

## ORIGINAL ARTICLE

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## Digit ratio (2D:4D) in Klinefelter's syndrome

## Keywords:

2D:4D, azoospermia, digit ratio, Klinefelter's syndrome, prenatal testosterone, testosterone sensitivity

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## SUMMARY

The ratio of second to fourth digit length (2D:4D) is a correlate of prenatal testosterone. High 2D:4D is associated with low prenatal testosterone, and reduced sensitivity to testosterone. Klinefelter's syndrome (KS; 47 XXY) affects the endocrine system, such that low testosterone levels are found in KS fetuses, new-borns and adults. To date, there are no published data regarding the pattern of 2D:4D in KS males. Here we consider 2D:4D in KS individuals ( $n = 51$ ), their relatives (16 fathers and 15 mothers) and an unaffected control sample of 153 men and 153 women. Adult KS individuals were taller than their fathers and had shorter fingers than fathers and male controls. Compared with fathers, male controls and mothers, KS males had shorter fingers relative to height. With regard to 2D:4D, KS individuals had higher 2D:4D than fathers (right and left hands), male controls (right and left hands) and mothers (left hands). Among KS males older than 13 years there were 34 individuals currently prescribed testosterone and nine not prescribed. In comparison to the former, the latter individuals had higher right 2D:4D and higher right-left 2D:4D. We conclude that KS males have mean 2D:4D values similar to those found in female population norms. In addition, testosterone supplementation in KS males may be most common for individuals with low right 2D:4D.

## INTRODUCTION

The relative lengths of the second (index finger) digit and fourth (ring finger) digit (2D:4D) is a sexually dimorphic trait in humans (male 2D:4D < female 2D:4D: Manning, 2002; Peters *et al.*, 2002). It has been suggested that 2D:4D is a biomarker of prenatal sex steroids such that low 2D:4D correlates with high testosterone and low oestrogen in the foetus and high 2D:4D with low foetal testosterone and high foetal oestrogen (Manning *et al.*, 1998; Manning, 2002). Empirical evidence gathered since Manning *et al.* (1998) suggests that there are lateralized effects concerning the link between 2D:4D and sex steroids. Thus, right 2D:4D tends to show stronger associations with hormonally influenced target traits than left 2D:4D and a greater sex difference than left 2D:4D (Hönekopp & Watson, 2010). Therefore, right 2D:4D is thought to be more sensitive to foetal sex hormones than left 2D:4D (Manning, 2008; Hönekopp & Watson, 2010). In addition a further measure, right-left 2D:4D, often shows strong links to hormonally influenced traits (e.g. in sports performance, Bennett *et al.*, 2010).

Experimental evidence from rodent models has lent support to the hypothesized links between 2D:4D and foetal sex hormones. Zheng & Cohn (2011) demonstrated that 2D:4D in mice

is dependent on a balance of androgen and oestrogen signalling during a narrow foetal window. In this regard, they have shown that inactivation of androgen receptors, or blocking androgen receptors or increasing foetal oestrogen leads to 'female-type' 2D:4D in offspring. Whereas, inactivation of oestrogen receptors or blocking their function or increasing prenatal testosterone levels results in a 'male-type' mean 2D:4D in murine offspring (see Manning, 2011 for an overview of the significance of Zheng & Cohn's work, 2011). In humans, we cannot manipulate foetal hormone receptors or foetal levels of sex steroids and therefore, we must seek indirect evidence for the link between 2D:4D and foetal androgen. One approach to this issue is to consider the 2D:4D of individuals who are likely to have been exposed to abnormal androgen levels in early foetal development. With regard to abnormally high foetal androgen levels, low mean 2D:4D has been reported in individuals with congenital adrenal hyperplasia (e.g. Brown *et al.*, 2002; Okten *et al.*, 2002) and individuals with autism (e.g. Manning *et al.*, 2001). Here, for the first time, we investigate the effects of low foetal testosterone by considering mean 2D:4D in Klinefelter individuals.

