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No effects of prenatal hormone transfer on digit ratio in a large sample of same- and opposite-sex dizygotic twins

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Abstract

Hormone-transfer theory proposes that the hormonal milieu of one twin may influence the other. In opposite-sex twins it has been proposed that hormonal transfer may produce a masculinizing effect in females. The second-to-fourth-finger ratio (2D:4D) has been proposed as an index of the level of prenatal androgens and could, theoretically, be used to detect hormone transfer. Previous examinations in smaller samples provide conflicting evidence. The present study attempted to clarify these results in a large sample of same-sex and opposite-sex dizygotic twins (867 individuals). No significant differences were found in the means, variances or covariance of same- and opposite-sex twins. Female 2D:4D was significantly higher than male (in both hands), as expected, but no sex effects were found on the variance or covariance. Thus, no evidence of hormonal transfer was found in this large twin sample.

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1. Introduction

The ratio of second-to-fourth finger length (2D:4D) has been hypothesized to be influenced by hormonal exposure during the period of gestation in which the digits develop (Manning, 2002). The 2D:4D ratio is sexually dimorphic, with males typically showing a lower ratio than females, a difference which can be observed by the third month of pregnancy (Malas, Dogan, Evcil, & Desdiclioglu, 2006). Since Manning and his colleagues first proposed that the 2D:4D ratio might serve as a non-invasive window into prenatal hormonal conditions (Manning, Scutt, Wilson, & Lewis-Jones, 1998), there have been over 100 studies reporting associations between 2D:4D and a variety of later behavioral and physiological outcomes (McIntyre, 2006). Many of these studies were done with fairly small samples, and there have been numerous failures of replication (see Putz, Gaulin, Sporter, & McBurney, 2004), along with enough successes to encourage further pursuit of the method.

It has also been suggested that the comparison of same-sex and opposite-sex twins could offer evidence on the effects of prenatal hormone exposure (Miller, 1994). This method capitalizes on the natural occurrence of same-sex (SS) and opposite-sex (OS) twins, and the differential hormonal milieux of male and female foetuses. Based on the hypothesis that the hormones of one twin may influence the co-twin, the SS/OS paradigm could provide evidence of a masculinizing effect on the female member of an OS pair, or a feminizing effect on the male member, or both. Such hormonaltransfer effects have been demonstrated in rodents and other litter-bearing species, in which the presence of male foetuses on either side of a female foetus can result in various anatomical and behavioral changes suggesting that the female has been to some degree masculinized by the androgens of the adjacent males (Ryan & Vandenbergh, 2002). Whether such effects occur in humans remains in dispute. There have been reports of positive findings for spontaneous otoacoustic emissions (McFadden, 1993), sensation seeking (Resnick, Gottesman, & McGue, 1993), and number of children in historic populations (Lummaa, Pettay, & Russell, 2007); whereas other reports suggest that such effects, if they exist at all in humans, must be very small in magnitude (the variables assessed included age at first pregnancy and number of children in modern populations, and psychological femininity—Loehlin & Martin, 1998, 2000; Rose et al., 2002).

There have been at least three recent studies in which the 2D:4D ratios of dizygotic (DZ) twins with same- and opposite-sex co-twins have been compared. Two studies, van Anders, Vernon, & Wilbur (2006) and Voracek & Dressler (2007), reported the masculinization of females with a male co-twin, i.e., a 2D:4D ratio lower than that of females with a female co-twin. The third study, Cohen-Bendahan (2005), did not find a difference. Both of the positive studies were based on very small samples: 8 pairs of female same-sex DZ twins and 9 pairs of opposite-sex DZ twins in the van Anders et al. study, and 9 and 10 pairs, respectively, in Voracek and Dressler (it should be noted that testing the hormonal hypothesis was not the primary focus of this study). The van Anders et al. result was significant only for the left hand; Voracek and Dressler used the mean of both hands. Both studies compared both members of same-sex pairs to single opposite-sex twins, treating the two same-sex twins statistically as if they were independent. Given the substantial heritability of 2D:4D (Paul, Kato, Cherkas, Andrew, & Spector, 2006) and the resulting correlation between siblings, this tends to inflate estimates of statistical significance¹. Cohen-Bendahan

¹ Non-independence within the same-sex group decreases the effective sample size, but not the variance, biasing the denominator of the *t*-test.

avoided this problem by using only one girl from each same-sex pair, and she had somewhat larger samples, a total of 26 girls from same-sex and 29 from opposite-sex pairs.

