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The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen*

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The differentiation of the urinogenital system and the appendicular skeleton in vertebrates is under the control of *Hox* genes. The common control of digit and gonad differentiation raises the possibility that patterns of digit formation may relate to spermatogenesis and hormonal concentrations. This work was concerned with the ratio between the length of the 2nd and 4th digit (2D:4D) in humans. We showed that (i) 2D:4D in right and left hands has a sexually dimorphic pattern; in males mean 2D:4D = 0.98, i.e. the 4th digit tended to be longer than the 2nd and in females mean 2D:4D = 1.00, i.e. the 2nd and 4th digits tended to be of equal length. The dimorphism is present from at least age 2 years and 2D:4D is probably established *in utero*; (ii) high 2D:4D ratio in right hands was associated with germ cell failure in men ($P = 0.04$); (iii) sperm number was negatively related to 2D:4D in the right hand ($P = 0.004$); (iv) in men testosterone concentrations were negatively related to right hand 2D:4D and in women and men LH (right hand), oestrogen (right and left hands) and prolactin (right hand) concentrations were positively correlated with 2D:4D ratio and (v) 2D:4D ratio in right hands remained positively related to luteinizing hormone and oestrogen after controlling for sex, age, height and weight.

Key words: 2nd digit:4th digit ratio/luteinizing hormone/oestrogen/spermatozoa/testosterone

Introduction

The vertebrate *Hox* gene family is essential for limb and genital development (Herault *et al*., 1997; Peichel *et al*., 1997). The *Hox* gene family is organized into four clusters *Hoxa* to *Hoxd* and the posterior-most *Hoxd* and *Hoxa* genes are required for the growth and patterning of digits and the differentiation of the genital bud (Kondo *et al*., 1997). In humans anatomical defects in digits and genitalia occur in the hand–foot–genital syndrome which results from a mutation within *Hoxa* (Mortlock and Innis, 1997). In mice deregulation of *Hoxd* expression may alter the relative lengths of digits and affect the growth of the genital bud (Kondo *et al*., 1997; Peichel *et al*., 1997). These observations raise the possibility that patterns of digit growth may be related to fertility.

In the human hand the 2nd and 4th digits present a pattern of approximate symmetry around the central axis of the 3rd digit. However, there is considerable variation in the ratio of the length of the 2nd digit to 4th digit (2D:4D). Many individuals have longer 2nd digits than 4th (2D:4D $\geqslant 1$) and many have longer 4th digits compared to 2nd (2D:4D $\leqslant 1$). We present evidence that the former ratio is more common in females and the latter more common in males and that 2D:4D is fixed at an early age. Low values of 2D:4D in the right hand are associated with high sperm numbers and high concentrations of testosterone in males. High values of 2D:4D are correlated with high concentrations of luteinizing hormone (LH), oestrogen and prolactin in men and women.

Materials and methods

Digit length was measured on the ventral surface of the hand from the basal crease of the digit to the tip, using vernier callipers measuring to 0.05 mm. This measurement is known to show a high degree of repeatability (Manning, 1995; Scutt and Manning, 1996).

We have used parametric tests (unpaired *t*-tests, simple linear and multiple regression tests) for all analyses. Means and standard errors are reported as measures of central tendency and dispersion. All variables were transformed (log $1+x$ transformation was used because some values were zero) in order to achieve normality and homoscedasticity (Zar, 1984). Prior to transformation relationships between variables were examined for evidence of curvilinear associations.

Study I

The 2nd and 4th digits were measured in the right and left hands of 800 subjects (400 males and 400 females) from the Merseyside area. Subjects were recruited from preschool groups, primary and secondary schools and Liverpool University students. Age ranged from 2 years to 25 years and 40 subjects (20 males and 20 females) were measured for each year group from 2 to 18 years, i.e. 40 subjects aged 2 years, 40 subjects aged 3 years etc. (340 males and 340 females), with 120 subjects (60 males and 60 females) in the age group 19 to 25 years. The first 100 subjects were measured twice with the repeated measure done by the same investigator in order to establish the reliability of our measurements. We used Model II single factor analysis of variance (ANOVA) tests to calculate the repeatabilities (r_1) of our measurements (Zar, 1984):

r_1 = (groups MS – error MS)/(groups MS + error MS)

where MS = mean squares. Repeated measures ANOVA tests (Palmer

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and Strobeck, 1984) were used to calculate the ratio (F) between groups MS (i.e. differences between individuals) and error MS (i.e. measurement error):