Klinefelter's syndrome (KS) is found in men with an additional X chromosome (47,XXY: Klinefelter *et al.*, 1942). The prevalence

of KS in the general male population is about one in 1000. However, the symptoms are often mild and very variable, thus many KS males are not diagnosed and it is thought the true prevalence may be about four in 1000 (Lanfranco *et al.*, 2004; Forti *et al.*, 2010). During childhood the morphological and behavioural traits of KS are not marked and therefore, the diagnosis is often made during adolescence or in adults. Almost all KS men are infertile, seminiferous tubule degeneration begins in the foetus and progresses through infancy and accelerates in puberty (Aks-glaede *et al.*, 2006). Thus, the frequency of KS rises to about 3–4% among male patients that are infertile and to about 10–12% in male patients that are azoospermic (Forti *et al.*, 2010). In such individuals, testicular biopsies may yield spermatozoa for successful intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization treatment (Giltay & Maiburg, 2010).

Klinefelter's syndrome is caused by the presence of an additional X chromosome in males and it manifests itself as a disorder of the endocrine system (Zitzmann *et al.*, 2004). The most obvious features of KS are caused by testosterone deficiency and include elevated levels of gonadotropins, small testes, hypogonadism and gynecomastia. There are also perturbations in body proportions (greater than average height which is dependent on greater leg length), and sometimes cognitive problems (which may include delayed acquisition of language). Low testosterone has been found in foetuses and infants with KS. Thus, testosterone levels similar to female concentrations have been described in a foetus with KS (Künzig *et al.*, 1977). Also, in comparison to controls, infants and young boys (1–23 months) with KS have been reported to have low testosterone, and reduced penile and testicular size (Ross *et al.*, 2005). With regard to adolescents, testosterone in KS individuals is low to low-normal (Forti *et al.*, 2010).

Coupled with low levels of testosterone, KS phenotypes are correlated with sensitivity to testosterone. The androgen receptor gene (AR) is highly polymorphic for a trinucleotide repeat (CAGn) in exon 1. Normal variation in CAGn is about 10–30, with sensitivity to testosterone negatively related to CAGn (La Spada *et al.*, 1991). The presence of long CAGn is predictive of phenotypic effects in KS e.g. gynecomastia, small testes and above-average height (Zitzmann *et al.*, 2004; Bojesen *et al.*, 2011). In addition responsiveness to testosterone therapy may be determined by CAGn. Thus, Nielsen *et al.* (1988) reported variation in responsiveness to testosterone therapy in KS males, with most (77%) showing improvements in mood, energy and drive but some showing little effect.

In this report, we compare 2D:4D in KS individuals, unaffected relatives and unaffected male controls and female controls from the general population. In general, our predictions are that patterns of 2D:4D in KS reflect low levels of foetal testosterone. In particular, we predict that KS individuals will have higher mean 2D:4D than their unaffected fathers and male population norms. Comparisons with female patients are more difficult to predict, but we would expect that mean 2D:4D in KS would be as high if not higher than mean 2D:4D of mothers and female population norms. With regard to patterns of testosterone use in KS individuals, it is to be expected that beneficial effects will be found in patients with the greatest sensitivity to testosterone. There is some evidence that male patients with low 2D:4D have experienced high foetal testosterone and are particularly sensitive to testosterone (Manning *et al.*, 2003; Butovskaya *et al.*, 2012;

Knickmeyer *et al.*, 2011; but see Hurd *et al.*, 2011; Hampson & Sankar, 2012; Loehlin *et al.*, 2011). Given that this is so, we predict that KS individuals using testosterone will have lower 2D:4D than KS men, who are currently not prescribed testosterone.

## MATERIALS AND METHODS

The protocol of the study was essentially that for a similar study of 2D:4D in families with children with autism (Manning *et al.*, 2001). Klinefelter's Syndrome individuals, together with their unaffected relatives, were recruited in the UK from members of the Klinefelter's Syndrome Association (KSA). Information regarding the Study was sent to members in the form of a KSA newsletter. They were asked to provide photocopies of the hands for KS individuals, and their fathers, mothers, brothers and sisters. The photocopy instructions were as follows: 'Place the palm down GENTLY on the photocopier screen, keeping the fingers straight and together (see example of hands below). Then press "copy". Also, please check that the photocopy is light enough to see the finger creases (where the finger joins the palm)'. Participants were asked to provide information regarding their status (KS, Father, Mother, Brother, Sister), and their age, height, and ethnicity (White, East-Asian, Black, Mixed). In addition, information concerning hormone replacement was requested 'Use of hormone replacement: Yes or No? If yes, which type? i.e. Testosterone or Oestrogen'. The photocopies and the associated information were then sent anonymously by post to one of us (JTM). Informed consent was provided and the protocol for the study was approved by the local ethics committee and by the Executive Committee of the KSA.

