

The Second to Fourth Digit Ratio (2D:4D) in a Japanese Twin Sample:  
Heritability, Prenatal Hormone Transfer, and Association with Sexual Orientation

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**ABSTRACT**

The 2nd to 4th digit ratio has been argued to reflect prenatal hormonal influences and is reportedly associated with various psychological and behavioral traits, such as sexual orientation, cognitive abilities, and personality. We examined genetic and environmental influences on the 2nd to 4th digit ratio (2D:4D) using a Japanese twin sample ( $N = 300$ ). The genetic analysis showed substantial additive genetic influences for both right and left hand 2D:4D. The rest of the variance was explained mainly by environmental influences not shared within twin pairs. These findings were, in general, in accordance with preceding studies with primarily Caucasian twin samples. The bivariate genetic analysis revealed that the additive genetic influences were largely shared between the right and left hand, while the non-shared environmental influences were largely unique to each hand. Results from a comparison of opposite-sex and same-sex twins were not significant, although they were in the predicted direction according to the prenatal hormone transfer hypothesis. Female monozygotic twin pairs discordant in sexual orientation showed significant within-pair differences in left hand 2D:4D, where non-heterosexual twins had lower (more masculinized) 2D:4D. In addition, we found that non-heterosexual male MZ twins had larger (more feminized) 2D:4D than their heterosexual co-twins. These results suggest the existence of non-shared environmental influences that affect both 2D:4D and sexual orientation.

**Keywords:** 2D:4D, finger-length ratios, heritability, prenatal hormone transfer, sexual orientation, twins

## INTRODUCTION

The second to fourth digit ratio (2D:4D), the relative length of the index finger to that of the fourth finger, has been reported to be smaller for males than for females. The effect of sex has been reported consistently from a wide range of age and ethnic groups (Loehlin, Medland, & Martin, 2009; Manning, Churchill, & Peters, 2007; Manning et al., 2000; Manning, Henzi, Venkatramana, Martin, & Singh, 2003; Manning, Stewart, Bundred, & Trivers, 2004; Putz, Gaulin, Sporter, & McBurney, 2004; Williams et al., 2000). It has been argued that higher levels of prenatal exposure to androgens, such as testosterone, and sensitivity to these androgens results in lower 2D:4D in males (Manning, Bundred, Newton, & Flanagan, 2003; Manning, Scutt, & Lewis-Jones, 1998; Williams et al., 2000). Given this premise, large numbers of studies have been conducted to examine the relationship between 2D:4D and traits which are presumably related to prenatal androgen exposure, such as athletic ability (Hönekopp, Manning, & Müller, 2006; Manning & Taylor, 2001), spatial ability (Loehlin et al., 2009; Peters, Manning, & Reimers, 2007; Putz, McDaniel, Jordan, & Breedlove, 2008; Putz et al., 2004), personality traits such as aggression and sensation seeking (Austin, Manning, McInroy, & Mathews, 2002; Lippa, 2006; Loehlin et al., 2009), and sexual orientation (Grimbos, Dawood, Burriss, Zucker, & Putz, 2010; Lippa, 2003; Manning et al., 2007; McFadden & Shubel, 2002; McFadden et al., 2005; Peters et al., 2007; Putz et al., 2004; Williams et al., 2000).

It should be noted that the putative effects of prenatal androgen on 2D:4D can be both genetic and environmental. Clarifying the degree of genetic and environmental influences can enhance our understanding of the source of individual difference in 2D:4D and of the etiology of the association between 2D:4D and behavioral and psychological traits. For instance, if prenatal hormones, thus 2D:4D, are greatly influenced by genetics, it is possible that the same genetic

factor has effects on certain psychological traits through a pathway other than prenatal hormones. If this is the case, the putative effects of prenatal androgen on psychological traits would be in question. Therefore, it is important to examine genetic and environmental influences on individual differences in 2D:4D. In fact, several behavioral genetic studies with twin samples have been conducted in the West (Gobrogge, Breedlove, & Klump, 2008; Hall & Love, 2003; Medland & Loehlin, 2008; Medland, Loehlin, & Martin, 2008; Paul, Kato, Cherkas, Andrew, & Spector, 2006; van Anders, Vernon, & Wilbur, 2006; Voracek & Dressler, 2007). Those studies can be roughly classified into three types.

### **Classic Twin Method**

The first type employs the classic twin method, which decomposes the covariance of twin traits into variances of three independent parameters: additive genetic factors (A), environmental factors shared by twin siblings (C), and environmental factors not shared by twin siblings (E) (Neale & Maes, 2002; Plomin, DeFries, McClearn, & McGuffin, 2008). In some cases, dominant genetic factors (D) are substituted for shared environmental factors (C).

Paul et al. (2006) were the first to conduct a twin study on 2D:4D. They analyzed data from 148 identical (monozygotic, MZ) twin pairs and 308 fraternal (dizygotic, DZ) twin pairs from the St Thomas' Adult Twin Registry in London. All of the participants were female. They reported that A explained 66% of right hand 2D:4D variances and 71% of left hand 2D:4D variances. The rest of the variance was best explained by E.

Voracek and Dressler (2007) were the second to report a twin study on 2D:4D. They analyzed data from 114 Austrian twins. Using data from 20 female MZ pairs and 9 female same-sex DZ pairs, they reported that A explained 81% of the individual differences in mean 2D:4D of the right hand and left hand while E explained 19% of them.

Gobrogge et al. (2008) analyzed data from 300 male and female same-sex twins from the Michigan State University Twin Study of Behavioral Adjustment. They concluded that the estimates for A, C, and E parameters for right hand were 43%, 15%, and 42%, respectively. For left hand 2D:4D, they reported that genetic and environmental influences were different between the sexes. For males, the estimates were 81%, 0%, and 19% for A, C, and E, respectively. For females, the estimates were 51%, 0%, and 49%, respectively.

Medland and Loehlin (2008) conducted a study with a large twin sample ( $N = 1741$  individuals from 757 families) in Australia. In contrast to Gobrogge et al. (2008), they reported that genetic and environmental influences were not different between the sexes both for the right and left hand. They employed two procedures to measure finger length from photocopies of the hands: direct measurements with calipers and computer-assisted measurements. They incorporated the data from the two procedures in a multivariate analysis and showed that A was 71% for the right hand and 80% for the left hand. The rest of the variances were explained by E. They also reported that the majority of the covariation between the right and left hand was explained by A. In addition to twin studies, Voracek and Dressler (2009) analyzed data from 235 Austrian families and reported A estimates to be 58% for the right hand 2D:4D and 48% for the left hand.

Like twin studies on behavioral and psychological traits (Turkheimer, 2000), the twin studies on 2D:4D have revealed relatively strong genetic influences and small shared environmental influences. However, given the argument that 2D:4D is influenced by the prenatal environment, the small shared environmental effects are somehow puzzling. If the prenatal environment has some influence on 2D:4D, we would expect that even DZ co-twins are similar to each other because of the shared prenatal environment. The small shared environmental

influences do not support the prediction. This suggests the possibility that twins do not share their prenatal hormonal environment. To test prenatal hormonal influences more directly, a second type of twin study compared twins from opposite-sex pairs (OS pairs) to those from same-sex pairs (SS pairs).

### **OS/SS Comparison**

Van Anders et al. (2006) compared 2D:4D among nine girls from OS pairs and 16 girls from SS pairs. They reported that 2D:4D of the OS pair girls was significantly lower than that of SS pair girls, suggesting prenatal hormone transfer from male co-twins to their female co-twins in OS pairs. Hereafter, we will call this the OS/SS paradigm.