F = (groups MS)/(error MS)

Repeatabilities were high and the variance of our between-subject measures was significantly greater than the variance of our repeated measures (males: $n = 50$, 2nd digit, $r_1 = 0.96$, $F = 50.87$, $P = 0.0001$; 4th digit, $r_1 = 0.97$, $F = 61.54$, $P = 0.0001$; females: $n = 50$, 2nd digit, $r_1 = 0.96$, $F = 5183$, $P = 0.0001$; 4th digit, $r_1 = 0.96$, $F = 52.32$, $P = 0.0001$). This indicated that our measurements showed real differences between subjects.

Study II

In this prospectively designed study we measured 131 subjects (69 males and 62 females) attending the Reproductive Medicine Unit in Liverpool Women's Hospital. Digits 2 and 4 of the right and left hand were measured as before and all subjects were measured twice. Repeatabilities of measurements were high (males: right hand, 2nd digit, $r_1 = 0.99$, 4th digit, $r_1 = 0.99$; left hand, 2nd digit, $r_1 = 0.93$, 4th digit, $r_1 = 0.99$; females: right hand, 2nd digit, $r_1 = 0.98$, 4th digit, $r_1 = 0.99$; left hand: 2nd digit, $r_1 = 0.99$, 4th digit, $r_1 = 0.99$) and between-subject variance was higher than variance within our repeated measures (repeated measures ANOVA; males: right hand: 2nd digit, $F = 226.49$, $P = 0.0001$, 4th digit, $F = 258.63$, $P = 0.0001$; left hand: 2nd digit, $F = 29.36$, $P = 0.0001$, 4th digit, $F = 216.73$, $P = 0.0001$; females: right hand: 2nd digit, $F = 121.29$, $P = 0.0001$, 4th digit, $F = 170.01$, $P = 0.0001$; left hand: 2nd digit, $F = 145.45$, $P = 0.0001$; left hand: 2nd digit, $F = 145.45$, 4th digit, $F = 145.45$, $P = 0.0001$, 4th digit, $F = 165.17$, $P = 0.0001$). We concluded that our measurements reflected real differences in finger length between our subjects.

Sixty-seven males provided semen samples by masturbation and these were investigated for ejaculate characteristics including sperm number, % motility, sperm speed i.e. average speed, % progressive, and the sperm migration test (SMT). A Makler counting chamber was used for sperm concentration and motility evaluation. The SMT is a measure of sperm migration from an aliquot of liquefied semen into a layer of culture medium (for our protocol for semen analysis and the SMT see Biljan *et al*., 1994). Sperm numbers and the SMT, but not motility or normal morphology, are correlated with fertilization rate for in-vitro fertilization (IVF) patients (Biljan *et al*., 1994). Men who had unsuccessful reversals of vasectomies or pelvic injuries resulting in retrograde ejaculation were excluded from the sample, leaving 58 males.

Blood samples were taken between 10:00 and 15:00 h. Testosterone concentrations were assayed in males (58 subjects) and LH, follicle stimulating hormone (FSH), oestrogen and prolactin concentrations were measured in both males (58 subjects) and females (40 subjects).

Results

Study I

The right hand 2D:4D (mean ± SE) was 0.99 ± 0.002, range 0.83–1.31. Males had a lower 2D:4D ratio than females (males: 0.98 ± 0.002, range 0.83–1.19; females: 1.00, range 0.88–1.31, see Figure 1). We tested the difference between male and female means using an unpaired *t*-test. The means showed a highly significant difference ($n = 800$, $t = 3.79$, $P = 0.0002$).

The left hand 2D:4D (mean ± SE) was 0.99 ± 0.002, range 0.84–1.2. As with the right hand, males had a lower 2D:4D ratio than females (males: 0.98 ± 0.002, range 0.86–1.2; females: 1.00 ± 0.002, range 0.88–1.15). The difference between the male and female mean 2D:4D was highly significant (unpaired *t*-test, $n = 800$, $t = 4.42$, $P = 0.0001$).