On receipt of the photocopies, each was coded with a number from a Table of random numbers. The photocopies were randomized such that family groups were dispersed throughout the sample. Finger lengths were then measured blind to the status of the individual (i.e. whether they were KS or a relative of a KS individual). All fingers were measured twice, with a period of time of at least 24 h between measurements, and the second measurement was made blind to the first.

A control sample of men and women was recruited from a sample of members of the general public. Recruitment was from Libraries, Museums, Recreational Centres and Schools in the North and North-West of England. Photocopies of the right and left hands were made and age and height recorded. The total sample was 627 individuals. As with KS patients and their relatives, finger lengths were measured twice (Manning *et al.*, 1998). From this sample we age-matched ( $\pm 3$  years) three male and three female control participants to each KS individual.

## RESULTS

There were responses from 55 families. The photocopies from two families were too dark for accurate measurement and were removed from the sample. With regard to the KS sample, the parents of two KS individuals judged them to be too young for their hands to be photocopied, and one photocopy (a left hand) from an adult KS individual was not sufficiently clear enough to measure finger length, and this was removed from the study. The remaining sample consisted of 51 KS individuals (51 right-hand photocopies and 50 left-hand photocopies) together with 16 fathers, 15 mothers, 3 brothers and 6 sisters, i.e. a total of 91 participants. The fathers and mothers tended to be for young KS

males, presumably because for adult KS their parents may have not been readily available. In addition, there were 153 male and 153 female controls. Thus, there were 397 individuals in the sample (397 right-hand photocopies and 396 left hand photocopies).

All participants were Caucasian (there was one Black KS participant, but the photocopies for this individual were too dark for accurate measurement).

### Repeatability of 2D:4D

We pooled the complete sample and calculated the intra-class correlation coefficient ( $r_1$ ) for the first and second values for right hand 2D:4D and left hand 2D:4D. For the right hand  $r_1 = 0.91$  and for the left hand  $r_1 = 0.96$ . A repeated measures ANOVA showed the between-individual differences in right 2D:4D were significantly higher than the error [ $F(1396) = 22.3$ ,  $p = 0.0001$ ] and this was also the case for left 2D:4D [ $F(1395) = 50.70$ ,  $p = 0.0001$ ]. Therefore, we calculated the means for right and left 2D:4D and used these in all subsequent analyses.

### Descriptive statistics

The descriptive statistics of the sample [mean (SD)] were as follows:

Age – KS individuals 31.94[17.53], range 6–71 years, the sample included 36 individuals who were 18 years or older [mean age 39.14(15.34)]; Fathers 58.00[11.93], range 31–78 years; Mothers 53.6[12.00], range 24–77 years; Brothers 23.00[5.57], range 17–28 years; Sisters 26.33[13.32], range 13–48 years; Control males 33.25[17.56], range 6–72 years, including 113 individuals who were 18 years or older [mean age 40.27(14.96) years]; control females 32.87[17.41], range 6–71 years, including 113 individuals who were 18 years or older [mean age 39.89(14.91)].

Height – KS individuals 179.37[15.86] cm, >17 years 183.29 [8.92] cm; Fathers 178.27[8.95] cm; Mothers 165.71[6.29] cm; Brothers 174.27[7.51] cm; Sisters 165.50[6.12] cm; control males 174.07[17.08] cm, >17 years 180.67[9.47] cm; control females 161.73[12.02] cm, >17 years 165.15[7.61] cm.

Mean finger length – To consider finger lengths between individuals (rather than relative differences in finger length within individuals) we summed across all four fingers and obtained the mean finger length per individual (Sutcliffe *et al.*, 2010). These were: KS individuals 74.30[6.96] mm, >17 years 76.16[4.79] mm; Fathers 78.03[4.35] mm; Mothers 70.74[2.86] mm; Brothers [71.94] mm; Sisters [68.84] mm; control males 75.45[7.15] mm, >17 years 77.98[4.79] mm; control females 75.45[7.15] mm, >17 years 77.98[3.84] mm.