Consistent with van Anders et al. (2006), Voracek and Dressler (2007) reported significant differences between females from OS pairs (10 individuals) and females from SS pairs (18 individuals) on mean 2D:4D of the right and left hand. In contrast, Cohen-Bendahan (2005) failed to observe significant differences in a study of twin girls from the Netherlands (29 individuals from OS pairs and 26 individuals from SS pairs). Likewise, Medland et al. (2008) reported null results from a large sample twin study (867 individuals) in Australia.

As for male twins, van Anders et al. (2006) reported no significant differences in OS and SS pair boys. Medland et al. (2008) also reported no significant differences. Williams et al. (2000) reported that males who had two or more older non-twin brothers had a more masculine 2D:4D. However, this should be interpreted as an effect of progressive maternal immunization against male-specific antigens rather than prenatal hormone transfer (Blanchard, 2001; Blanchard & Klassen, 1997; Bogaert & Skorska, 2011).

### **Discordant MZ Comparison**

The third type of study employs the discordant MZ twin paradigm. A number of studies have been conducted on the association between sexual orientation and 2D:4D. A recent meta-analysis showed that non-heterosexual females, including both homosexuals and bisexuals, had smaller (more masculine) 2D:4D than heterosexual females (Grimbos et al., 2010). Hall and Love (2003) conducted a within-pair comparison of 2D:4D of seven female MZ twin pairs with discordant sexual orientation. In each pair, one co-twin had a heterosexual orientation and the other had a homosexual orientation. In agreement with Grimbos et al.'s meta-analysis, Hall and Love reported that female homosexual MZ co-twins had more masculine 2D:4D compared with their heterosexual co-twins. A within-pair comparison of five female MZ twin pairs with concordant sexual orientation was not significant.

Because MZ pairs share 100 percent of their genotypes, the differences between them must be explained by non-shared environmental influences. The environmental influences could include epigenetic effects. Previous results suggest that the same non-shared environmental factors that affect 2D:4D could also affect sexual orientation, indicating an overlap of environmental effect between the two phenotypes.

The present study was designed to test whether the findings of the preceding studies could be replicated with a Japanese twin sample. Reasons for conducting the current study were twofold. First, studies on 2D:4D have often produced contradictory results, partly because of relatively low statistical power (Loehlin et al., 2009). Therefore, it is important to test the replicability of preceding studies. This was especially the case with the discordant-twin study, which employed relatively small sample sizes. Second, there are large ethnic differences in 2D:4D (Manning, Bundred, & Mather, 2004; Manning et al., 2000, 2003, 2004, 2007; Manning & Robinson, 2003; McIntyre, Cohn, & Ellison, 2006). Given that preceding twin studies have

employed mainly Caucasian twin samples, we believed that it was worth replicating these studies with a Japanese (Asian) sample. Specifically, we conducted the three types of analyses with Japanese twin data.

### **Analysis 1: Classic Twin Method**

Previous studies have suggested a relatively large genetic influence (40% to 81%) and relatively small shared environmental influence (0% to 15%) on 2D:4D in same-sex twin pairs. In some studies, genetic influences were larger for the left hand than for the right. We conducted two univariate analyses separately for the left and right hands, consistent with previous studies. In addition, we ran a bivariate analysis to examine whether and to what degree the genetic and environmental influences overlapped or were independent between the two hands.

### **Analysis 2: OS/SS Comparison**

We predicted that female twins from OS twin pairs would have smaller (more masculine) 2D:4D than females from SS DZ pairs as shown by some of the preceding studies using the OS/SS paradigm. In addition, we examined whether the male twins from SS twin pairs, who might have been influenced by their male co-twin's androgen, had more masculine 2D:4D than males from OS pairs.

### **Analysis 3: Discordant MZ Comparison**

We analyzed whether MZ twin pairs discordant in their sexual orientation show discordance in 2D:4D. For female pairs, we predicted that non-heterosexual twins, including both bisexuals and homosexuals, would have lower (more masculine) 2D:4D than their heterosexual co-twins, as reported by Hall and Love (2003).

For males, a meta-analysis reported that non-heterosexual men had larger (more feminine) 2D:4D than heterosexual men in non-Caucasian samples (Grimbos et al., 2010).

Therefore, we predicted that non-heterosexual male co-twins would have larger 2D:4D than their heterosexual co-twins in our Japanese (Asian) twin sample.

## **METHOD**

### **Participants**

The Keio Twin Project (KTP) recruited twin participants ages 14-30 years through population-based registries in parts of the Tokyo area in 1998–2002 by selecting the names of two family members who lived together and had the same dates of birth. Participation in the project was voluntary. Of the 5,217 pairs of twins to whom we sent letters, 2,073 twin individuals agreed to participate in our research. The registrants were all native Japanese and the participation rate was 20% (for a detailed description of the KTP, see Shikishima, Ando, Ono, Toda, & Yoshimura, 2006). After recruitment, registrants were assessed by questionnaires delivered via mail surveys.

### ***Hand Photocopying Participants***

In 2004, the KTP requested that those registrants who had not mailed back their surveys attend an on-campus session held at Keio University where we administered several cognitive ability tests and personality questionnaires, and conducted physical measurements, including photocopying of the hands. A total of 300 twin individuals (149 complete pairs and 2 single twins), including 204 females and 96 males participated in the session. Ages ranged from 16 to 32 years ( $M = 22.48$ ,  $SD = 4.09$ ). Participants were paid ¥5000 (approximately 60 U.S. dollars) for their participation.

### ***Sexual Orientation Assessment Participants***

The Klein Grid (Klein, Sepekoff, & Wolf, 1985), a questionnaire that assesses sexual orientation, was included in a booklet sent to the registered twins who agreed to participate in the

KTP. The booklet contained questionnaires on topics such as social and economic status (e.g., educational background, occupation, family income), health (e.g., smoking, drinking), and eating behavior (e.g., food preferences, eating frequency, interest in dieting). We included instructions with the questionnaires stressing that all items should be answered individually without discussing or exchanging information with others, especially twin siblings. Participants returned the questionnaire booklet by mail. A total of 720 twins returned the questionnaire booklet containing the Klein Grid (171 males and 549 females; 516 MZ twins, 142 same-sex DZ twins, and 62 opposite-sex DZ twins). The ages ranged from 18 to 37 years ( $M = 25.37$ ,  $SD = 4.41$ ). Of the total, 223 provided photocopies of their hands.

The participants were informed of the purpose of the study, the research items, protection of their privacy, and their right to stop their participation at any time. Signed informed consent was obtained for all participants at the beginning of the on-campus session as well as in the postal survey.

## **Measures**

### ***Zygoty Determination***

Zygoty was initially determined using a questionnaire comprising three questions about the twins' physical resemblance (e.g., As a toddler, were you and your twin "as alike as two peas in a pod"?) that have been demonstrated to have 93.2% accuracy (Ooki, Yamada, Asaka, & Hayakawa, 1990). For twin pairs from whom DNA information was available through blood or buccal smears (86 pairs), zygoty was diagnosed by examining gene polymorphisms (for details, see Shikishima et al., 2006). The number of each type of twin pair who participated in photocopying of the hands was as follows: 75 identical female, 29 identical male, 22 fraternal female, 14 fraternal male, and 9 fraternal female-male opposite-sex pairs.

It should be noted that the twinning rate is low in Japan, about seven per 1,000 maternities (Imaizumi, 2001). The rate is about nine to 17 per 1,000 in the U.S., Australia, and Europe (Hoekstra et al., 2008). Given that the rate of monozygotic twinning is constant around the world (about four per 1,000 maternities), the difference between Japan and other areas is explained by the lower dizygotic twinning rate in Japan (about two to three per 1,000 maternities, depending on the year of birth) (Imaizumi, 2001).

