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2nd to 4th digit ratios, fetal testosterone and estradiol

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Abstract

Background: The ratio of 2nd to 4th digit length (2D:4D) is sexually dimorphic (mean 2D:4D is lower in males than females) and is thought to be fixed early in development. 2D:4D has been reported to be related to fetal growth, hand preference, autism, Asperger's syndrome, sperm counts, family size, age at myocardial infarction in men and breast cancer in women. There is indirect evidence that 2D:4D is established in utero and is negatively related to prenatal testosterone and positively with prenatal estradiol. However, there are no studies which show direct relationships between fetal testosterone (FT), fetal estradiol (FE) and 2D:4D. *Aims*: To investigate the relationships between 2D:4D ratios and FT and FE from amniotic fluid. *Study design*: Cohort study. *Subjects*: 33 children. *Outcome measures*: Radioimmunoassays of FT and FE obtained from routine amniocentesis; 2D:4D ratios calculated from 2nd and 4th digit length of the right and left hands at age 2 years. *Results*: A significant negative association between right 2D:4D ratio and FT/FE ratio, which was independent of sex. *Conclusions*: These preliminary findings lend support to an association between low 2D:4D and high levels of FT relative to FE, and high 2D:4D with low FT relative to FE.

Keywords: Digit ratio; Fetal testosterone; Fetal estradiol

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1. Introduction

The Homeobox genes *Hox a* and *d* control (a) the differentiation of the urinogenital system, and may therefore indirectly influence the prenatal production of testicular androgen and (b) the development of the digits [1,2]. This observation has led to the suggestion that patterns of digit formation may relate to gonad function [3]. One likely candidate for such a link is the ratio between the length of the 2nd (the ''index'' finger) and 4th (the ''ring'' finger) digit (2D:4D). The 2D:4D ratio is a sexually dimorphic trait which is lower in men than women [3,4], relative digit length is established as early as the 14th week [5], and the sex difference appears by two years and perhaps before birth [3].

2D:4D is associated with measures of size at birth in males [6], sperm counts [3], family size [7], age at breast cancer presentation [8] and age at myocardial infarction [9]. It is therefore important to understand the factors which lead to 2D:4D formation. There is indirect evidence that the sex difference in 2D:4D is causally related to relative concentrations of testosterone and oestrogen. Thus, (a) the waist/hip ratio of mothers, a positive correlate of testosterone and a negative correlate of estradiol, is negatively related to the 2D:4D ratio of their male and female children [10]; (b) some behavioural traits with an excess of males have been shown to be associated with low values of 2D:4D, e.g. left hand preference [11], good visual-spatial ability [12], autism and Asperger's syndrome [13]; (c) males and females with congenital adrenal hyperplasia, a trait associated with high prenatal testosterone, have low values of 2D:4D compared to controls [14,15]; and (c) traits which show an excess of females, e.g. high verbal fluency [16] and high levels of emotional behaviour [17], are associated with high 2D:4D. However, these and similar observations may result from interactions between 2D:4D and sex-related factors other than prenatal androgen and estradiol. The purpose of this work was to examine the association between 2D:4D and relative concentrations of fetal testosterone (FT) and estradiol (FE).

2. Subjects and methods

FT and FE may be obtained from amniocentesis [18]. This test is offered to mothers in the second trimester of their pregnancy when there is an elevated risk of a fetus with a chromosomal abnormality. The mothers in this study had undergone routine amniocentesis in the Cambridge area in 1996 and 1997, and had all given birth to healthy singleton infants. The protocol was agreed by the local Ethics Committees and informed consent was obtained from the mothers.

The amniotic fluid was analysed in the Department of Clinical Biochemistry of Adenbrooke's Hospital, Cambridge. Amniotic fluid was extracted with diethyl ether. Experiments have demonstrated 95% recovery of testosterone and estradiol by this method. The ether was evaporated to dryness at room temperature and the extracted material redissolved in assay buffer. A radioimmunoassay, the DPC 'Count-a-Coat' method (Diagnostic Products, Los Angeles, CA90045-5597), was used to measure