### Hormonal supplements

There were 50 KS individuals who reported information regarding hormonal supplements. Of these, 34 were taking testosterone and 16 were not. In general, testosterone supplementation is usually considered from about age 14 years. Consistent with this, all KS individuals reporting the use of testosterone in our sample were 14 years or older. Within the sample of individuals who were >13 years there were 34 individuals who were prescribed testosterone and nine who were not.

### Comparisons of means

Sample sizes for brothers and sisters were too small for meaningful analyses. Therefore, these data were discarded and

analyses restricted to comparisons between KS individuals and their parents and control males and females. Comparisons between KS individuals and their fathers are the most revealing of differences. However, we have only 16 fathers and two are not paired with KS children as the latter were too young to be photocopied. The next most informative comparison is that between KS individuals and male controls.

With regard to the type of comparisons, we first compare linear variables (mean heights and mean finger lengths) across the five groups. These linear dimensions are influenced by age, thus we restrict our samples to individuals older than 17 years. Next, we compare the group means for the relationship of finger length to height and for right 2D:4D, left 2D:4D and right–left 2D:4D. There is evidence that 2D:4D is not strongly influenced by age (Trivers *et al.*, 2006), therefore we consider the entire age range of the samples. We tested the difference in means using *t* tests (paired or non-paired as appropriate), when differences are significant we also give effect sizes (Cohen's *d*).

### Height

Regarding male height for individuals older than 17 years, we found a significant difference for KS men vs. father pairs ( $n = 8$ ) such that the mean for the former was greater than the mean for the latter (paired *t*-test,  $x - y = 7.74$  cm,  $t = 2.77$ ,  $p = 0.03$ ,  $d = 0.56$ ). A one-way ANOVA across all five groups (>17 years for KS men, fathers, male controls, mothers and female controls) showed significant differences,  $F(4, 288) = 61.71$ ,  $p = 0.0001$ . However, multiple *a posteriori* tests (Fisher's PLSD) showed significant differences were restricted to sex differences in height.

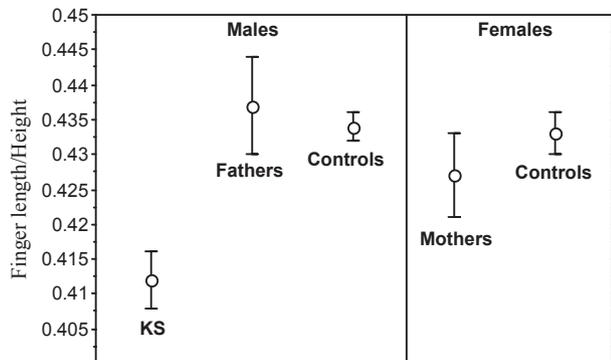
### Finger length

We calculated the mean of finger length summed across all four fingers for each individual (see Sutcliffe *et al.*, 2010) and compared these means across male patients that were older than 17 years. For KS men vs. father pairs ( $n = 7$ ) we found that the former had shorter fingers than the latter (paired *t*-test,  $x - y = 4.57$  mm,  $t = 3.58$ ,  $p = 0.009$ ,  $d = 0.41$ ). A one-way ANOVA across all five groups showed significant differences in finger lengths (>17 years,  $F(4.282) = 47.96$ ,  $p = 0.0001$ ). Among men, multiple *a posteriori* tests indicated a significant difference between mean KS finger length and male controls such that the former was shorter (KS vs. male controls,  $-1.87$  cm, Fisher's PLSD = 1.63,  $p < 0.05$ ,  $d = 0.38$ ). As expected, female patients had shorter mean finger lengths than male patients.

### Relative finger length (finger length/height)

Paired *t*-tests showed that KS individuals had shorter finger length relative to their height than fathers and mothers (Fathers;  $x - y = -0.04$ ,  $t = 6.65$ ,  $p = 0.0001$ ,  $d = 1.00$ ; Mothers;  $x - y = -0.03$ ,  $t = 3.36$ ,  $p = 0.006$ ,  $d = 0.78$ ). Across the groups KS individuals had shorter finger length relative to height than all other groups [KS 0.41(0.03), fathers 0.44(0.03), male controls 0.43(0.02), mothers 0.43(0.02), female controls 0.43(0.04); see Fig. 1]. A one-way ANOVA showed these group differences to be significant [ $F(4377) = 5.56$ ,  $p = 0.0002$ ]. Multiple *a posteriori* tests (Fisher's PLSD) showed a significant difference between mean relative finger length of KS individuals vs. fathers ( $x - y = -0.03$ , 0.02,  $<0.05$ ), KS vs. male controls ( $x - y = -0.02$ , 0.01,  $p < 0.05$ ) and KS vs. female controls ( $x - y = -0.02$ ,  $p < 0.05$ ).