### ***Finger-Length Measurement***

Finger-length ratios were calculated from measurements of photocopies of the left and right hands taken by a portable photocopy machine during the on-campus session. Research assistants explained that the hand should be put on the machine with fingers together. A white sheet covered the hand to help produce a higher contrast ratio. The left and right hand images were taken separately. When the photocopy images were vague, two or more photocopies were taken. Participants could mark out their fingerprints on the image if they wanted to do so. For each photocopy of a hand, two raters used steel vernier calipers to measure the finger length from the crease proximal to the palm at the base of the digit to the tip of the digit. The raters were blind to the twin type. The intraclass correlations between the raters were high: .93 and .95 for right hand second digit and fourth digit; .96 and .96 for left hand second digit and fourth digit. We used the means of the measurements taken by the two raters as the finger lengths.

### ***Sexual Orientation Assessment***

Sexual orientation was assessed with the Klein Grid (Klein et al., 1985), translated into Japanese by one of the authors of the present study. The Klein Grid consists of questions regarding seven dimensions at three different time periods (past, present, and future). The seven dimensions are 1) SA, sexual attraction; 2) SB, sexual behavior; 3) SF, sexual fantasy; 4) EM,

emotional closeness; 5) SP, social preference; 6) ID, sexual identification; and 7) LS, heterosexual/homosexual life style. Responses ranged from “other sex only” (1 point) to “own sex only” (7 points) for SA, SB, SF, EM, and SP dimensions. For ID and LS dimensions, response choices were “heterosexuals only” (1 point) to “homosexuals only” (7 points).

## RESULTS

### Sexual Dimorphism in 2D:4D

The photocopies of the left hands of two female participants were excluded because of poor photocopy quality. Both of them were from MZ pairs. Thus, we had 300 right hand 2D:4D (96 male and 204 female) and 298 left hand 2D:4D (96 male and 202 female).

Table 1  
Finger Lengths and Relative Finger-Length Ratios of Female and Male Twins

|                  | Female |      | Male  |      | Cohen's  |          |     |          |
|------------------|--------|------|-------|------|----------|----------|-----|----------|
|                  | M      | SD   | M     | SD   | <i>d</i> | <i>t</i> | df  | <i>p</i> |
| Right 2D         | 65.89  | 3.29 | 70.40 | 4.27 |          |          |     |          |
| Right 4D         | 68.72  | 3.47 | 74.38 | 4.81 |          |          |     |          |
| Right 2D:4D      | 0.96   | 0.03 | 0.95  | 0.03 | 0.42     | 3.46     | 298 | <.001    |
| Left 2D          | 65.52  | 3.19 | 70.06 | 4.49 |          |          |     |          |
| Left 4D          | 68.57  | 3.59 | 74.55 | 4.80 |          |          |     |          |
| Left 2D:4D       | 0.96   | 0.03 | 0.94  | 0.03 | 0.51     | 4.25     | 296 | <.0001   |
| Right 2D:4D      |        |      |       |      |          |          |     |          |
| Gobrogge et al.  | 0.99   | 0.05 | 0.97  | 0.04 | 0.44     | -4.30    | 298 | <.001    |
| M & L (caliper)  | 0.98   | 0.03 | 0.96  | 0.03 | 0.49     |          |     |          |
| M & L (computer) | 0.99   | 0.03 | 0.97  | 0.03 | 0.60     |          |     |          |
| Left 2D:4D       |        |      |       |      |          |          |     |          |
| Gobrogge et al.  | 1.00   | 0.05 | 0.97  | 0.04 | 0.66     | -5.00    | 298 | <.001    |
| M & L (caliper)  | 0.98   | 0.03 | 0.97  | 0.03 | 0.41     |          |     |          |
| M & L (computer) | 0.98   | 0.03 | 0.97  | 0.03 | 0.35     |          |     |          |
| Mean 2D:4D       |        |      |       |      |          |          |     |          |
| V & D            | 0.97   | 0.03 | 0.96  | 0.03 | 0.56     | -2.87    | 107 | =.005    |

*Note.* Mean and SD from preceding 2D:4D twin studies are provided for the comparison. The effect sizes (Cohen's *d*) for the preceding studies are computed by the authors of the present study. “M & L” denotes Medland and Loehlin (2008), who used two methods in measuring finger-length (caliper and computer assisted measurement). They provided the variances computed for male and female combined data, and conducted  $\chi^2$  tests instead of *t* tests. “V & D” denotes Voracek and Dressler (2007), who provided the data on the mean 2D:4D of the left and right hand.

We conducted a two-way mixed factorial ANOVA with sex as the between-participants factor and right-left hands as the within-participants factor. We excluded two participants for whom we had only left hand 2D:4D from the analysis. As expected, the main effect of sex was significant,  $F(1, 296) = 17.03, p < .0001$ , showing males had significantly smaller 2D:4D than females. The effect size (partial  $\eta^2$ ) was .054. The main effect of the hands was also significant,  $F(1, 296) = 13.31, p < .001$ , showing right hand 2D:4D was significantly larger than left hand 2D:4D. The effect size (partial  $\eta^2$ ) was .043. None of the interaction was significant. For comparison with preceding studies, Cohen's  $d$  indices of sex are separately reported for right and left hand in Table 1. The effect sizes were comparable to previous studies with twin samples.

### **Analysis 1: Classic Twin Method**

The classic twin method utilizes the fact that MZ pairs share all of their genes while DZ pairs share, on average, half of their genes with individual variations, assuming assortative mating among the parents is negligible. Both MZ and DZ pairs share their common family environment (i.e., shared environment) to the same extent. Given these assumptions, we can infer the presence of genetic influences if we observe a larger within-pair correlation for MZ compared with DZ pairs (Neale & Maes, 2002). Two types of genetic influences can be considered, assuming the effects of epistasis (gene-gene interaction) are negligible (Plomin et al., 2008). Additive genetic influences reflect variation in multiple genotypes whose influences are small but additive in forming a quantitative phenotype. Dominance genetic influences represent variation in interactions between alleles at the same locus. Additive genetic covariance is two times larger for MZ pairs than for DZ pairs, while dominance genetic covariance is four times larger for MZ pairs than for DZ pairs. Therefore, if we observe that phenotypic covariance of

MZ pairs is more than two times larger than that of DZ pairs, we can interpret this as evidence for the presence of dominance influences.

### *Intraclass Correlations*

First, we computed the intraclass correlations of 2D:4D for each sex by zygosity class. We found that the correlations were larger for MZ pairs than for DZ pairs on both hands, suggesting the presence of genetic influences (Table 2). The correlations for female MZ pairs were more than two times larger than those for female DZ pairs, suggesting dominance genetic influences on female 2D:4D. This finding suggested that genetic influences might differ between males and females.

Table 2  
Intraclass Correlations Between Co-twin Digit Ratios

|             | Right 2D:4D | <i>n</i> | Left 2D:4D | <i>n</i> |
|-------------|-------------|----------|------------|----------|
| MZ female   | .62         | 73       | .72        | 75       |
| DZ female   | .17         | 22       | .11        | 22       |
| MZ male     | .59         | 29       | .59        | 29       |
| DZ male     | .24         | 14       | .40        | 14       |
| DZ opposite | .39         | 9        | .38        | 9        |

*Note.* *n* = number of pairs for each sex by zygosity class. The photocopies of the left hands of two female participants were excluded because of poor photocopy quality.

