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Finger-Length Ratios in Female Monozygotic Twins Discordant for Sexual Orientation[1]

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The second to fourth finger digit ratio (2D:4D ratio) is a sex-dimorphic characteristic in humans that may reflect relative levels of first trimester prenatal sex hormones. Low interdigital ratio has been associated with high levels of androgens. It has been reported in unrelated women that low 2D:4D ratio is associated with lesbian sexual orientation, but because of the nature of those samples, it was not possible to conclude whether lower ratio (and hypothetically, higher androgen levels) in lesbians are due to differences in genetics as opposed to differences in environment. To test the hypothesis that low 2D:4D in lesbians is due to differences in environment, interdigital ratio data were analyzed in a sample of female monozygotic (MZ) twins discordant for sexual orientation (1 twin was lesbian, the other was heterosexual; $n = 7$ pairs). A control group of female MZ twins concordant for sexual orientation (both twins were lesbian) was used as a comparison ($n = 5$ pairs). In the twins discordant for sexual orientation, the lesbian twins had significantly lower 2D:4D ratios on both the right and left hands than their heterosexual cotwins. There were no significant differences for either hand in the twins concordant for sexual orientation. Because MZ twins share virtually the same genes, differences in 2D:4D ratio suggest that low 2D:4D ratio is a result of differences in prenatal environment.

KEY WORDS: lesbian; monozygotic twins; interdigital ratio; sexual orientation.

INTRODUCTION

The 2D:4D (second to fourth finger digit ratio) ratio is a sex-dimorphic characteristic in humans (Garn, Burdi, Babler, & Stinson, 1975; George, 1930; Phelps, 1952), which may reflect relative levels of first trimester prenatal testosterone. Low interdigital ratio (below 1) is typical of males, and has been associated with high levels of androgens, whereas higher ratio (1 or greater) is associated with females and high estrogen levels (Manning, Scutt, Wilson, & Lewis-Jones, 1998). Studies of 2D:4D ratio, testosterone level, and its correlates support the assumption that low 2D:4D ratio is associated with high testosterone levels. For example, Manning and his associates have found a positive correlation between low 2D:4D and sperm count, testosterone level, male cognitive skills, and athletic ability. Evidence from these studies supports the corresponding hypothesis that low testosterone level (high estrogen level) is positively associated with high 2D:4D ratio as indicated by female fertility markers (e.g. family size and waist-to-hip ratio), estrogen levels, female cognitive skills, and associations with diseases such as breast cancer (Austin, Manning, McInroy, & Mathews, in press; Lutchmaya, Baron-Cohen, Raggatt, Knickermeyer, & Manning, 2002; Manning, 2001, 2002; Manning et al., 1998; Manning & Bundred, 2000; Manning & Taylor, 2001; Manning, Trivers, Singh, & Thornhill, 1999). Other researchers have found an association between low 2D:4D ratio and congenital adrenal hyperplasia (Brown, Hines, Fane, & Breedlove, in press). It has also been reported that 2D:4D ratio is lower in homosexual women than in heterosexual women, suggesting

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that prenatal exposure to androgens was higher in homosexual women (Brown, Finn, Cooke, & Breedlove, 2002; Tortorice, 2001; Williams et al., 2000).

The first study to suggest that low 2D:4D ratio was associated with lesbian sexual orientation was done using a sample obtained from public street fairs in San Francisco (Williams et al., 2000). It was found that the right hands of homosexual women were masculinized compared with those of the heterosexual controls, but 2D:4D ratio was not significantly different between the lesbian sample and heterosexual male controls.

A second study of finger digit ratio examined the differences in 2D:4D between self-identified "butch" and "femme" lesbians (Brown et al., 2002). This study, which recruited samples from a Gay Pride event in Oakland, indicated that there were lower 2D:4D ratios on the right hands of butch lesbians than on those of femme lesbians. There were no differences in the left hands.