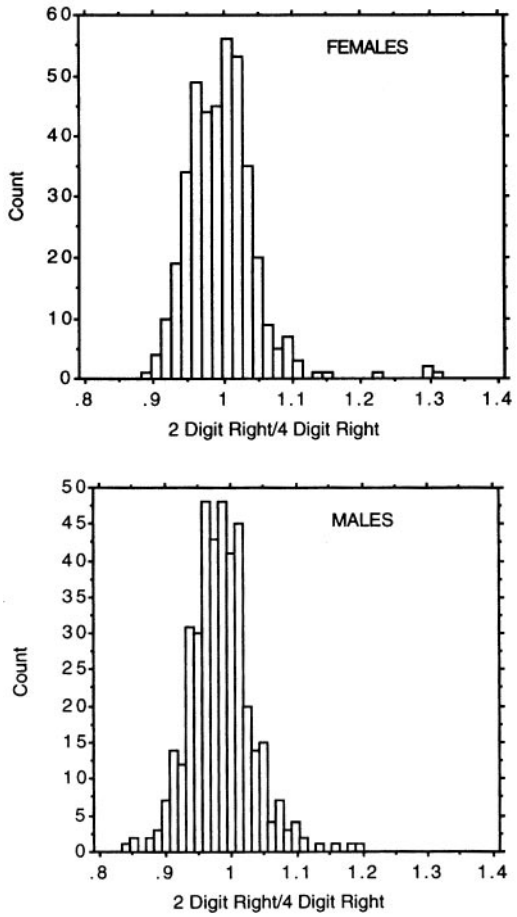

Figure 1. Histograms of the distributions of the 2D:4D ratio for 400 females and 400 males. Age range is 2–25 years. The female distribution has a mean of 1.00 and the male a mean of 0.98.

There was no evidence of a change in the 2D:4D ratio with age (log $1+x$ transformed 2D:4D regressed on age: right hand: males, $n = 400$, coefficient = –0.00003, $F = 0.0006$, $P = 0.94$; females, $n = 400$, coefficient = –0.0002, $F = 0.43$, $P = 0.51$; left hand: males, $n = 400$, coefficient = 0.0001, $F = 0.51$, $P = 0.47$; females, $n = 400$, coefficient = 0.0002, $F = 0.96$, $P = 0.31$). It is therefore likely that the ratio is determined *in utero* and/or in the first 2 years of life.

Study II

Means ± SE for age, height and weight of our sample were as follows: males, age 34.13 ± 0.74 years, height 178.24 ± 0.76 cm, weight 84.57 ± 1.87 kg; females, age 31.00 ± 0.49 years, height 161.95 ± 0.87 cm, weight 67.92 ± 2.15 kg.

Males had a significantly lower 2D:4D ratio in the right hand than females (males, 0.97 ± 0.003; females, 0.99 ± 0.004; unpaired *t*-test, $t = 2.52$, $P = 0.01$). Males also had a lower 2D:4D ratio in the left hand compared to females but the difference was not significant (males, 0.96 ± 0.003; females, 0.97 ± 0.004; unpaired *t*-test, $t = 0.98$, $P = 0.33$).

Regression of 2D:4D ratio on weight, height and age produced

Table I. The results of simple linear regression analyses (n = 58) of number of spermatozoa per ejaculate, motility, average speed, percentage progressive spermatozoa and sperm migration test (SMT) regressed on 2nd to 4th digit length ratio (2D:4D) of the right and left hands