### *Univariate Model Fitting*

To further analyze the relative contributions of genetic and environmental influences, univariate genetic models were fitted to the twins' variance-covariance matrices with structural-equation modeling using the maximum likelihood method (Neal & Maes, 2002). The analysis decomposes the covariance of twin traits into variances of four independent parameters: additive genetic factors (A), dominance genetic factors (D), shared environmental factors (C), and non-shared environmental factors and error (E) (Neale & Maes, 2002; Plomin et al., 2000). As mentioned above, the presence of both dominance genetic effects and sex-specific effects

Table 3

## Univariate Genetic Analysis of Digit Ratios for the Right and Left Hand

|           | Model<br>Constrai-<br>nts* | Males      |                   |     |            |            |                   | Females |            |     |            |     |            | $\chi^2$ (df)    | <i>p</i>  | AIC           |
|-----------|----------------------------|------------|-------------------|-----|------------|------------|-------------------|---------|------------|-----|------------|-----|------------|------------------|-----------|---------------|
|           |                            | A          | 95% CI            | D/C | 95% CI     | E          | 95% CI            | A       | 95% CI     | D/C | 95% CI     | E   | 95% CI     |                  |           |               |
| Right     | ADE Const.                 | .23        | [.00, .71]        | .40 | [.00, .72] | .38        | [.28, .51]        | -       |            | -   |            | -   |            | 16.24(13)        | NS        | -9.76         |
|           | Unconst.                   | .01        | [.00, .82]        | .68 | [.00, .83] | .31        | [.17, .60]        | .60     | [.00, .72] | .00 | [.00, .71] | .40 | [.28, .55] | 15.42(10)        | NS        | -4.58         |
| ACE       | Const.                     | .62        | [.24, .72]        | .00 | [.00, .34] | .38        | [.28, .51]        | -       |            | -   |            | -   |            | 16.72(13)        | NS        | -9.28         |
|           | Unconst.                   | .67        | [.13, .83]        | .00 | [.00, .39] | .33        | [.17, .64]        | .60     | [.00, .72] | .00 | [.00, .63] | .40 | [.28, .55] | 16.25(10)        | .09       | -3.75         |
| <b>AE</b> | <b>Const.</b>              | <b>.62</b> | <b>[.49, .72]</b> | -   |            | <b>.38</b> | <b>[.28, .51]</b> | -       |            | -   |            | -   |            | <b>16.72(14)</b> | <b>NS</b> | <b>-11.28</b> |
|           | Unconst.                   | .67        | [.36, .83]        | -   |            | .33        | [.17, .64]        | .60     | [.45, .72] | -   |            | .40 | [.28, .55] | 16.25(12)        | NS        | -7.75         |
| Left      |                            |            |                   |     |            |            |                   |         |            |     |            |     |            |                  |           |               |
| ADE       | Const.                     | .68        | [.00, .76]        | .00 | [.00, .76] | .32        | [.24, .44]        | -       |            | -   |            | -   |            | 7.85(13)         | NS        | -18.15        |
|           | Unconst.                   | .59        | [.00, .77]        | .00 | [.00, .75] | .41        | [.24, .68]        | .17     | [.00, .79] | .54 | [.00, .80] | .29 | [.20, .42] | 5.76(10)         | NS        | -14.24        |
| ACE       | Const.                     | .68        | [.22, .76]        | .00 | [.00, .43] | .32        | [.24, .44]        | -       |            | -   |            | -   |            | 7.85(13)         | NS        | -18.15        |
|           | Unconst.                   | .40        | [.00, .76]        | .19 | [.00, .69] | .42        | [.24, .70]        | .70     | [.24, .80] | .00 | [.00, .44] | .30 | [.20, .43] | 6.00(10)         | NS        | -14.00        |
| <b>AE</b> | <b>Const.</b>              | <b>.68</b> | <b>[.56, .76]</b> | -   |            | <b>.32</b> | <b>[.24, .44]</b> | -       |            | -   |            | -   |            | <b>7.85(14)</b>  | <b>NS</b> | <b>-20.15</b> |
|           | Unconst.                   | .59        | [.32, .77]        | -   |            | .41        | [.24, .68]        | .70     | [.57, .80] | -   |            | .30 | [.20, .43] | 6.17(12)         | NS        | -17.83        |

*Note.* Standardized path estimates are shown with 95% confidence intervals in parentheses. The D/C columns show estimates of dominance genetic influences for the ADE models and shared environmental influences for the ACE models. For constrained models, estimates are shown only for male columns. Statistical tests showed no significant differences between constrained models and unconstrained models. The best fit models are indicated in bold. The  $\chi^2$  test of each model shows whether the model is significantly different from the saturated model. Non-significance of the tests shows good fit of the model to the data. AIC index is computed by subtracting 2 times of df from the  $\chi^2$  value. It shows the relative fitness of the models controlling for the number of parameters in each model. The smaller the AIC is, the better the model is compared to other models.

\* “Const.” denotes constrained model. “Unconst.” denotes unconstrained models.

were considered. Because we can estimate at most three parameters when we have only three equations in a model, we constructed three (ACE, ADE, and AE) by two (constrained and unconstrained) models for each hand. That gave us 12 models in total (Table 3). We compared the fitness of the models using the Akaike Information Criteria or AIC (Akaike, 1987). In the constrained models, all the parameters were set to be equal among the sexes, whereas in the unconstrained models, parameters were allowed to vary between the sexes. Mx software (Neale, Boker, Xie, & Maes, 2004) was used for the analysis.

For both hands, the constrained AE model was not significantly worse than more complex models, and it was the best according to the AIC, which takes both good fit and parsimony into account (bold font in Table 3). For the right hand, A was estimated to be 68% (95% CI, 56–76%), and E was estimated to be 32% (95% CI, 24–44%). The second-best model was the constrained ADE model followed by the constrained ACE model.

For the left hand, A was estimated to be 62% (95% CI, 49–72%), and E was estimated to be 38% (95% CI, 28–51%). Again, the second-best models were the constrained ADE and constrained ACE models.

In addition, we constructed partially unconstrained models where a selected parameter (e.g., A, D, C, or E) was set to be equal between the sexes with the other parameters allowed to vary. All of the partially unconstrained models provided worse fits to the data than the constrained models.

### ***Bivariate Genetic Analysis***

To clarify the genetic and environmental overlap between the two hands, bivariate genetic analysis using Cholesky decomposition was conducted (Neale & Maes, 2002).