A third study also indicated differences between butch and femme lesbians recruited through flyers from the New Jersey area (Tortorice, 2001, 2002). This study also examined 2D:4D in male-identified transgendered individuals (genetic females), and found no difference between them and the heterosexual female controls, suggesting that sexual orientation and gender identity may have a different etiology or timing.

Other studies have also linked possible differences in prenatal androgenization to lesbian sexual orientation in women. Like the 2D:4D studies, some of this research also comes from the analysis of sex-dimorphic traits. Some functional portions of the ear region in humans are sex-dimorphic. Females are more sensitive with regard to the ability to produce click-evoked otoacoustic emissions (related to the relative masculinization or feminization of the cochlea; McFadden & Pasanen, 1998), and with regard to auditory evoked potentials (McFadden & Champlin, 2000). Lesbians in these studies had more masculine development in these hearing areas than did heterosexual controls. Sex differences in otoacoustic emissions and auditory evoked potentials are seen at birth and are presumed to be the result of differences in prenatal exposure to masculinizing hormones; thus, the masculine response in lesbians suggests that they may be androgenized during prenatal development (McFadden, 2002).

The nature of these studies makes it difficult to ascertain whether differences in lesbians (as compared with heterosexual controls) are due to differences in genes as opposed to environment. This is significant, because there have been twin studies that suggest that there is a genetic basis for lesbian sexual orientation (Bailey, Pillard, Neale, & Agyei, 1993; Hershberger, 1997). These studies report higher concordance rates in monozygotic (MZ) twins than in dizygotic (DZ) twins and nontwin siblings. However, the lack of 100% concordance between MZ twins indicates that environment is also likely to be a contributing factor. Studies, such as those cited earlier, that investigate the relation between characteristics associated with prenatal sex hormone levels may shed some light on the nature of environmental contributions to sexual orientation, especially if they can be designed to control for genetics.

This study takes advantage of data collected previously to investigate the prenatal environmental contribution to sexual orientation in MZ twin pairs to address the issue of genetics versus environment with regard to 2D:4D ratio. Thus, it represents an expansion of a previous study that indicated that differences in prenatal environment were associated with differences in sexual orientation between MZ twins. The previous dermatoglyphic study showed that MZ twins discordant for sexual orientation (one heterosexual, one homosexual) had experienced differences in second trimester environment as reflected in ridge count differences (Hall, 2000a, 2000b). Twins concordant for sexual orientation (both homosexual) did not show any indication of differing prenatal environment. That study also showed that prenatal environment was reflected differently in the dermatoglyphics of males discordant for sexual orientation, and females discordant for sexual orientation, suggesting that sexual orientation has a different etiology in males compared with females (Hall, 2000a). In males, significant differences were observed in directional asymmetry between cotwins with heterosexual males showing higher ridge counts on their right hands than their cotwins, and homosexual males showing higher ridge counts on their left hands than their cotwins (Hall, 2000a). Because total finger ridge count (TFRC) was the same, this produced a "mirror imaging" effect, which reflected a trend towards left-directed asymmetry (but not necessarily left-greater asymmetry) in the homosexual twin of a set. In females, significant differences were seen in TFRC, with the lesbian twin showing a lower ridge count than her heterosexual cotwin (Hall, 2000b). Again, differences were significant primarily from the standpoint of intratwin relationships.

Differences in the male twins may be related to differences in prenatal testosterone levels, as high levels of testosterone are associated with leftward asymmetry (Sorenson-Jamison, Meier, & Campbell, 1993). Several other studies have also suggested that prenatal testosterone levels may be high in homosexual males. Robinson and Manning (2000) reported that homosexual males had low 2D:4D ratios compared with heterosexual controls. Weaker support for this was found in another study

(Williams et al., 2000) that indicated lower 2D:4D in males with two or more older brothers (the more older brothers a man has, the greater the likelihood of him being homosexual; Blanchard, 1997). However, no differences were found between homosexual men and heterosexual controls (Williams et al., 2000). McFadden and Champlin (2000) also found an indication of high testosterone levels in homosexual males when they examined the auditory evoked potentials of homosexual and heterosexual males. These findings are counterintuitive to many researchers, and are not in keeping with the findings of feminized structures in the brains of homosexual males (Allen & Gorski, 1992; Byne et al., 2001; LeVay, 1991). However, it should be noted that masculinization may occur via different pathways in the brain as opposed to in the skin and peripheral organs (such as the ear region and the finger digits).