**Figure 1** The mean finger length divided by height (standard error bars) of KS individuals, fathers of KS individuals, male controls, mothers of KS individuals and female controls. Non-overlap of standard error bars indicates that means are significantly different (for men; KS men against fathers of KS  $p < 0.01$  and male controls  $p < 0.0001$ ; for women; KS men against mothers of KS  $p < 0.01$  and female controls  $p < 0.0001$ ).



**2D:4D**

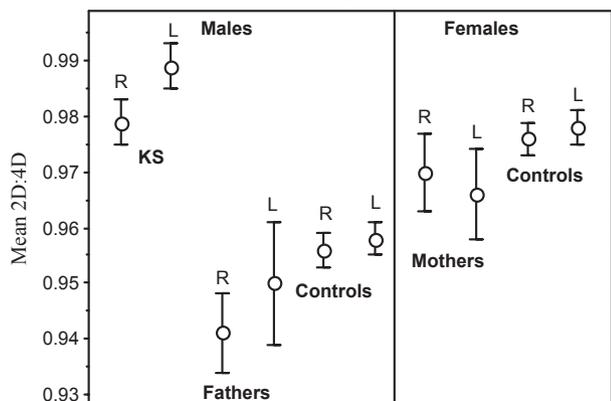
Mean right and left hand 2D:4D across the groups is given in Table 1 and Fig. 2. Paired *t*-tests showed KS individuals had significantly higher mean 2D:4D than their fathers and the differ-

**Table 1** Means and standard deviations (SD) for right and left hand 2D:4D in 51 KS individuals, 16 Fathers of KS, 153 male controls, 15 mothers of KS and 153 female controls. The *p* values (non-paired *t*-tests) refer to comparisons of the means for KS men against means for fathers, male controls, mothers and female controls

	KS males <i>n</i> = 51	Fathers of KS <i>n</i> = 16	Male controls <i>n</i> = 153	Mothers of KS <i>n</i> = 15	Female controls <i>n</i> = 153
Right 2D:4D (SD)	0.979 (0.028)	0.941** (0.025)	0.956*** (0.034)	0.969 (0.028)	0.976 (0.034)
Left 2D:4D (SD)	0.989 (0.031)	0.950* (0.044)	0.958*** (0.041)	0.966* (0.033)	0.978* (0.035)

\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.0001.

**Figure 2** Mean 2D:4D (standard error bars) for the right (R) and left (L) hands of KS individuals, fathers of KS individuals, male controls, mothers of KS individuals and female controls. Non-overlap of standard error bars indicates that means are significantly different [for men; KS men against fathers of KS (right hand  $p < 0.01$ , left hand  $p < 0.05$ ) and male controls (right hand and left hand  $p < 0.0001$ ): for women; KS men against mothers of KS (left hand  $p < 0.05$ ) and female controls ( $p < 0.5$ )].



ence was greater for the right hand than the left (right hand  $x - y = 0.04$ ,  $t = 4.00$ ,  $p = 0.002$ ,  $d = 1.43$ ; left hand  $x - y = 0.035$ ,  $t = 2.48$ ,  $p = 0.03$ ,  $d = 1.03$ ). With regard to comparisons between KS individuals and their mothers, paired *t*-tests showed the former had higher 2D:4D than the latter, but this was only significant for the left hand (right hand;  $x - y = 0.01$ ,  $t = 1.14$ ,  $p = 0.27$ ; left hand;  $x - y = 0.03$ ,  $t = 2.71$ ,  $p = 0.02$ ,  $d = 0.72$ ). Considering all groups, a one-way ANOVA showed significant differences for the right hand [ $F(4383) = 11.39$ ,  $p = 0.0001$ ]. Multiple *a posteriori* tests (Fisher's PLSD) showed KS individuals had higher right 2D:4D compared with that of fathers ( $x - y = 0.04$ ,  $0.02$ ,  $p < 0.05$ ,  $d = 1.43$ ) and male controls ( $x - y = 0.03$ ,  $0.01$ ,  $p < 0.05$ ,  $d = 0.74$ ). With regard to the left hand, there were also significant differences across the groups [ $F(4382) = 10.32$ ,  $p < 0.05$ ]. Multiple *a posteriori* tests (Fisher's PLSD) showed KS individuals had significantly higher left 2D:4D than that of fathers ( $x - y = 0.04$ ,  $0.02$ ,  $p < 0.05$ ,  $d = 1.03$ ), male controls ( $x - y = 0.03$ ,  $0.01$ ,  $p < 0.05$ ,  $d = 0.85$ ) and mothers ( $x - y = 0.02$ ,  $0.02$ ,  $p < 0.05$ ,  $d = 0.72$ ).