Trait	Coefficient	*F*	*P*
Right hand			
No. spermatozoa	–44.97	8.59	0.004
Motility	–8.57	0.49	0.48
Average speed	–19.64	3.65	0.06
% progressive	–24.12	3.13	0.08
SMT	–20.27	3.34	0.07
Left hand			
No. spermatozoa	–25.69	2.65	0.11
Motility	–9.10	0.61	0.44
Average speed	–0.27	0.001	0.98
% progressive	–8.25	0.37	0.55
SMT	–4.73	0.18	0.67

one significant relationship, i.e. that between weight and 2D:4D ratio in the right hand of males (coefficient = 0.02, F = 4.16, P = 0.04). All other relationships were non-significant (males: right hand, height coefficient = 0.03, F = 0.03, P = 0.86, age coefficient = 0.001, F = 0.02, P = 0.90; left hand, height coefficient = 0.0001, F = 0.0001, P = 0.97, weight coefficient = 0.02, F = 3.39, P = 0.07, age coefficient = –0.01, F = 0.98 P = 0.33; females: right hand, weight coefficient = 0.001, F = 0.02, P = 0.90; height, coefficient = –0.08, F = 2.43, P = 0.12, age coefficient = 0.002, F = 0.22, P = 0.64; left hand, weight coefficient = 0.01, F = 0.82, P = 0.37, height coefficient = 0.02, F = 0.10, P = 0.76, age coefficient = 0.02, F = 0.10, P = 0.76).

There were 12 male subjects with germ cell failure (GCF), i.e. they produced no spermatozoa or few spermatozoa which were non-motile. These subjects had a significantly higher 2D:4D ratio (mean ± SE) in their right hand than those males with active spermatozoa (GCF males, 1.00 ± 0.005, n = 12; non-GCF males, 0.97 ± 0.004, n = 46; unpaired t-test, t = 2.11, P = 0.04). Similar but non-significant differences were seen in the left hand 2D:4D ratio (GCF males, 0.98 ± 0.01; non-GCF males, n = 46, 0.96 ± 0.004, unpaired t-test, t = 0.83, P = 0.41).

Table I shows the relationship between the 2D:4D ratio (right and left hands) and measures of sperm number per ejaculate, motility, two measures of speed and SMT. Sample size is n = 58 males for sperm number and n = 46 for all other measures (men with GCF are removed). All show negative relationships with 2D:4D. The relationship between 2D:4D and sperm number was significant for the right hand (Figure 2). The relationships in the smaller samples were non-significant but quite strong (P = 0.06 to 0.08) for average speed, SMT and % progressive.

Table II shows that sperm number remains significantly related to 2D:4D ratio in the right hand when the effects of weight, height and age are controlled for in multiple regression tests. Height and weight are strongly related to each other, therefore the independent variables were forced simultaneously into the analysis.

There were testosterone assays available for 58 male subjects and LH, oestrogen, prolactin and FSH assays for 98 subjects

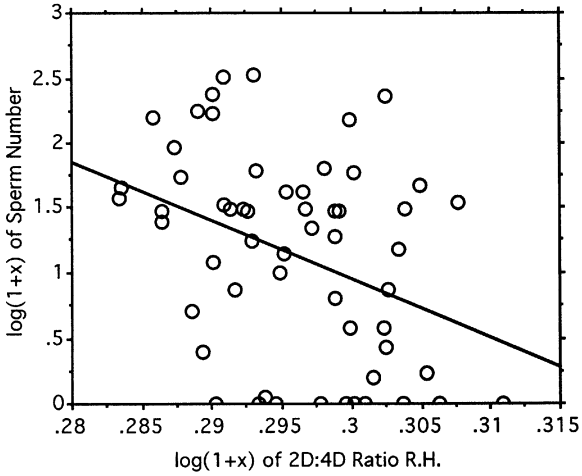

Figure 2. The relationship between number of spermatozoa per ejaculate [log(1+x)] and 2D:4D [log(1+x)] in the right hand (R.H.) of 58 men.

Table II. The results of multiple regression analyses of the relationships between independent variables 2nd to 4th digit length ratio (2D:4D; right and left hands), age, height and weight and the dependent variable number of spermatozoa per ejaculate. The independent variables were forced into the analysis simultaneously

Trait	Coefficient	*t*	*P*
Right hand			
2D:4D	–42.33	2.58	0.01
Age	–1.07	0.77	0.44
Height	–0.65	0.09	0.93
Weight	–0.62	0.38	0.70
Left hand			
2D:4D	–23.36	1.38	0.17
Age	–1.49	1.02	0.31
Height	0.46	0.06	0.95
Weight	–1.09	0.65	0.52