testosterone. This assay uses an antibody to testosterone coated onto propylene tubes and a 125-I-labelled testosterone analogue. The detection limit of the assay is approximately 0.1 nmol/l. The method therefore measures total extractable testosterone. Amniotic fluid (0.6 ml) was extracted with 3 ml diethyl ether. The estradiol was measured by fluorescence-labelled immunoassay. The Wallac-Delfia method was used (Wallac OY, Turku, Finland). This assay uses a polyclonal rabbit antibody to estradiol in a competitive format in which sample estradiol competes with europium-labelled estradiol analogue for the antibody binding sites. A second antibody directed against rabbit IgG is coated to the microtitre plate and is used to capture the first antibody and its bound estradiol analogue. After washing, the europium is measured by time-resolved fluorescence. Calibration is with pure 17β-estradiol. The detection limit is 25 pmol/l. The cross reactivity with steroids other than 17β-estradiol is very low. It should be noted that 16 hydroxy and 16 oxo-steroids, steroids that are formed in the foeto-placental unit, cross react to less than 0.9% by weight. Intra-assay coefficients of variation (i.e. 1 standard deviation expressed as a percentage of the mean value) were 5.2% at 180 pmol/l and 3.9% at 875 pmol/l.

The digit lengths were recorded at follow-up when the children were 2 years old. Participants with injuries to the digits were excluded from the study. Initially, we used a flat-bed scanner to record the ventral surface of the left and right hands. However, movement of the hands made measurement difficult so that photocopies of the palms of the hand were taken. Measurements of 2nd and 4th digit length were made with vernier callipers measuring to 0.05 mm. The 2D:4D ratio was calculated from digit length measured from the basal crease of the digit proximal to the palm to the tip of the digit. It is known that this measurement can be made with high repeatability from photocopies of the hand and it correlates strongly with 2D:4D calculated from total digit length measured from X-rays of the fingers [11]. All digits were measured independently by two measurers who were unaware of the hormonal levels.

3. Results

There were 33 children, 18 males and 15 females, in the sample. Means for hormonal concentrations were FT=0.72±0.46 nmol/l, FE=1013±379.05 pmol/l and FT/FE ratio 0.001±0.001. Male children had significantly higher levels of FT than females (males 0.91±0.31, females 0.52±0.53, t=2.62, p=0.01). There were no significant differences in FE between the sexes (males 979.77±372.30, females 1058.73±407.02, t=0.57, p=0.57). FT/FE ratios showed significantly higher values for males compared to females (males 0.001±0.001, females 0.0005±0.0004, t=3.32, p=0.002).

The landmarks for measurement of 2D:4D were clear for 29 right hands and 29 left hands. Repeatabilities or intra-class correlation coefficients (r_1) of 2D:4D ratios were calculated using Model II single factor ANOVA tests [19]:

$$r_1 = (\text{groups MS} - \text{error MS})/(\text{groups MS} + \text{error MS})$$

Table 1
The relationships between the 2D:4D ratio of 29 children and their levels of FT, FE and FT/FE ratio

Hormone	Regression coefficient (*b*)	*F*	*p*
FT			
Right hand 2D:4D	−3.74	3.05	0.09
Left hand	−1.09	0.17	0.68
FE			
Right hand 2D:4D	2499.43	2.44	0.13
Left hand 2D:4D	1271.47	0.45	0.51
FT/FE ratio			
Right hand	−0.007	10.14	0.004*
Left hand	−0.003	0.76	0.39

* Bonferroni correction for two tests, p=0.008.

Values of r_1 were high for both right and left hands (right hand r_1=0.95, left hand r_1=0.93). The ratio (F) between the differences between individuals (groups MS) and measurement error (error MS) was calculated using repeated measures ANOVA tests:

$$F = (\text{groups MS})/(\text{error MS})$$

Values of F were high and significant (right hand F=35.13, p=0.00010; left hand F=27.99, p=0.0001). We concluded that the between-individuals difference in 2D:4D was greater than