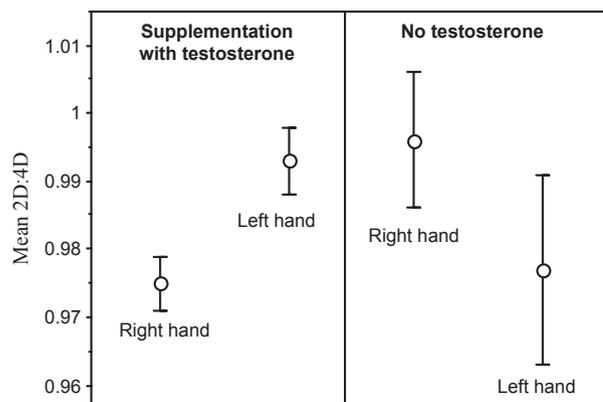
**Right-left 2D:4D**

The group means for right-left 2D:4D were as follows: KS  $-0.008[0.03]$ , fathers  $-0.009[0.04]$ , control males  $-0.002[0.03]$ , mothers  $0.004[0.02]$ , control females  $-0.002[0.03]$ . A one-way ANOVA showed no significant differences across the groups [ $F(4382) = 0.84$ ,  $p = 0.50$ ].

**Testosterone supplementation**

Here we considered KS individuals who were >13 years old, i. e.  $n = 43$ . The KS participants who reported use of testosterone supplements (79%) did not significantly differ from those who were not using such supplements (21%) in their age [testosterone (T) 36.63(16.65) years vs. no testosterone (NT) 30.67(15.73) years,  $t = 0.96$ ,  $p = 0.34$ ], height [T 183.10(9.08) cm vs. NT 185.11 (11.14) cm,  $t = 0.56$ ,  $p = 0.58$ ], mean finger length [T 75.83 (4.86) mm vs. NT 76.09 (4.75) mm,  $t = 0.14$ ,  $p = 0.89$ ], or finger length relative to height [T 0.41(0.03) vs. NT 0.41(0.03),  $t = 0.12$ ,  $p = 0.90$ ]. However, compared with individuals who were not taking testosterone, the testosterone group had significantly lower values of right 2D:4D and right-left 2D:4D [Fig. 3: right 2D:4D T 0.975(0.03) vs. NT 0.996(0.03),  $t = 2.12$ ,  $p = 0.04$ ,

**Figure 3** A comparison of mean right and left hand 2D:4D [SE bars] in KS men who were taking testosterone supplements ( $n = 34$ ) and those who were not ( $n = 9$ ). Non-overlap of standard error bars indicates that means are significantly different (for right hand, testosterone supplementation against non-supplementation,  $p < 0.05$ ).



$d = 0.70$ : left 2D:4D  $T\ 0.993(0.03)$  vs. NT  $0.977(0.04)$ ,  $t = 1.39$ ,  $p = 0.17$ : right–left 2D:4D  $T\ -0.016(0.03)$  vs.  $0.019(0.03)$ ,  $t = 3.42$ ,  $p = 0.002$ ,  $d = 1.17$ ].

## DISCUSSION

We have found that KS individuals tended to be taller than their fathers and had shorter fingers than their fathers and male controls. In addition, KS males differed from relatives and controls in two important respects. First, KS men had shorter fingers relative to their height than did their fathers, control males, mothers and control females. These differences showed medium to large effect sizes. Secondly, mean 2D:4D of KS men was higher than that for their fathers (right and left hands), male controls (right and left hands), and mothers (left hand only). The effect sizes for differences in 2D:4D varied from medium to large. In addition, with regard to testosterone supplementation, we found that KS individuals prescribed testosterone had lower right hand 2D:4D relative to left hand 2D:4D (high effect size  $d = 1.17$ ) and lower right hand 2D:4D (medium effect size  $d = 0.70$ ) than KS males not prescribed testosterone.