Table III. The relationships between the 2nd to 4th digit length ratio (2D:4D; right and left hands) and testosterone in 58 men and luteinizing hormone (LH), oestrogen, prolactin and follicle stimulating hormone (FSH) in 58 men and 40 women

Trait	Coefficient	*F*	*P*
Testosterone			
Right hand 2D:4D	–7.90	5.02	0.03
Left hand 2D:4D	–6.10	3.11	0.08
LH			
Right hand 2D:4D	10.26	8.99	0.004
Left hand 2D:4D	7.00	3.85	0.049
Oestrogen			
Right hand 2D:4D	16.27	10.73	0.002
Left hand 2D:4D	7.89	2.04	0.16
Prolactin			
Right hand 2D:4D	6.27	5.13	0.03
Left hand 2D:4D	2.58	0.81	0.37
FSH			
Right hand 2D:4D	2.73	0.42	0.52
Left hand 2D:4D	1.41	0.11	0.75

(58 males and 40 females). Table III gives the relationships between 2D:04D and hormonal concentrations and Figures 3 and 4 show the associations between 2D:4D and testosterone and LH respectively. Testosterone was significantly negatively

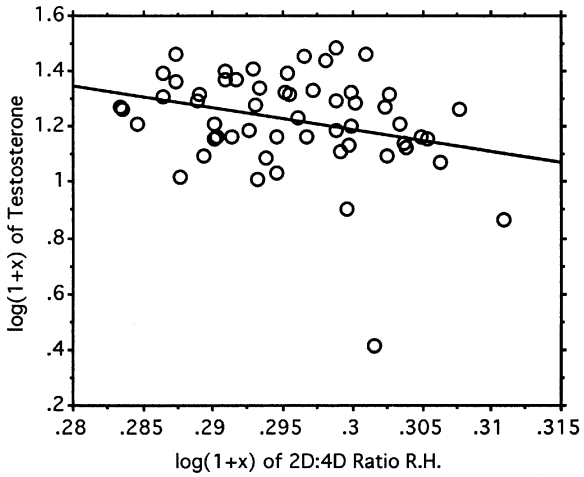

Figure 3. The relationship between testosterone concentration [log(1+*x*)] and 2D:4D [log(1+*x*)] in the right hand (R.H.) of 58 men.

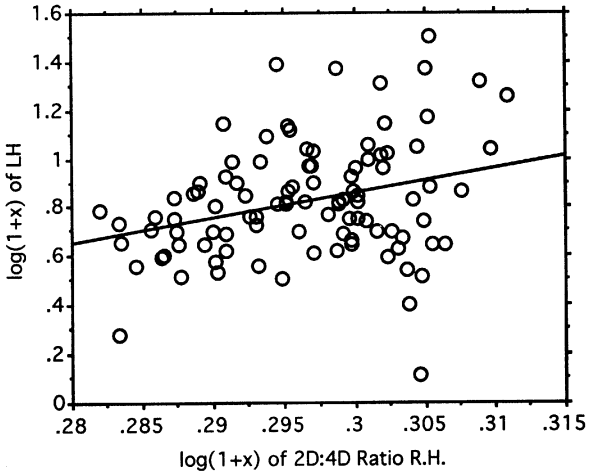

Figure 4. The relationship between LH [log(1+*x*)] and 2D:4D [log(1+*x*)] in the right hand (R.H.) of 98 subjects (58 men and 40 women).

related to 2D:4D in the right hand i.e. males with longer 4th digits than 2nd digits had more testosterone ($P = 0.03$). There were also statistically significantly positive relationships between 2D:4D and LH (right and left hands; $P = 0.004$ and 0.049), oestrogen (right hand; $P = 0.002$), and prolactin (right hand; $P = 0.03$).

The relationship between 2D:4D in the right hand and testosterone lost statistical significance when controlled for weight, height and age. However, 2D:4D remained a stronger predictor of testosterone than the other variables ($P = 0.07$) (Table IV). Relationships between 2D:4D in the right hand and LH and oestrogen remained statistically significant after sex (coded as a dummy variable, males = 0, females = 1), weight, height and age were controlled for (Table V).