Given the conflicting outcomes in these small studies, it seems important to evaluate the hypothesis of hormonal-transfer effects on 2D:4D with larger samples. We had collected 2D:4D measurements from a large sample of adolescent twins as part of a different study (Wright & Martin, 2004). Thus we were in position to test the hypothesis in a much larger sample, using a method equipped to model the non-independences intrinsic within twin data, structural equation modelling. To this end, we compared the means, variances, and covariances of 2D:4D among 687 individuals who were members of either same-sex or opposite-sex DZ pairs. The chief interest is in a comparison of mean 2D:4D in the same and opposite-sex groups, but it is conceivable that the hormone exposure might affect other statistics as well. For example, hormone transfer might increase the correlations between male same-sex DZs, by decreasing within-pair differences as each twin's androgens affect the other. This could lead to covariance differences between male and female same-sex DZs. Or variances might be affected among male same-sex DZs by the mutual hormonal influences. Tests of such hypotheses can readily be made within a structural modelling framework. If effects on variances and covariances are negligible, one can proceed with confidence to the tests of means that are of primary interest. If the effects are not negligible, one can evaluate them in their own right.

2. Materials and methods

2.1. Participants

Adolescent twins were recruited from the general population, in the context of ongoing studies of melanoma risk factors and studies of cognition. The protocols have been described in detail elsewhere (Wright & Martin, 2004). Twins were enlisted by contacting the principals of primary schools in the greater Brisbane area, media appeals and by word of mouth. It is estimated that approximately 50% of the eligible birth cohort were recruited into the study, which began in 1992. The sample is predominantly Caucasian, with over 95% of participants reporting the ancestry of at least 3 of their 4 grandparents as being English, Irish or Scottish. The sample appears representative with respect to mole count (Zhu et al., 1999) and IQ (Wright et al., 2001). Age range for the sample was 11–24 (mean = 15.46, SD = 3.27) and did not differ between the SS and OS groups (see Table 1).

This paper concerns data collected from twins between July 2002 and January 2007. Photocopies of the participants' hands were taken when the participants attended clinical sessions at the Queensland Institute of Medical Research (Brisbane, Australia). Participants were asked to place their hands flat on the glass with their fingers slightly apart, and were instructed not to press down on the glass. Twins and their siblings who had participated in the study prior to July 2002 were sent a letter asking them to send in photocopies of their hands. Of the 1335 individuals who were approached 323 sent in photocopies, a response rate of 24%. Informed consent was obtained from all participants and parents prior to testing. The protocol used was approved by the Queensland Institute of Medical Research Ethics Board. Initial zygosity diagnoses were determined by typing eight highly polymorphic DNA microsatellite markers and three blood groups (ABO, MNS, Rh). This has subsequently been confirmed by microsatellite and/or whole genome single nucleotide polymorphism scans for this sample.

| Sex | SS/OS | N | Age | | Left hand | | Right hand | |
|--------|----------|----------------------|-------|------|-----------|------|------------|------|
| | | | Mean | SD | Mean | SD | Mean | SD |
| Female | SS | 237 | 15.77 | 3.27 | .984 | .035 | .982 | .032 |
| | OS | 212 | 15.98 | 3.51 | .984 | .033 | .981 | .034 |
| | Combined | 449 | | | .984 | .034 | .981 | .033 |
| Male | SS | 218/219 ^a | 14.87 | 2.93 | .970 | .034 | .964 | .029 |
| | OS | 199 | 15.27 | 3.17 | .972 | .035 | .967 | .031 |
| | Combined | 417/418 | | | .971 | .034 | .965 | .031 |

Table 1
Mean and standard deviation of left and right hand 2D:4D in male and female same (SS) and opposite sex (OS) twins

Measurements of the 2nd and 4th digits were available for 875 DZ twin individuals. The data of 8 individuals were excluded due to outlying 2D:4D (±3SD). These 8 individuals included 2 same-sex (SS) and 2 opposite-sex (OS) females, and 3 SS and 1 OS males. Following the exclusion of outliers, 2D:4D was available for 237 females from SS pairs, 219 males from same-sex DZ pairs, and 212 females and 199 males from opposite-sex DZ pairs. One SS male participant did not return a photocopy of his left hand, so only his right-hand data were included in the analyses.

2.2. Procedure

Finger length was ascertained from the photocopied hands by measuring the distance from the most basal crease of the finger to the tip using callipers that recorded to 0.01 mm. All measurements were made by a trained research nurse.

