivity to fetal androgen signaling is sexually dimorphic due to sex-specific epigenetic marks with XX fetuses being less sensitive than XY ones. To support this claim, the authors point out that in both rats and humans, about 5% of XX fetuses have testosterone levels that are in the lower end of the male range during the prenatal testosterone surge, which is important for genital development. Since the incidence of discordance between the genitals and gonads is much lower than they would be if testosterone levels were the only determinant, the authors conclude that there is sexual dimorphism in sensitivity levels to testosterone. The third pillar of this model is that by sex-reversing sensitivity to androgen, sexual orientation will be sex reversed as well. This means that feminizing epigenetic marks will make XY fetuses less sensitive to the effects of androgens and therefore more likely to develop as homosexual men. We will not be discussing arguments about why homosexuality continues to exist despite this penalty as it is beyond the scope of this paper. Interested readers can refer to the following references (Bobrow

& Bailey, 2001; Camperio-Ciani, Corna, & Capiluppi, 2004; Rahman et al., 2008; Zietsch et al., 2008).

We find the first two pillars of Rice et al.'s model to be sound. We believe it is very likely that sex-specific epigenetic marks are (at least partly) responsible for sexually dimorphic traits including sexual orientation. Although a causal link between sexually dimorphic epigenetic marks and traits is yet to be directly shown, there is extensive evidence (the majority from animal studies) of sex-specific epigenetics markers that are correlated with sex differences in the brain (Lister et al., 2013; Morgan & Bale, 2011; Morgan & Bale, 2012; Murray, Hien, de Vries, & Forger, 2009; Schwarz, Nugent, & McCarthy, 2010). Our own work on the effect of perinatal testosterone on the mouse brain also agrees with this (Ghahramani et al., 2014). Although not as extensive as the animal literature, the evidence concerning epigenetics and human sexual orientation that we have presented strongly suggests a link between the two. The case for differential sensitivity to androgen is also well made and we do not disagree with the overall claim.

However, we disagree with the third major component of this model, which is that sex-reversing sensitivity to androgen signaling via epigenetic markers will result in homosexuality in both sexes. One of the fundamental assumptions of Rice et al.'s model is that the biological factors affecting sexual orientation are the same in both sexes. The data do not necessarily support this view. For instance, linkage to Xq28 for sexual orientation only holds true for men. The manifestation and expression of sexual orientation in men is not the mirror image of this process in women. We've already pointed out one fundamental difference earlier, namely, that the percentage of nonheterosexual women who are attracted to both sexes is much higher than in nonheterosexual men (Hamer et al., 1993; Hu et al., 1995; Vrangalova & Savin-Williams, 2012). Men also appear to be highly target-specific and only aroused by their stated preference (Cerny & Janssen, 2011; Chivers, Rieger, Latty, & Bailey, 2004). Additionally, sexual orientation appears to be much more fluid (more movement between categories) in women than in men (Diamond, 2000; Peplau & Garnets, 2000).

Perhaps most importantly, prenatal androgen levels have not been shown to play a role in male sexual orientation although they have been implicated in female sexual orientation. Variations in the gene that encodes the androgen receptor do not appear to be related to male sexual orientation (Macke et al., 1993). In addition, there are no reports showing that hypovirilized XY individuals experience an increased attraction to other men. Regarding animal studies, it is true that manipulation of prenatal and/or perinatal hormonal

levels in rodents has been shown to lead to changes in mating/sexual behavior (Dominguez-Salazar, Portillo, Baum, Bakker, & Paredes, 2002; Stockman, Callaghan, & Baum, 1985). Lordosis (the female-typical mating behavior) can be induced in males. However, changing sex-stereotyped mating behavior is not the same as changing the sexual orientation or partner preference of that animal. Inducing lordosis in a male does not make it prefer males over females. It will accept mounts from females as well. Further, these changes in behavior are only induced by hormonal changes far outside the natural variation in androgen levels (Phoenix, Goy, Gerall, & Young, 1959).

On the other hand, prenatal androgen exposure in women could affect their sexual orientation. The strongest data for this view comes from women who have a genetic disorder known as congenital adrenal hyperplasia (CAH). Female fetuses that have CAH experience increased levels of androgen exposure, which greatly exceed female-typical levels. In some cases, androgen levels are high enough to cause masculinization of their external genitalia. Because CAH can be fatal if uncontrolled (for reasons unrelated to the level of circulating testosterone), these girls start treatment immediately after birth, which brings their postnatal testosterone levels back into the female-typical range. The proportion of adult CAH women who identify as lesbian is many times higher than in the general population and is correlated with prenatal androgenization (Dittmann, Kappes, & Kappes, 1992; Hines, Brook, & Conway, 2004; Meyer-Bahlburg, Dolezal, Baker, & New, 2008). Studies of CAH girls have repeatedly shown that they are masculinized on other sexually dimorphic cognitive and behavioral traits. These include play behavior (Hines, 2011; Nordenstrom, Servin, Bohlin, Larsson, & Wedell, 2002), spatial cognition (Mueller et al., 2008), and aggression (Pasterski et al., 2007).