Some of the organizing effects of the brain are the result of the conversion of testosterone to estradiol via the aromatase pathway. This results in the binding of estradiol to estrogen receptors that trigger the organizing effects of the hormone. Testosterone is converted to 5-α-dihydrotestosterone (DHT) in the skin via 5-α-reductase (LeVay, 1996). One could theorize that levels of testosterone in the brain would be influenced by differences in aromatase levels or in aromatase conversion rates that could be independent of levels of 5-α-reductase. Thus, feminization might occur in the brain, whereas high levels of DHT are simultaneously resulting in masculinization of the ear region or the finger digits and dermal ridges. Another possibility is that timing in development of the brain and the hands is different.

Differences in TFRC between female cotwins discordant for sexual orientation suggests differences in overall fetal size (Bracha, Torrey, Gottesman, Bigelow, & Cunniff, 1992). This cannot be clearly linked with differences in hormones. Because ridge count develops during the second trimester of prenatal development (Babler, 1991), and because twins share virtually the same genes, differences in ridge count can be attributed to differences in prenatal environment (Hall, 2000a) but not directly to differences in hormones. Low 2D:4D ratio, on the other hand, appears to be associated with high levels of androgens during fetal development (Manning et al., 1998). Thus, if lesbian MZ twins had lower 2D:4D ratio than did their straight cotwins, it could be an indication that higher androgen levels are associated with lesbian sexual orientation. It would certainly be an indication that differences in 2D:4D were due to environment as opposed to genetics.

To test the hypothesis that lesbian sexual orientation is associated with lower 2D:4D ratio (and thus indirectly to higher levels of androgen exposure during prenatal development), female MZ twins were analyzed for differences in 2D:4D ratio. The hypothesis that 2D:4D ratio differences are due to environmental factors rather than genetic factors would be supported if 2D:4D ratios differed between cotwins.

METHOD

Subjects

A sample of MZ twins in which at least one was homosexual were previously recruited from cities throughout the United States in 1996–97 for the purpose of examining prenatal environmental contributions to sexual orientation. Twins were recruited through advertisements in homophile publications and through gay-related Internet sites. All data were collected by the first author through in-person interviews.

Seven sets of female twins discordant for sexual orientation and five sets of female twins concordant for sexual orientation were obtained (Hall, 2000a). Table I shows that the age range for the entire sample was 24–56 with a mean age of 39.6 SD(9.8). The first seven pairs of twins represent the twins discordant for sexual orientation, whereas the last five pairs represent the twins concordant for sexual orientation. Only two sets of twins identified as something other than Caucasion. One set was of mixed racial background, the other was Hispanic.

Measures

Sexual orientation was assessed with the Kinsey Scale (Kinsey, Pomeroy, & Martin, 1948) and the Klein Grid (Klein, Sepekoff, & Wolf, 1985). Assignment based on Kinsey and Klein Grid scores are shown in Table I. A Kinsey score of 5 or 6 was used to define homosexuality. A Kinsey score of 0 or 1 was used to define heterosexuality. In all of the heterosexual participants, Kinsey scores were either 0 or 1, with the majority being 0. In all of the homosexual participants, Kinsey scores were between 5 and 6. Bisexuals were screened out during initial interviews. In the twins discordant for sexual orientation, homosexual twins were assigned to a category X, whereas their heterosexual cotwin was assigned to a corresponding Y category. Twin pairs in which both twins were homosexual were also assigned to an X and Y category on the basis of the same criteria. Klein Grid scores were used to assign twins concordant for sexual orientation into two categories. That is, the twin with the high Klein Grid score was placed in X, whereas the cotwin with the low score was placed in Y. Klein Grid scores were used because they