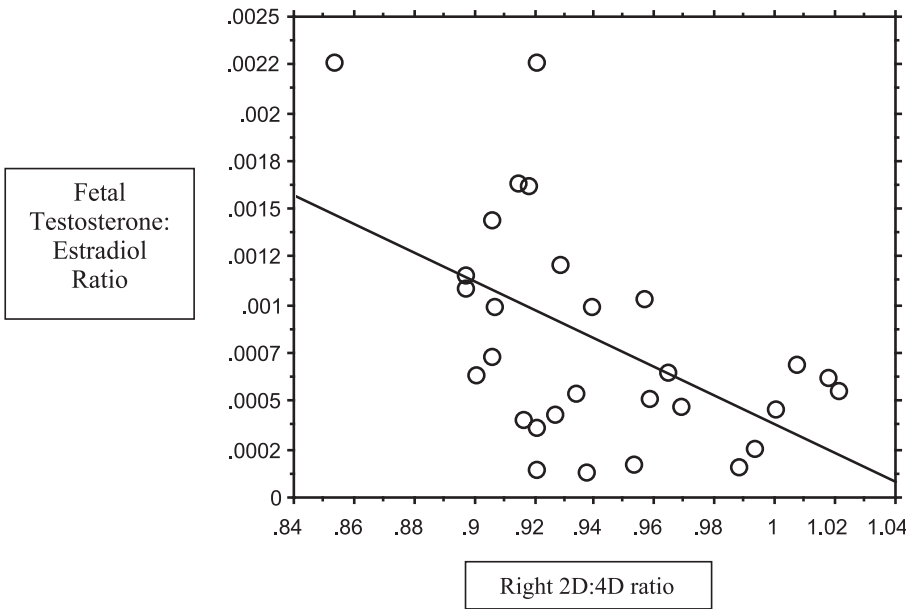

Fig. 1. The relationship between FT/FE ratio (the ratio of fetal testosterone to fetal estradiol) and right hand 2D:4D ratio in 29 children. The equation for the line is $y = -0.007x + 0.008$, r^2=0.27.

our measurement error and therefore the ratios reflected real differences between individuals. Means of first and second measurements of 2D:4D were used in the analyses.

As expected male children had lower mean 2D:4D ratios than females but the difference was not significant (right hands: males 0.936±0.05, females 0.946±0.04, t=0.69, p=0.49; left hands: males 0.926±0.04, females 0.929±0.04, t=0.22, p=0.83).

Our predictions were that 2D:4D would be negatively associated with FT, positively associated with FE and negatively related to FT/FE ratio. The direction of slope of all six associations was found to be consistent with predictions (Table 1). Non-significant negative associations were found for right and left hand between 2D:4D and FT. Also non-significant positive associations were found for 2D:4D and FE. For FT/FE ratio, there was a significant negative association with right hand 2D:4D (simple linear regression, b=−0.007, F=10.14, p=0.004, Fig. 1). This remained significant after correction for multiple tests within the 2D:4D and FT/FE association (two tests, Bonferroni correction, p=0.008). A non-parametric correlation test (Spearman rank test) also showed a significant negative association between right 2D:4D and FT/FE (r_s=−0.47, Z=2.49, p=0.01). This indicated that the association was not an artifact of outliers of FT/FE ratio. Removal of the effect of sex (dummy coded males=1, females=2) did not affect the p-value of the right 2D:4D and FT/FE association (multiple regression test, right 2D:4D b=−0.007, t=3.16, p=0.004; sex b=−0.0005, t=2.79, p=0.01).

4. Discussion

We have found that low 2D:4D ratios are associated with high FT in relation to FE levels, and high values of 2D:4D with low FT and high FE. The relationship is likely to reflect testosterone produced by the fetal gonads and adrenals as the fetus is isolated from maternal androgen by its conversion to estradiol within the placenta by the enzyme aromatase [20]. It may be noteworthy that all relationships between 2D:4D and fetal sex steroids were stronger in the right hand than the left. Traits that differ between the sexes show a tendency for the male form of the trait to be most strongly expressed on the right side of the body [21]. This may mean that right 2D:4D is more sensitive to the effects of relative FT and FE concentration. A number of studies have shown the right 2D:4D to be a stronger predictor of index traits (e.g. testosterone and sperm counts [3], fetal growth [6], athletic ability [12] and MI [9]) than the left.

The finding of a negative association between 2D:4D and FT/FE is further evidence that prenatal sex steroids have an influence on digit development. We suggest that low values of FT/FE are related to impaired fetal growth in males, while high values of FT/FE are associated with left handedness and autism, high sperm counts, athletic ability in men, protection against early MI in men and protection against early breast cancer in women.

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