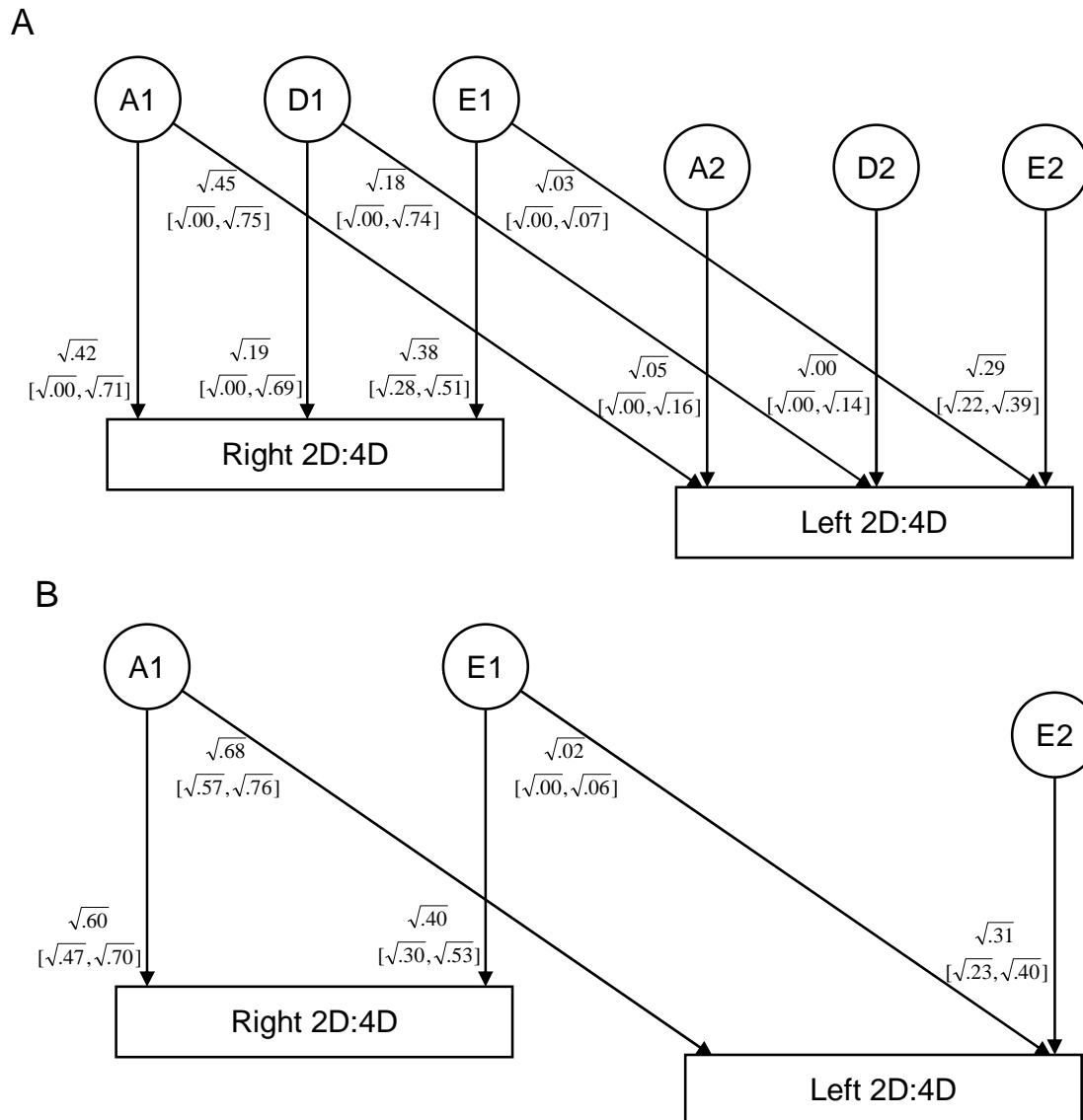


Fig 1. Bivariate Cholesky decomposition models. The above model (A) is the full ADE model (model 1 in Table 4). The below model (B) is the best model (model 19 in Table 4). “A1” and “A2” indicate the additive genetic factors. The path from A1 to right hand 2D:4D is expressed as A1R in Table 4. Likewise, the path from A1 to left hand 2D:4D is expressed as A1L in Table 4. “D1” and “D2” indicate the dominance genetic factor. “E1” and “E2” indicate the combined effects of the non-shared environmental factors and errors. The descriptions of the paths from these factors in Table 4 follow in the same manner as the additive genetic factors. The path coefficients were expressed so that the relative influence of each factor to each phenotype was apparent (95% confidence intervals in the parentheses). For instance, in model A, the additive genetic factor explained 42% of the phenotypic variation in right hand 2D:4D, while the path coefficient was actually a square root of .42. The genetic and environmental correlations between the two hands can be calculated by multiplying the corresponding path coefficients that bridge the two phenotypes. For example, in model A, the additive genetic correlation between the right and left hand can be acquired by multiplying the square root of .42 (A1R) and square root of .45 (A1L) which is .43.

Table 4

## Comparison of the Full ADE Model and Submodels

| Model     | Paths      |            |     |     |     |     |            |            |            | $\Delta\chi^2$ | $\Delta df$ | $p$         | $\Delta AIC$ |
|-----------|------------|------------|-----|-----|-----|-----|------------|------------|------------|----------------|-------------|-------------|--------------|
| 1         | A1R        | A1L        | A2L | D1R | D1L | D2L | E1R        | E1L        | E2L        |                |             |             |              |
| 2         | A1R        | A1L        | A2L | D1R | D1L | D2L | E1R        |            | E2L        | 10.30          | 1           | 0.00        | 8.30         |
| 3         | A1R        | A1L        | A2L | D1R | D1L |     | E1R        | E1L        | E2L        | 0.00           | 1           | 1.00        | -2.00        |
| 4         | A1R        | A1L        | A2L | D1R | D1L |     | E1R        |            | E2L        | 10.30          | 2           | 0.01        | 6.30         |
| 5         | A1R        | A1L        | A2L | D1R |     | D2L | E1R        | E1L        | E2L        | 0.57           | 1           | 0.45        | -1.43        |
| 6         | A1R        | A1L        | A2L | D1R |     | D2L | E1R        |            | E2L        | 11.48          | 2           | 0.00        | 7.48         |
| 7         | A1R        | A1L        | A2L | D1R |     |     | E1R        | E1L        | E2L        | 0.57           | 2           | 0.75        | -3.43        |
| 8         | A1R        | A1L        | A2L | D1R |     |     | E1R        |            | E2L        | 11.48          | 3           | 0.01        | 5.48         |
| 9         | A1R        | A1L        | A2L |     |     |     | E1R        | E1L        | E2L        | 0.57           | 3           | 0.90        | -5.43        |
| 10        | A1R        | A1L        | A2L |     |     |     | E1R        |            | E2L        | 11.48          | 4           | 0.02        | 3.48         |
| 11        | A1R        | A1L        |     | D1R | D1L | D2L | E1R        | E1L        | E2L        | 0.41           | 1           | 0.52        | -1.59        |
| 12        | A1R        | A1L        |     | D1R | D1L | D2L | E1R        |            | E2L        | 10.30          | 2           | 0.01        | 6.30         |
| 13        | A1R        | A1L        |     | D1R | D1L |     | E1R        | E1L        | E2L        | 0.41           | 2           | 0.81        | -3.59        |
| 14        | A1R        | A1L        |     | D1R | D1L |     | E1R        |            | E2L        | 10.30          | 3           | 0.02        | 4.30         |
| 15        | A1R        | A1L        |     | D1R |     | D2L | E1R        | E1L        | E2L        | 1.00           | 2           | 0.61        | -3.00        |
| 16        | A1R        | A1L        |     | D1R |     | D2L | E1R        |            | E2L        | 11.48          | 3           | 0.01        | 5.48         |
| 17        | A1R        | A1L        |     | D1R |     |     | E1R        | E1L        | E2L        | 1.00           | 3           | 0.80        | -5.00        |
| 18        | A1R        | A1L        |     | D1R |     |     | E1R        |            | E2L        | 11.48          | 4           | 0.02        | 3.48         |
| <b>19</b> | <b>A1R</b> | <b>A1L</b> |     |     |     |     | <b>E1R</b> | <b>E1L</b> | <b>E2L</b> | <b>1.44</b>    | <b>4</b>    | <b>0.84</b> | <b>-6.56</b> |
| 20        | A1R        | A1L        |     |     |     |     | E1R        |            | E2L        | 11.48          | 5           | 0.04        | 1.48         |
| 21        | A1R        |            | A2L | D1R | D1L | D2L | E1R        | E1L        | E2L        | 2.18           | 1           | 0.14        | 0.18         |
| 22        | A1R        |            | A2L | D1R | D1L | D2L | E1R        |            | E2L        | 12.53          | 2           | 0.00        | 8.53         |
| 23        | A1R        |            | A2L | D1R | D1L |     | E1R        | E1L        | E2L        | 2.18           | 2           | 0.34        | -1.82        |
| 24        | A1R        |            | A2L | D1R | D1L |     | E1R        |            | E2L        | 12.53          | 3           | 0.01        | 6.53         |
| 25        | A1R        |            | A2L | D1R |     | D2L | E1R        | E1L        | E2L        | 80.80          | 2           | 0.00        | 76.80        |
| 26        | A1R        |            | A2L | D1R |     | D2L | E1R        |            | E2L        | 421.72         | 3           | 0.00        | 415.72       |
| 27        | A1R        |            | A2L | D1R |     |     | E1R        | E1L        | E2L        | 80.80          | 3           | 0.00        | 74.80        |
| 28        | A1R        |            | A2L | D1R |     |     | E1R        |            | E2L        | 421.74         | 4           | 0.00        | 413.74       |
| 29        | A1R        |            | A2L |     |     |     | E1R        | E1L        | E2L        | 80.80          | 4           | 0.00        | 72.80        |
| 30        | A1R        |            | A2L |     |     |     | E1R        |            | E2L        | 428.70         | 5           | 0.00        | 418.70       |
| 31        | A1R        |            |     | D1R |     |     | E1R        | E1L        | E2L        | 85.70          | 4           | 0.00        | 77.70        |
| 32        | A1R        |            |     | D1R |     |     | E1R        |            | E2L        | 576.75         | 5           | 0.00        | 566.75       |
| 33        | A1R        |            |     |     |     |     | E1R        | E1L        | E2L        | 85.70          | 5           | 0.00        | 75.70        |
| 34        | A1R        |            |     |     |     |     | E1R        |            | E2L        | 583.71         | 6           | 0.00        | 571.71       |
| 35        |            |            |     |     |     |     | E1R        | E1L        | E2L        | 87.88          | 6           | 0.00        | 75.88        |
| 36        |            |            |     |     |     |     | E1R        |            | E2L        | 811.63         | 7           | 0.00        | 797.63       |