Manning et al. have reported that measures from photocopies yield lower mean 2D:4D than live measures (Manning, Fink, Neave, & Caswell, 2005), but the opposite has also been reported (Voracek & Dressler, 2006). This is not likely to be critical in any case, as 2D:4D studies using multiple forms of measurement typically find a general consistency of results among them (e.g., Burris, Little, & Nelson, 2007; Voracek & Dressler, 2006).

Measurements of finger lengths tend to be quite reliable. Voracek, Manning, and Dressler (2007) showed that the inter-rater reliability (measured by intra-class correlation, ICC) for measurements of the fingers from the same set of photocopies by 17 experts ranged between .94 and .96. The reliability of the 2D:4D ratio was lower, ICC .72 and .76 for left and right hands, respectively. The present data set does not contain repeated measures of the same photocopy, but we do have test–retest data from measures made by the same research nurse from different photocopies.

Seventy individuals (including 12 MZ twins and 3 non-siblings) who had attended a clinical visit also sent in photocopies allowing us to assess the reliably of the photocopies received by mail. ICCs for individual finger measurements made using the digital callipers ranged between .95 and .93; ICC for the left and right 2D:4D were .72 and .69, respectively. These figures are comparable to those reported by Voracek et al. (2007) for the same photocopy rated by different judges. In another reliability study, 372 individuals (which included 127 MZ twins and 4 non-siblings) attended a second clinical session and had their hands photocopied again 2 years after the first photocopy was made. ICCs for individual finger measurements ranged between .67 and .65. However, the ICCs for the left

^a Note: N for SS Males is 218 for the Left hand and 219 for the right.

and right 2D:4D ratios were .74 and .68, respectively, suggesting that while the measurement of the individual fingers was influenced by the growth of the fingers during two years of adolescence, the ratio remained relatively stable. In cases where two measurements were available for a participant we used the clinical photocopy rather than the one supplied by the participant, and in cases where two clinical measures were available the later of the two measures was used.

2.3. Statistical analysis

Our primary analyses used the structural modelling techniques typically used with twin data. However, because we were making a direct comparison with van Anders et al. (2006) and Voracek and Dressler (2007), we also report results from independent-samples *t*-tests. A model-fitting approach has two advantages over *t*-tests. First, it can take into account in a natural way the non-independence of observations between twin pair members, or between the left and right hands of individuals; and second, it is more informative. As previously noted, it can assess the possible effects of hormone transfer on variances and covariances, as well as on means. Mx, a matrix algebra program (Neale & Maes, 2002) that has been widely used for the analysis of twin and family data was used for the structural equation modelling. The *t*-tests were conducted using SPSS 13.0.

In the Mx analyses the data from DZ twins were modelled as a three-group problem (female-female pairs, male-male pairs, and opposite-sex pairs). Common means and variances were initially assumed within the following four sets of individuals: members of female same-sex pairs, members of male same-sex pairs, female members of OS pairs, and male members of OS pairs. Initially, these were allowed to differ for left and right hands, for a total of 8 means and 8 variances. Covariances were allowed to be different for female SS twins, male SS twins, and OS twins. This resulted in a total of 13 covariance estimates: 4 within persons (between the left and right hands of individuals in the four sets mentioned above), 6 across twins for the same hand (for left hands and right hands in the three twin groups), and 3 across twins and hands (left hand of one twin with right hand of the other in the three groups). Thus, the base model contained 8 mean, 8 variance, and 13 covariance estimates. Essentially, the analyses consisted in testing whether certain of these could be equated without significantly worsening the fit of the model.

Prior to analysis, the 2D:4D values were multiplied by 100 to avoid computational problems associated with the analysis of data having numerically small variances. The rescaled data were used in all Mx analyses.

3. Results

Summary statistics of left and right hand 2D:4D are given by group in Table 1 (in their original scaling). The means and standard deviations of 2D:4D ratios observed in this sample are within the range of those reported in other samples (Manning, 2002; McFadden et al., 2005).

3.1. Independent samples t-tests

When both members of the same-sex pairs were used in the analysis, as in the van Anders et al. and Voracek and Dressler studies, the *t*-tests showed no differences between the 2D:4D of females

born of SS or OS pairs (right 2D:4D $t_{447} = 0.534$, p = 0.59; left $t_{447} = 0.084$, p = 0.93). Similarly no effect was seen for males born of SS or OS pairs (right 2D:4D $t_{415} = -1.277$, p = 0.20; left $t_{416} = -0.801$, p = 0.42). Thus these data failed to confirm the van Anders et al. and the Voracek and Dressler result of SS and OS differences, using their statistical method. However, in agreement with typical results in the literature, 2D:4D was significantly lower in males than females (right 2D:4D $t_{865} = 7.314$, p < 0.001; left $t_{864} = 5.720$, p < 0.001).