Discussion

Our findings suggest that the 2D:4D ratio is laid down very early in development i.e. at least before age 2 years. Prenatal testosterone concentrations are thought to modify developmental rate (McEwen, 1981; MacLusky and Naftolin, 1981; Bardin and Catterall, 1981; Geschwind and Galaburda, 1985). One example of this modification is found in the development of the epidermal ridges of the digits. Jamison *et al.* (1993) have found that dermatoglyphic asymmetry and testosterone concentrations are positively correlated in adult males. They have also argued that adult testosterone concentrations are likely to be correlated with fetal concentrations.

Our finding of a negative relationship between testosterone concentrations in men and their 2D:4D ratio suggests the following model. *Hox* genes control development of the digits and testes. With the differentiation of Leydig cells within the testes the male fetus begins to produce testosterone at ~8 weeks until mid-gestation (George *et al.*, 1981). Testosterone affects the development of the digits including the 2D:4D ratio and digit dermatoglyphics. High concentrations of fetal testosterone lead to a low 2D:4D ratio which therefore indicates

Table IV. The results of a multiple regression analysis with testosterone as the dependent variable and 2nd to 4th digit length ratio (2D:4D; right hand), age, height and weight as independent variables. The independent variables were forced into the analysis simultaneously

Trait	Coefficient	*t*	*P*
Right hand 2D:4D	–6.93	1.85	0.07
Age	–0.01	0.05	0.96
Height	0.81	0.47	0.64
Weight	–0.33	0.91	0.37

Table V. The results of multiple regression analyses in which dependent variables were luteinizing hormone (LH), oestrogen and prolactin and independent variables were 2nd to 4th digit length ratio (2D:4D; right hand and left hand for LH and right hand only for oestrogen and prolactin), sex, age, height and weight

Trait	Coefficient	*t*	*P*
LH			
Right hand			
2D:4D	8.49	2.37	0.02
Sex	0.13	1.56	0.12
Age	–0.20	0.60	0.55
Height	0.34	0.21	0.83
Weight	0.001	0.003	0.99
Left hand			
2D:4D	5.62	1.55	0.12
Sex	0.13	1.55	0.12
Age	–0.21	0.61	0.54
Height	0.15	0.09	0.93
Weight	0.07	0.22	0.83
Oestrogen			
Right hand			
2D:4D	9.13	2.20	0.03
Sex	0.49	5.39	0.0001
Age	0.59	1.57	0.12
Height	2.02	1.12	0.27
Prolactin			
Right hand			
2D:4D	4.69	1.68	0.09
Sex	0.11	1.79	0.07
Age	–0.55	2.13	0.04
Height	1.21	0.99	0.33
Weight	–0.08	0.36	0.72

high prenatal testicular activity. As a result adult testicular activity is correlated with the 2D:4D ratio.

Our results suggest that the relationship between 2D:4D ratio and testosterone is particularly strong in the right hand. We are unable to explain why this should be so. However, laterality in the association between dermatoglyphic asymmetry and testosterone has been noted by Jamison *et al*. (1993). Their data strongly showed lower ridge counts and pattern intensity in left hands compared to right when testosterone is low and the opposite was true of the high testosterone group. Also Geschwind and Galaburda (1985) have suggested that testosterone may slow the growth rate of the left side of the brain while enhancing growth of the right side. Therefore testosterone effects could often show laterality.

Measures of sperm function may also be associated with *Hox* genes. Erickson (1990) has reviewed evidence that homeobox genes including *Hox* genes are expressed in spermatozoa after meiosis. This expression may indicate a role for the products of *Hox* genes in some aspects of sperm structure and/or activity. If 2D:4D predicts testosterone concentrations, sperm numbers and perhaps sperm function, it could also predict fertility in men. The resolution of this question requires further work.

High 2D:4D ratios are characteristic of females and LH and oestrogen are found in large amounts in individuals with 2D:4D > 1. Maternal testosterone may cross the placenta and affect the differentiation of the ovaries and the digits. This could explain why females with low 2D:4D have low concentrations of oestrogen. Further work is needed to examine the relationship between the 2D:4D ratio and fertility in women.

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