*Note.* The paths set in each model are indicated. For instance, A1R indicates that the path from the A1 parameter to right hand 2D:4D was set in the model. The full ADE model was Model 1. The  $\Delta\chi^2$  values show the differences between the  $\chi^2$  value of a reduced model (Model 2 to 36) and the full model (Model 1). Non-significance of the  $\chi^2$  tests shows that the particular model and the full model are statistically not significantly different, thus suggesting the good fit of the target model. The AIC indices are computed by subtracting 2 times of the  $\Delta df$  from the  $\Delta\chi^2$ . The smaller AIC indicates the better fitting of the model. The best-fitting model with the lowest AIC is indicated in bold print (Model 19). Because E parameters reflected not only the non-shared environmental influences but also the measurement errors, models without either E1R or E2L were not tested. D parameter estimation is appropriate only when an A parameter is given in the model. Therefore, DE models (e.g., models with D2 while A2 was absent) were not tested.

The univariate analyses showed that constrained models were always better than the corresponding unconstrained models. Therefore, we assumed that the genetic and environmental effects were equal for the two sexes. Because the second-best models for the univariate analyses were ADE models for both hands, we first constructed the full ADE Cholesky model (Fig. 1a) and compared it to all the possible 36 submodels in which certain paths and factors were omitted (Table 4). Because the full AE model was a submodel of the full ADE model, we could search for wider model space with this approach.

The model with the best-fitting index (the lowest AIC) was the AE Cholesky model lacking the unique additive genetic factor contributing only to the left hand 2D:4D (Fig. 1b). We also constructed the full ACE Cholesky model and compared it to all the submodels. Again, the same model was the best model.

In the best model, the common additive genetic factor was estimated to explain 60% and 68% of the phenotypic variance in the right hand and left hand 2D:4D, respectively. The environmental overlap was relatively small: only 2% of phenotypic variance of the left hand 2D:4D was explained by common non-shared environmental factors. However, there were significant unique non-shared environmental factors for the left hand (31%). In other words, these results suggested that environmental but not genetic influences were instrumental in making the two hands dissimilar.

## **Analysis 2: OS/SS Comparison**

To examine the hypothesized effect of prenatal hormone transfer among OS twin pairs, we compared the 2D:4D of twins from the OS pairs to those from the SS pairs. We analyzed data from the DZ twin pairs with whom we had 2D:4D data. Therefore, we had nine females from OS

pairs and nine males from OS pairs who were the co-twins of the aforementioned females. We had 46 females from SS pairs and 28 males from the SS pairs.

Table 5  
Digit Ratios of OS and SS Dizygotic Twins

| Pair type* | Own sex | Partner sex | <i>n</i> | Right |      | Left |      |
|------------|---------|-------------|----------|-------|------|------|------|
|            |         |             |          | M     | SD   | M    | SD   |
| SS         | female  | female      | 46       | 0.97  | 0.02 | 0.97 | 0.03 |
| OS         | female  | male        | 9        | 0.97  | 0.03 | 0.96 | 0.04 |
| SS         | male    | male        | 28       | 0.95  | 0.04 | 0.94 | 0.03 |
| OS         | male    | female      | 9        | 0.95  | 0.02 | 0.94 | 0.03 |

*Note.* SS = same-sex DZ twin. OS = opposite-sex DZ twin.

### *Comparison of Females from OS and SS Pairs*

Based on the prenatal hormone transfer hypothesis (van Anders et al., 2006), we conducted one-tailed *t*-tests assuming OS females had smaller 2D:4D than SS females. The statistical comparisons were not significant for either hand (Table 5; right hand,  $t(53) < 1$ ; left hand,  $t(53) < 1$ ).

### *Comparison of Males from OS and SS Pairs*

If there were prenatal hormone transfer between twin siblings, males from SS pairs might have smaller and more masculine 2D:4D than males from OS pairs because the former might have experienced hormone transfer from their male co-twins. Therefore, we conducted one-tailed tests to compare OS and SS males. The statistical tests were not significant for either hand (Table 5; right hand,  $t(35) < 1$ ; left hand,  $t(53) < 1$ ).

### **Analysis 3: Discordant MZ Pairs Comparison**

First, we assessed the sexual orientation of twins using the Klein Grid (Klein, Sepekoff, & Wolf, 1985). Then we compared the MZ twin pairs who were discordant in their sexual orientation separately for female and male pairs. Specifically, we compared heterosexual co-twins and non-heterosexual co-twins. The latter included both homosexuals and bisexuals.

Table 6  
Principal Component Analysis of Klein Grid Scores from the Twin Sample ( $N = 720$ )

| Dimension         | Time    | Loadings    |             |              |              |
|-------------------|---------|-------------|-------------|--------------|--------------|
|                   |         | PC1         | PC2         | PC3          | PC4          |
| Sexual Attraction | past    | 0.13        | -0.03       | -0.30        | <b>-0.65</b> |
|                   | present | 0.12        | -0.02       | -0.30        | <b>-0.69</b> |
|                   | future  | 0.13        | -0.02       | -0.30        | <b>-0.70</b> |
| Sexual Behavior   | past    | 0.05        | -0.03       | -0.10        | <b>-0.20</b> |
|                   | present | 0.05        | -0.03       | -0.10        | <b>-0.20</b> |
|                   | future  | 0.05        | -0.02       | -0.11        | <b>-0.23</b> |
| Sexual Fantasy    | past    | 0.10        | -0.04       | -0.14        | <b>-0.41</b> |
|                   | present | 0.10        | -0.04       | -0.15        | <b>-0.41</b> |
|                   | future  | 0.10        | -0.05       | -0.15        | <b>-0.42</b> |
| Emotional         | past    | 0.75        | -0.50       | <b>-0.84</b> | 0.31         |
| Closeness         | present | 0.75        | -0.54       | <b>-0.82</b> | 0.27         |
|                   | future  | 0.75        | -0.55       | <b>-0.81</b> | 0.23         |
| Social Preference | past    | <b>1.37</b> | 0.10        | 0.56         | -0.11        |
|                   | present | <b>1.39</b> | 0.15        | 0.60         | -0.04        |
|                   | future  | <b>1.28</b> | 0.10        | 0.60         | -0.04        |
| Identity          | past    | 0.06        | 0.04        | <b>-0.16</b> | -0.12        |
|                   | present | 0.05        | 0.05        | <b>-0.17</b> | -0.17        |
|                   | future  | 0.06        | 0.05        | <b>-0.18</b> | -0.17        |
| Life Style        | past    | 0.21        | <b>1.10</b> | -0.49        | 0.13         |
|                   | present | 0.24        | <b>1.09</b> | -0.47        | 0.10         |
|                   | future  | 0.23        | <b>1.02</b> | -0.46        | 0.10         |
| Eigenvalues       |         | 7.37        | 4.33        | 4.19         | 2.38         |

*Note.* Variables with highest loadings on principal components are indicated in bold print.