3.2. Structural Equation Analysis

The results of the structural equation analyses are given in Table 2. As shown in models 2–6, the variances of left and right 2D:4D in SS and OS twins could be equated, and there were no significant differences between the male and female variances. However, the variances of left and right 2D:4D could not be equated $\chi_1^2 = 5.438$, p = .02; 2D:4D variance, left = .001135, right = .001005) and were allowed to differ in all further models.

Table 2
Results of structural equation modelling in Mx

| Model | vs model | Δ -2LL | Δdf | p |
|--|----------|---------------|-------------|------|
| 1. Saturated Model (-2LL 8620.75; df 1704) | | | | |
| Variance tests | | | | |
| 2. Within hand: Female $SS = Female OS$ | 1 | 2.588 | 2 | .274 |
| 3. Within hand: Male $SS = Male OS$ | 2 | 1.572 | 2 | .456 |
| 4. Left hand: Male = Female | 3 | 0.003 | 1 | .956 |
| 5. Right hand: Male = Female | 4 | 3.117 | 1 | .077 |
| 6. Male = Female Left = Right | 5 | 5.438 | 1 | .020 |
| Covariance tests | | | | |
| 7. Within twin: Female $SS = Female OS$ | 5 | 0.920 | 1 | .337 |
| 8. Within twin: Male $SS = Male OS$ | 7 | 0.453 | 1 | .501 |
| 9. Within twin: Females = Males | 8 | 0.464 | 1 | .496 |
| 10. Left hand: Cross twin & sex | 9 | 0.138 | 2 | .933 |
| 11. Right hand: Cross twin & sex | 10 | 0.980 | 2 | .613 |
| 12. Cross twin: Left = Right | 11 | 7.664 | 1 | .006 |
| 13. Cross twin, sex & trait | 11 | 4.191 | 2 | .123 |
| Means tests | | | | |
| 14. Left hand: Female $SS = Female OS$ | 13 | 0.002 | 1 | .964 |
| 15. Right hand: Female SS = Female OS | 14 | 0.350 | 1 | .554 |
| 16. Left hand: Male $SS = Male OS$ | 15 | 0.725 | 1 | .395 |
| 17. Right hand: Male $SS = Male OS$ | 16 | 0.850 | 1 | .357 |
| 18. Females: Left = Right | 17 | 4.505 | 1 | .034 |
| 19. Males: Left = Right | 17 | 13.740 | 1 | .000 |
| 20. Left hand: Female = Male | 17 | 33.600 | 1 | .000 |
| 21. Right hand: Female = Male | 17 | 51.690 | 1 | .000 |

The change in model fit (Δ -2LL), degrees of freedom (Δ df), and p-value of each comparison is given, models resulting in a significant loss of fit are indicated with italics.

Similarly, the within-person covariances between left and right 2D:4D in SS and OS twins could be equated (models 7 and 8), and there were no significant differences between the male and female within-person covariances (model 9). In addition, for both the left and right hand, the cross-twin covariance could be equated across SS male and female twins and OS twins (models 10 and 11). This indicates an absence of sex differences in the correlations of DZ twins and suggests that the magnitude of genetic and environmental effects influencing 2D:4D do not differ between the sexes. This is relevant to the hypothesis that the androgen receptor (located on the X chromosome) is associated with this trait (Manning, Bundred, & Flanagan, 2002). A significant X-linked gene effect would produce a distinctive pattern of sex differences (Jardine & Martin, 1984; Lynch & Walsh, 1997). The left and right hand cross-twin covariances could not be equated $\chi_1^2 = 7.664$, p = .006, model 12); however, the cross-twin-cross-trait covariances could be equated across SS male and female twins and OS twins (model 13).

The third section of Table 2 shows the tests of central interest: those on means. These tests were performed equating all non-significant variances and covariances (i.e., adopting model 13). Models 14–17 tested for differences between the SS and OS means for females and males and for the left and right hand. These tests confirm there were no differences between SS and OS twins for either hand in either sex. Consistent with the previous literature, there were, however, significant differences between the right- and left-hand 2D:4D of both sexes (models 18 and 19), and between male and female 2D:4D for both hands (models 20 and 21). The raw and equated means and their 95% confidence intervals are presented graphically in Fig. 1. The hand and sex differences are evident, and the overlapping confidence intervals confirm the lack of difference between SS and OS twins.