Table I. Twin Assignment Based on Kinsey/Klein Grid Scores

		Twin with high Kinsey/Klein (X_i)		Twin with low Kinsey/Klein (Y_i)	
Pair #	Age	Kinsey score	Klein Grid score	Kinsey score	Klein Grid score
1.	24	6	4.5	0	1.1
2.	26	5.5	3.9	1	0.6
3.	44	6	5.2	0	0.85
4.	47	6	4.1	0	1.0
5.	56	6	4.8	1	2.0
6.	44	6	5.5	0	1.8
7.	30	6	4.2	0	0.8
8.	52	6	5.2	5	4.7
9.	40	6	5.1	6	4.3
10.	34	6	5.6	6	4.6
11.	39	5.5	5.0	6	4.3
12.	39	5.5	4.7	5	4.7

are more likely to vary slightly between twins concordant for sexual orientation.

This categorization of twins with high scores in X and twins with low scores in Y was based on the concept that prenatal environment effected the degree of homo/heterosexuality just as it affected ridge count. Therefore, if the direction of a ridge count was associated with sexual orientation in the discordant pairs, the relative degree of homosexuality in the concordant twins would also be reflected in the ridge count. This distinction was a necessary logical assumption for statistical analysis. Twins were always compared only to their cotwins. Thus, all of the data are on individual intratwin set variation, not on intertwin group variation, with the exception of the mean ratios. Blood was drawn to assess zygosity, but not all twins were willing to give blood, so twin zygosity was assessed using a questionnaire (Cederlof, Friberg, Johnson, & Kaji, 1961; Nichols & Bilbro, 1966).

Inked prints were obtained using ink and a roller provided by Faurot following the guidelines of the American Dermatoglyphics Association (1990). Ink was rolled onto the hand that was placed on paper resting on a foam cushion with a towel wrapped around it. Equal pressure was applied to each joint of the hand and across the metacarpals to insure that all of the features of the hand were represented in the ink print. All twins were printed by the same investigator using the same techniques. Prints were not originally obtained for the purpose of analyzing finger digit ratio, so no bias was present when digits were printed. Finger digit measurements were obtained later from the ink prints of the participants' hands, and ratios were determined by the first author blind to the data, and a blinded interrater chosen for technical skill with metrics.

All of the prints were measured in centimeters using the same calipers. Measurements were made to 1/10th cm. To obtain a measurement, a line was drawn through the crease between the metacarpal and the proximal phalange. For many participants, more than one crease was apparent, and the crease most proximal to the metacarpal was always used. A second line was drawn at the distal tip of the distal phalange. The tip of the calipers was always placed at the central point of the digit. Ratios were derived for each hand. The interrater measurements were compared using t tests. With an alpha level of .05, they were highly correlated at $r = .993$, $p \leq .0001$, $df = 47$, and not statistically different ($t < 1$).

Previous research on the methodology of obtaining 2D:4D ratios using ink prints indicates that although ink printing consistently results in a slightly longer measure of finger length, the relationship between the digits (2D:4D ratio) is consistent and is not statistically different from ratios obtained from photocopied hands (Hall, 2001b).

The Wilcoxon Rank Sum Matched Pairs statistic was used to compare variation between cotwins. All tests were performed for one-tailed analysis at $\alpha = .05$.