### *Principal-component Analysis of the Klein Grid*

We conducted a principal-component analysis for Klein Grid data from the twin sample ( $N = 720$ ) as a substitute for a factor analysis. Four principal components with eigenvalues larger than 1.0 (Table 6) were extracted. Among them, the fourth component comprised sexual attraction to, sexual behavior with, and sexual fantasy involving same-sex individuals. Also, identity dimensions had loadings on the fourth component. Therefore, the fourth component was considered to be the component regarding sexual orientation. We computed the mean scores of sexual attraction, sexual behavior, and sexual fantasy and used them as the sexual orientation

(SO) scores. Because identity dimensions loaded on both the third and fourth components, we did not include the identity score when computing the SO scores.

We classified individuals with SO scores of 3 points (“other sex somewhat”) and above as having non-heterosexual orientation. Individuals who had scores of 3 to 5 points were considered to have a bisexual orientation. Thus, we included them as non-heterosexual rather than homosexual. We had eight MZ female pairs and four MZ male pairs who were discordant in their sexual orientation, that is, one co-twin was non-heterosexual while the other co-twin was heterosexual.

Table 7  
Sexual Orientation Scores and 2D:4D of Discordant Monozygotic Twin Pairs

| Female<br>Pair #                 | Non-hetero |       |      | Hetero |       |      | Difference    |               |
|----------------------------------|------------|-------|------|--------|-------|------|---------------|---------------|
|                                  | SO         | Right | Left | SO     | Right | Left | Right         | Left          |
| 1                                | 6.3        | 0.92  | 0.95 | 1.0    | 0.99  | 0.97 | <b>-0.07</b>  | <b>-0.02</b>  |
| 2                                | 4.0        | 0.88  | 0.92 | 1.3    | 0.92  | 0.93 | <b>-0.03</b>  | <b>-0.01</b>  |
| 3                                | 3.5        | 0.95  | 0.94 | 1.0    | 0.92  | 0.91 | 0.02          | 0.03          |
| 4                                | 3.3        | 0.96  | 0.91 | 2.0    | 0.91  | 0.95 | 0.04          | <b>-0.04</b>  |
| 5                                | 3.3        | 0.96  | 0.95 | 1.0    | 0.96  | 0.96 | <b>-0.00</b>  | <b>-0.01</b>  |
| 6                                | 3.0        | 0.94  | 0.93 | 2.6    | 0.95  | 0.95 | <b>-0.01</b>  | <b>-0.02</b>  |
| 7                                | 3.0        | 0.94  | 0.94 | 2.0    | 0.94  | 0.98 | <b>-0.00</b>  | <b>-0.05</b>  |
| 8                                | 3.0        | 0.94  | 0.96 | 2.0    | 0.98  | 1.02 | <b>-0.04</b>  | <b>-0.07</b>  |
| Within pairs t-test (one-tailed) |            |       |      |        |       |      | <i>p</i> =.19 | <i>p</i> <.05 |

| Male<br>Pair #                   | Non-hetero |       |      | Hetero |       |      | Difference    |               |
|----------------------------------|------------|-------|------|--------|-------|------|---------------|---------------|
|                                  | SO         | Right | Left | SO     | Right | Left | Right         | Left          |
| 1                                | 7.0        | 0.95  | 0.94 | 1.0    | 0.95  | 0.94 | -0.00         | <b>0.00</b>   |
| 2                                | 4.0        | 0.97  | 0.95 | 1.0    | 0.97  | 0.94 | <b>0.00</b>   | <b>0.01</b>   |
| 3                                | 3.0        | 0.96  | 0.97 | 1.0    | 0.97  | 0.94 | <b>0.01</b>   | <b>0.03</b>   |
| 4                                | 3.0        | 0.94  | 0.89 | 2.4    | 0.91  | 0.87 | <b>0.03</b>   | <b>0.03</b>   |
| Within pairs t-test (one-tailed) |            |       |      |        |       |      | <i>p</i> =.14 | <i>p</i> <.05 |

*Note.* The sexual orientation scores (SO) and the right and left hand 2D:4D for individuals from monozygotic twin pairs discordant in sexual orientation. Individuals with SO scores equal to or higher than 3 points were classified as non-heterosexuals. The “Difference” columns show the within pair differences of 2D:4D (non-hetero minus hetero). For female pairs, the differences were predicted to be negative, as indicated in bold print. For male pairs, the differences were predicted to be positive, as indicated in bold print. Results of the within-pair one-tailed *t*-tests are presented.

### ***Comparison of Female MZ Pairs Discordant for Sexual Orientation***

We conducted a one-tailed test on the female data assuming that non-heterosexual female co-twins would have smaller 2D:4D than their heterosexual co-twins. For the right hand 2D:4D, the within-pair  $t$ -test was not significant,  $t(7) < 1$ . For the left hand, the within-pair  $t$ -test was significant, showing that the non-heterosexual female co-twins had lower left 2D:4D than did their heterosexual co-twins,  $t(7) = -2.24$ ,  $p < .05$ , one-tailed (Table 7).

### ***Comparison of Male MZ pairs Discordant for Sexual Orientation***

We conducted a one-tailed within-pair  $t$ -test on the data assuming that non-heterosexual male co-twins would have larger 2D:4D than their heterosexual co-twins. The within-pair comparison was not significant for the right hand,  $t(3) = 1.31$ . For the left hand, the within-pair comparison was significant, showing that non-heterosexual male twins had larger left hand 2D:4D than did their heterosexual co-twins,  $t(3) = 2.54$ ,  $p < .05$ , one-tailed.

## **DISCUSSION**

### **Classic Twin Study**

We observed relatively large additive genetic influences on 2D:4D ratios. These genetic influences were slightly larger for the left hand (68%) than for the right hand (62%). Shared environment influences were relatively small compared with A and E. In general, these results were consistent with previous studies conducted with mainly Caucasian twin samples (Gobrogge et al., 2008; Medland & Loehlin, 2008; Paul et al., 2006; Voracek & Dressler, 2008). Thus, it appears that genetic and environmental influences on 2D:4D are not very different between Caucasians and Asians. However, we cannot be certain that the genes regulating 2D:4D are the same in the Caucasian and Asian samples.

Some similarities and differences from preceding studies are worth noting. First, we did not observe significant differences in genetic and environmental differences across sex. In the univariate genetic analyses, constrained models always showed a better fit to the data than corresponding unconstrained models. This was in agreement with Medland and Loehlin (2008) while contradicting Gobrogge et al. (2008). It should be noted, however, that the statistical power might not have been strong enough to detect the effect of sex. Given the lack of statistical power, it is more noteworthy that the sex differences were opposite to what Gobrogge et al. reported. They reported that A was larger for males (81%) than for females (51%) for left hand 2D:4D. The current data showed that even when we looked at the unconstrained models, A was larger for females (70%) than for males (60%) for the left hand (Table 3). Gobrogge et al. argued that the relatively smaller genetic influence on the right hand might reflect the larger sensitivity of the right hand to prenatal androgen exposure. However, they reported that sex differences were larger for the left hand than for the right hand (Cohen's  $d = 0.66$  for left,  $0.44$  for right) as in the current study ( $0.51$  for left,  $0.42$  for right). It is noteworthy that Medland and Loehlin (2008) reported larger sex differences for the right hand (see Table 1). They reported that sex-constrained models showed better fit with sample sizes larger than the current study and Gobrogge et al.