4. MOLECULAR MECHANISMS UNDERLYING THE LONG-TERM EFFECTS OF HORMONES

The long-term changes in CAH women seem to originate from the prenatal exposure to high levels of testosterone. How does this one early experience continue to have ramifications throughout that individual's life? Although we do not have a definitive answer to this question yet, recent studies in animal models have begun to shed light on this issue and strongly implicate the involvement of epigenetics.

The long-term effects of prenatal hormone exposure have been studied in animal models for decades. Collectively, these effects are termed

"organizational" because they appear to organize affected tissues and behaviors to develop in a particular way (Ngun, Ghahramani, Sanchez, Bocklandt, & Vilain, 2011). On the other hand, the acute actions of hormones that rely on their continued presence (and often on an earlier organizational effect) are termed "activational." The initial experimental demonstration of organizational effects was a seminal study where pregnant guinea pigs were injected with testosterone resulting in their daughters showing masculinized mating behavior in adulthood (Phoenix et al., 1959). This study demonstrated the main concepts of the organizational theory of hormonal action: differentiation along sex-specific lines, apparent effects much later in life, and sensitivity during a small developmental period (this so-called critical period is usually perinatal). Since then, sex steroids have been shown to lead to sex differences in brain gene expression, neural anatomy and morphology, and behavior (Arai & Matsumoto, 1978; Barraclough & Gorski, 1961; Fleming & Vilain, 2005; Hines, Allen, & Gorski, 1992; Kauffman et al., 2007; Murakami & Arai, 1989; van Nas et al., 2009; Rissman, Wersinger, Taylor, & Lubahn, 1997; Tang & Wade, 2012).

Many testosterone-related effects with regards to brain sexual differentiation in rodents are actually dependent on its conversion to estradiol via aromatization (Naftolin, 1994). For instance, the large sex difference seen in the sexually dimorphic nucleus of the preoptic area results from the prevention of neuronal apoptosis by aromatized testosterone (Tsukahara, 2009). Testosterone and estradiol promote sexual differentiation by acting on a wide variety of cellular processes such as cell division, migration, growth, and survival to synaptic patterning (Ngun et al., 2011). It is important to keep in mind that the active hormone in organizing the brain sexually differs between humans and most animal models. In humans (and other primates), androgens (and not estradiol) are the primary hormonal differentiators (Wallen, 2005).

There is compelling evidence implicating the involvement of epigenetic mechanisms in mediating the long-term effects of hormones and sexual differentiation of the brain in animal models. Adult methylation patterns at the promoters of the two canonical estrogen receptors and the progesterone receptor are affected by perinatal hormones (Schwarz et al., 2010). Levels of histone acetylation in the developing cortex/hippocampus are sexually dimorphic (Tsai, Grant, & Rissman, 2009). In addition, regulation of histone acetylation is crucial to sexual differentiation of the principal nucleus of the Bed Nucleus of the Stria Terminalis (BNST) (Murray et al., 2009). A large number of micro-RNAs show sexually dimorphic expression in the

neonatal mouse brain and early prenatal stress can lead to transgenerational dysmasculinization of miRNA expression (Morgan & Bale, 2011; Morgan & Bale, 2012). Our own data (Ghahramani et al., 2014) suggest that molecular organization by testosterone in the mouse brain occurs via early programming on relatively few genes and that this small initial effect is what sets up the brain to respond in a particular fashion to other events during postnatal development.

Presently, direct demonstration of epigenetic mechanisms in mediating the long-term effects of hormones in humans has not been achieved. However, there are strong indications that environmental factors can exert long-lasting effects on the brain through DNA methylation (Hernandez et al., 2011; Ladd-Acosta et al., 2007; McGowan et al., 2009), and we know that the methylome of the human brain shows many sex differences (Lister et al., 2013).

5. CONCLUSION

The preponderance of evidence from sexual orientation research strongly suggests that human sexual orientation has biological underpinnings and that it is tightly regulated at the molecular level. Although the "gay genes" are yet to be identified, there is little doubt that genetics plays a role in this trait. Epigenetics appears to be another important contributor, particularly in mediating environmental effects, such as the intrauterine milieu. However, much work remains to be done on both fronts to identify which genes are involved in the control of sexual orientation. Rice, Friberg, and Gavrilets (2013) have proposed steps to test their epigenetic hypothesis. Our group is currently testing the hypothesis that discordance in sexual orientation between MZ twins is related to discordance in epigenetic marks.