RESULTS

Table II shows the results for the Wilcoxon analysis of the left hand and right hand of the twins discordant for sexual orientation. In the twins discordant for sexual orientation ($n = 7$), there were significant differences in 2D:4D ratio for both hands. For the right hand, the results for the Wilcoxon were $T = 0$, $p \leq .05$, $n = 5$ (note that in two pairs of twins the ratios were identical, so n was reduced by 2). The results for the left hand were $T = 3$, $p \leq .05$, $n = 7$. In the twins concordant for sexual orientation, shown in Table III, there were no statistically significant differences for either hand. The results for the

Table II. Wilcoxon Rank Sum Analysis of Right and Left Hand 2D:4D Ratio for Twins Discordant for Sexual Orientation

	X (Homo.) ratio		*Y* (Hetero.) ratio		+/−		Difference		Rank	
Pair #	R	L	R	L	R	L	R	L	R	L
1.	0.90	0.97	0.93	0.935	−	+	0.03	0.03	3	3
2.	0.98	0.96	0.98	1.00		−	0	0.04	no	4.5
3.	0.97	0.92	1.03	1.00	−	−	0.06	0.08	5	7
4.	1.00	1.05	1.05	1.06	−	−	0.05	0.01	4	1.5
5.	1.01	1.02	1.02	1.03	−	−	0.01	0.01	1.5	1.5
6.	0.99	0.98	1.00	1.03	−	−	0.01	0.05	1.5	6
7.	1.00	1.01	1.00	1.05		−	0	0.04	no	4.5

Wilcoxon were $T = 4$, $n = 4$, for the right hand. For the left hand, the results were $T = 1$, $n = 3$.

DISCUSSION

Although results are not reported here, data on male MZ twins were analyzed, but no differences were found in 2D:4D between homosexual males and their heterosexual cotwins. In males, low 2D:4D ratio was associated only with high drinking/drug use scores in twins regardless of sexual orientation (Hall, 2001b).

The average ratios for the lesbian participants in this study (0.98–0.99) were consistent with population averages for European males, whereas the averages for the heterosexual participants (1.0–1.01) were consistent with those given for European females (Manning et al., 1998). Although it has been reported that there is ethnic variation in 2D:4D that may be greater than sex variation (Martin et al., 2001), the females in this study were all of White European descent with two exceptions. One pair of twins concordant for sexual orientation was Hispanic, and one pair was of mixed descent. Thus, this sample more closely corresponds to that of the Manning et al. (1998) study, and because this is a U.S. sample that drew from large urban areas across the country, it corresponds demographically with the previous studies of 2D:4D in lesbians done in the United States (Brown et al., 2002; Tortorice, 2001; Williams et al., 2000).

These results support previous findings that lesbian sexual orientation is associated with lower interdigital ratio, and by extrapolation, higher androgen levels during prenatal development. Although ratios were lower on average on the right hands than the left hands of lesbians, there were no statistically significant differences between mean right and left hand ratios for any of the groups.

Although the results should be interpreted with caution because of small sample size, this study makes an important contribution to the understanding of prenatal development in sexual orientation because the use of an MZ twin design allows differences in 2D:4D ratio to be interpreted primarily as a component of prenatal environment. In contrast, population studies comparing unrelated individuals cannot discriminate between differences in environment as opposed to differences in genetics. The differences observed here between cotwins indicate that there were differences in the prenatal environment during the first trimester of development. These differences are associated with differences in sexual orientation, suggesting that prenatal environment should be considered as an etiological factor in the development of this trait.

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Table III. Wilcoxon Rank Sum Analysis of 2D:4D Ratio in Twins Concordant for Sexual Orientation

	X Twin ratio		*Y* Twin ratio		+/−		Difference		Rank	
Pair #	R	L	R	L	R	L	R	L	R	L
1.	0.98	1	1	1.02	−	−	0.02	0.02	1	1
2.	0.96	0.97	0.96	0.93		+	0	0.04	no	2
3.	1.04	1.08	0.98	1.01	+	+	0.06	0.07	4	3
4.	0.97	1	1	1	−		0.03	0	2	no
5.	0.92	0.96	0.96	0.96	−		0.04	0	3	no

University and analyzed at Brown University. The authors acknowledge the work of Laurel McCrudden in providing interrater reliability measurements.

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