Second, we did not observe significant influences of C. The best models were AE models for both univariate and bivariate genetic analyses and for both the left and right hand. Even when we looked at the ACE models, the influence of shared environment was estimated to be small. These results were similar to Medland and Loehlin (2008), Paul et al. (2006), and Voracek and Dressler (2008), but differed from Gobrogge et al. (2008), who reported that C explained 15% of the phenotypic variance of the right hand for both males and females. Therefore, currently, we

have one study showing significant influences of C, while the other three studies did not find them to be significant. It should be noted that the absence of significant shared environmental influences does not necessarily mean the total absence of these influences.

The bivariate genetic analyses showed that models assuming only A and E were better than models assuming D or C. These results were congruent with the univariate genetic analyses and the multivariate analysis by Medland and Loehlin (2008). More importantly, we found that A was largely shared by the right hand and left hand, while E was not. The models without unique A showed better fit than those with it. Even when we assumed unique A for each hand, common A had much larger estimates than unique A (Fig. 1A). As for the non-shared environmental influences, unique E for left hand 2D:4D had larger estimates than common E. These results indicate that the within-individual differences between right and left hand 2D:4D can be largely explained by environmental influences, especially, environmental influences unique to each individual. These findings were consistent with the multivariate analysis by Medland and Loehlin (2008) that the majority of covariation between the right and left hand was due to additive genetic influence in an Australian twin sample. Because E parameters included measurement error, there is a possibility that within-individual differences were caused by these errors. However, the intraclass correlations between the two raters of finger length were quite high, suggesting relatively small measurement errors. In addition, even if we assumed that E was largely due to measurement error, it remains noteworthy that genetic influences were largely common between the two hands.

The exact details of the non-shared environment factors on 2D:4D cannot be deduced from the current data. One possibility is that MZ pairs experienced different prenatal environments. In fact, even MZ co-twins can experience different prenatal environments if they

are dichorionic-diamniotic twins or monochorionic-diamniotic twins. The former share neither a placenta nor an amniotic sac. The latter share a placenta but not an amniotic sac. Unfortunately, we could not examine this possibility because information about the placenta and chorion were not available for the current sample.

### **OS/SS Comparison**

We did not find any significant differences in 2D:4D between female twins from the OS and SS pairs. We did not find any significant differences between males from OS and SS pairs either. These findings were in accord with two of the four previous studies that examined the effects of prenatal hormone transfer (Cohen-Bendahan, 2005; Medland et al., 2008), but contradicted the studies by van Anders et al. (2006) and Voracek and Dressler (2008).

It should be noted that the pattern of the results showed some similarity with the study by van Anders et al. (2006). They reported that the OS/SS comparison was significant only for the female left hand. Similarly, we observed that the effect size was the largest for the OS/SS female comparison on left hand 2D:4D (Cohen's  $d = 0.24$ ) among all OS/SS comparisons. Additionally, the effect size of sex on the left hand 2D:4D was larger for van Anders et al. (2006) (Cohen's  $d = 0.68$ ) than for the current data (Cohen's  $d = 0.51$ ). Therefore, it is possible that the current sample showed a relatively small effect of androgen-related influences on 2D:4D. That might have been reflected in the smaller effect size of sex and the non-significant differences between OS and SS females. Given these considerations, we suppose that the absence of significant OS/SS differences does not necessarily indicate the complete absence of prenatal hormone transfer among Japanese/Asian twins. Future studies with larger Japanese/Asian samples might be worth pursuing.

### **Discordant MZ Comparison**

We observed significant within-pair differences for MZ twin pairs discordant for sexual orientation. The differences were observed for both females and males, although the direction of the differences was opposite. For female discordant pairs, co-twins with non-heterosexual orientation had smaller (more masculine) left hand 2D:4D compared with their heterosexual co-twins. This replicates the findings of Hall and Love (2003) with a unique sample of Japanese MZ twins.

For males, co-twins with non-heterosexual orientation had larger (more feminine) left hand 2D:4D than their heterosexual co-twins. To our knowledge, this is the first study to show within-pair 2D:4D differences for male MZ pairs discordant in sexual orientation. Results were in agreement with a meta-analysis by Grimbos et al. (2010) indicating that non-heterosexual males had larger 2D:4D than heterosexual males in samples with fewer white individuals.

The results imply the existence of environmental influences that affect both left hand 2D:4D and sexual orientation. Furthermore, the data suggest that environmental influences were not shared between the twin siblings. This is because MZ twin pairs share all of their genes and, by definition, their shared environments. Therefore, the within-pair differences among MZ pairs could inevitably be explained by non-shared environments.

It should be noted that Hall and Love (2003) reported significant differences for both right and left hand 2D:4D. The current analysis reached significance only for left hand 2D:4D. However, the within-pair differences were generally in the predicted direction for the right hand both for females (non-heterosexuals had lower 2D:4D) and for males (non-heterosexuals had larger 2D:4D).

The differences between left hand 2D:4D and right hand 2D:4D should be treated cautiously. It is possible to argue that we would have found significant differences for the right

hand if we had employed larger sample sizes. In fact, Hall and Love (2003) reported significant within-pair differences for both hands with a sample of 12 female pairs. As for the current study, six out of the eight female discordant pairs and four out of the five male discordant pairs showed the predicted within-pair differences for the right hand. However, the bivariate genetic analysis showed that non-shared environmental influences were largely unique to each hand. This suggests the possibility that there were non-shared environmental influences that affected sexual orientation and left hand 2D:4D but not right hand 2D:4D. These points should be clarified in further studies.

### **Stronger Effects for the Left Hand**

Looking over the three analyses reported here, we found an interesting characteristic of the current data. The predicted effects were stronger, though not necessarily significant, for the left hand. For instance, the effect size of sex was larger for the left hand and the effect size of OS/SS difference was largest for the female left hand. The discordant MZ comparisons were significant only for the left hand. There are arguments for the right hand being more sensitive to prenatal androgen exposure, resulting in a larger sex difference for that hand (Loehlin et al., 2009; Manning et al., 2007; McFadden & Shubel, 2002; Williams et al., 2000). In fact, a recent meta-analysis reported that sex differences are larger for right hand 2D:4D (Hönekopp & Watson, 2010). We do not know what brought about the larger effect for the left hand found in the current study. We suspect that it was because we looked at a twin sample. In fact, Gobrogge et al. (2008) also reported a larger sex difference for left hand 2D:4D in their twin sample (Table 1). However, two other twin studies with male and female samples reported larger effect sizes for the right hand (Medland & Loehlin, 2008; Voracek & Dressler, 2007). It could also be because we looked

at a Japanese (Asian) sample. To date, the primary ethnic differences observed in 2D:4D have been in the values of the means, not in sex differences or hand differences.

The field of 2D:4D study has often produced contradictory results, partly because of relatively small statistical power (Loehlin et al., 2009). In addition, large ethnic differences have been reported (Manning et al., 2000, 2003, 2004, 2007; Manning, Bundred, & Mather, 2004; Manning & Robinson, 2003). Given the situation, we believe that the current study with a Japanese (Asian) twin sample adds important data for the advancement of this field.

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