4. Discussion

In a large twin sample we sought to replicate the finding of van Anders et al. (2006) and Voracek and Dressler (2007), who found lower 2D:4D for females from opposite-sex DZ pairs than females from same-sex DZ pairs. We found no such difference. Nor was there a corresponding difference for male pairs. In addition, there were no differences in the variances or covariance of same-sex and opposite-sex twins, which might have provided alternative evidence that the hormonal milieux of co-twins were interacting. In short, there was no evidence for hormone-transfer effects on 2D:4D in this large sample of twins. In this, our results agree with those of Cohen-Bendahan (2005).

What, then, may account for the fact that van Anders et al. and Voracek and Dressler obtained positive results, whereas we and Cohen-Bendahan did not? The ages of SS and OS twins did not differ, suggesting that this is unlikely to explain the current null result. However, hand changes do occur with growth, and there were some age differences among the studies' samples. van Anders et al. studied children, in the age range 4–15 years. Voracek and Dressler's twins ranged in age from 10 to 80 years, but most were adult (mean overall age 36.1 years). Cohen-Bendahan's twins were 13-year-olds. Our twins overlapped all three groups in age: about three-fourths were in the age range 12–16 when measured, with the remaining between 17 and 24. It is mathematically possible that intermediate-age groups might fail to replicate results from older and younger age-overlapping groups, but it is not especially plausible, particularly for a presumably prenatal effect.

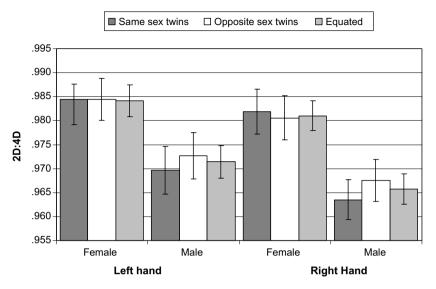


Fig. 1. Mean 2D:4D for male and female SS and OS twins, and the equated mean from model 17. Error bars show 95% confidence intervals.

Ethnicity is known to have a substantial effect on mean 2D:4D, but male-female differences tend to be fairly robust within ethnic groups, and are found in most such groups (Manning, Stewart, Bundred, & Trivers, 2004; Peters, Tan, Kang, Teixeira, & Mandal, 2002). Although details are not always available, the samples in the four studies appear to have been predominantly Caucasian: the Canadian twins of van Anders et al., Voracek and Dressler's Austrian twins, Cohen-Bendahan's twins from the Netherlands, and the Australian twins in our sample. Although it is possible that more subtle ethnic differences may exist among the samples, we know of no evidence that the permeability of placental membranes is influenced by such factors in a way that might explain the current null results.

Could procedural differences have masked a true effect? van Anders et al. (2006) collected a single photocopy of each hand, measured with a ruler to the nearest .5 mm. Some of their participants were visited by an experimenter with a portable photocopier while others were asked to return a photocopy by mail (numbers not given). Voracek and Dressler (2007) collected a single digital scan of each hand that was measured with digital callipers (to the nearest .01 mm) three times by the same investigator; the mean of these measures was used. Cohen-Bendahan's (2005) participants were asked to send in photocopies of their hands, which were then measured by vernier callipers. The data collection procedure used in the current study was perhaps most similar to that used by van Anders et al., but digital callipers were used to measure the finger lengths rather than a ruler. In addition, the current study was conducted over a longer period of time than the other studies, but the reliability of its measurement of 2D:4D ratios were comparable with previous reports (Voracek & Dressler, 2007). As each of the four studies used the same method of data collection for both OS and SS twins and Voracek and Dressler (2006) have previously found that the choice of data collection method is unlikely to explain the results of a given study, there are no obvious methodological explanations of the discrepant findings.

The failure in agreement of two methods, both purporting to assess the effect of prenatal androgens, does not, of course, indicate which one may be to blame, although there is a larger body of prior evidence in support of 2D:4D as an index of prenatal hormone effects. However, there remains a theoretical possibility that both methods are efficacious, but reflect the action of androgens at different times prenatally (McFadden & Champlin, 2000). It is also possible that both methods do glimpse a common phenomenon, but so weakly that to detect their agreement dependably would require much larger samples than ours (let alone those of the previous studies). In either case, research on both methods remains appropriate, but any practical application should be attended by some doubts.

In summary, we did not find in a large twin sample any evidence that hormone transfer between opposite-sex twins affects 2D:4D ratios measured at adolescence. This may mean that such hormone transfer does not occur in humans; or that it occurs, but not in conjunction with the prenatal events affecting 2D:4D; or that the effects are too slight to be dependably detectable.

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