Although we believe that the biological factors that affect sexual orientation differ between the sexes, we believe that the genetic network that underlies this trait is common to them. That is, we believe it to be unlikely that there are "straight male genes," "straight female genes," "gay genes," and "lesbian genes." Instead, we hypothesize that a network of genes underlies sexual attraction, and that this network can predispose for attraction to men, women, or both (Figure 8.2). Due to the tight correlation between biological sex and sexual orientation, it is likely that the same factors that trigger sex-typical development in other areas (such as sex-specific genes and hormones) are also responsible for initiating the development of sexual orientation in a particular fashion. In most individuals, this network

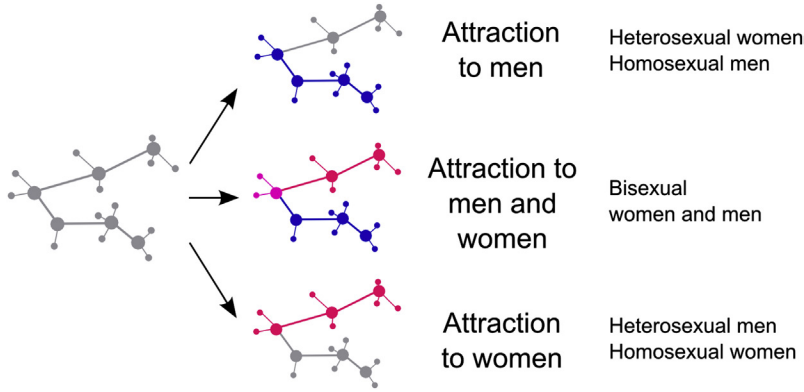


Figure 8.2 ***Genetic regulation of sexual orientation.*** We hypothesize that sexual orientation is regulated by a genetic network that is present in both sexes. This network predisposes an individual to be attracted to men, women, or both. The genetic cascade leading to development of sexual orientation is likely triggered by sex-specific factors such as sex-specific genes or hormones so that sexual orientation is concordant with biological sex. Adult sexual orientation depends on the interplay between this network and other factors (both genetic and nongenetic). If the activity of genes predisposing to attraction to men is dominant in this network, the outcome is a heterosexual woman or gay man (top row). Alternatively, if genes predisposing to attraction to women have higher levels of activity, the result is a heterosexual man or lesbian woman (bottom row). In situations where both types of genes have similar levels of activity, bisexual individuals are produced (middle row). (See the color plate.)

canalizes neural development such that they are predisposed to be sexually attracted to the opposite sex. However, at various points along this network, various factors (both genetic and nongenetic) can interact with it and alter the final outcome. For instance, the high level of testosterone exposure in utero that is experienced by females with CAH may alter the activity of this genetic network by affecting epigenetic marks so that male-typical development (attraction to women) is more likely to occur.

If our overall hypothesis is correct, this implies that there are different subtypes of nonheterosexual men and women if we categorize them based on the biological origin of their sexual orientation. The evidence indicates that this may be the case for male homosexuality. For example, linkage to Xq28 may help explain homosexuality in families where this trait is maternally loaded. All the studies that have identified linkage to Xq28 thus far have selected for maternal (but not paternal) linkage and coincidence of homosexuality in brother pairs. In contrast, the studies that did not find linkage to Xq28 (Drabant et al., 2012; Rice et al., 1999) did not select their subjects based on those criteria.

The slow and halting progress in this field is likely obvious even to the most casual reader of this review. Discoveries here have lagged far behind many other areas of behavioral genetics. We believe that there are two main reasons for this, which are intertwined. The first is that although research into the biology of sexual orientation attracts enormous public interest, this field has been experiencing a severe lack in funding for many years. As homosexuality is now considered a normal variation of human behavior, it is extremely difficult to get funding bodies (both public and private) to see how this line of research aligns with their missions, which are often heavily focused on health and pathological processes. More generally, research into human sexual behavior is still considered highly controversial. As such, grant applications on sexual orientation research have to overcome many significant hurdles that other types of applications do not.

The second major reason for this lack of progress is the complexity—in the genetic sense and in other ways—of sexual orientation. Researchers are usually dependent on self-identification, which may be inaccurate due to the continued social stigmatization of homosexuality. Even assuming a negligible effect from inaccurate self-reporting, it seems increasingly likely that there may be multiple genetic roots and thus subtypes of male homosexuality as discussed above. Given the current funding situation, it is highly unlikely that a GWAS with sufficient power will be performed (such a study would probably need an *n* far north of the 7887 men in the 23andme study) nor is that necessarily the most efficient method given the limited success of GWAS (Visscher, Brown, McCarthy, & Yang, 2012). Moving forward, our efforts and limited resources may be more effectively applied by designing studies that focus on particular subtypes of homosexuality.

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