Our finding concerning short fingers relative to height in KS males is similar to the pattern of finger length reported in children conceived by ICSI. Sutcliffe *et al.* (2010) compared finger length relative to height in 211 children conceived by ICSI and 195 control children. They reported that the former had shorter fingers after correction for height compared with the control group. They suggested that presence of short fingers relative to height is a marker for low foetal androgen. Thus, men with azoospermia may have experienced low foetal levels of androgen, and their children conceived by ICSI may also tend to show low foetal androgen. Therefore, a pattern of short fingers relative to height found in both KS individuals and children conceived by ICSI may indicate low prenatal testosterone in both groups.

With regard to 2D:4D, KS men were found to have mean 2D:4D that was higher than that of their fathers and male controls, and close to or greater than mean 2D:4D of their mothers or female controls. We think these ‘feminized’ values of 2D:4D are best interpreted as the effect of low prenatal testosterone on the formation of 2D:4D in KS males. Künzig *et al.* (1977) reported that male levels of foetal testosterone obtained from 226 samples reached their peak between the 12th and 15th week of pregnancy. Female foetal values did not significantly fluctuate during gestation. At 12–15 weeks testosterone levels were a highly significant predictor of foetal sex, but not at birth. However, in the case of a KS foetus, female levels of testosterone were found at 12–15 weeks. Low prenatal testosterone is associated with a reduction in the rate of growth of the fourth digits in foetuses (Manning, 2002; Zheng & Cohn, (2011), and for overview see Manning, 2012). Thus, high 2D:4D in KS individuals is evidence for a link between low prenatal testosterone and high 2D:4D. In this regard, it is also of interest that high 2D:4D is related to perturbations in spermatogenesis in samples of men from the general population (Auger & Eustache, 2011). However, it is azoospermic males that have the highest 2D:4D (Manning *et al.*, 1998) and success rates of sperm retrieval in ICSI are reduced in men with high 2D:4D compared with individuals with low 2D:4D (Wood *et al.*, 2003). Thus, low foetal testosterone, azoospermia and high 2D:4D are related to one another and are characteristic of KS men. It is to be noted that effect sizes for

differences in 2D:4D between KS men and male controls are of medium size (right hand  $d = 0.74$ , left hand  $d = 0.85$ ). This means that 2D:4D is unlikely to be of use for diagnosis of KS but may be used in this regard in conjunction with other KS traits such as greater than average height.

In comparison to KS males not taking testosterone (21%), we found KS individuals who were prescribed testosterone supplementation (79%) had low right 2D:4D and low right–left 2D:4D. This effect may arise because low 2D:4D is linked to high sensitivity to testosterone. Nielsen *et al.* (1988) reported variation in responsiveness to testosterone therapy in a sample of KS males, with 23% showing little or no improvement in mood, energy and drive, but significant cognitive improvements in 77% of the sample. The androgen AR shows polymorphism for CAG repeat number in exon 1 of the gene. The number of CAG repeats ranges from CAG<sub>n</sub> = 10 to about CAG<sub>n</sub> = 30, and sensitivity to testosterone is highest when CAG<sub>n</sub> is low (La Spada *et al.*, 1991). In KS, high CAG<sub>n</sub> is linked to gynecomastia, small testes and above-average height (Zitzmann *et al.*, 2004; Bojesen *et al.*, 2011). There is some evidence that 2D:4D may be positively correlated with CAG<sub>n</sub>. Manning *et al.* (2003) have reported that right 2D:4D and right–left 2D:4D were positively correlated with CAG<sub>n</sub> in men, and Butovskaya *et al.*, 2012 found a similar relationship between male left 2D:4D and CAG<sub>n</sub>. These relationships were not replicated by Loehlin *et al.*, (2011), and Hurd *et al.*, (2011) in men, but Loehlin *et al.* (2011) reported a significant positive correlation between left 2D:4D and CAG<sub>n</sub> in women. In addition, Knickmeyer *et al.* (2011), considering 2D:4D in newborns, reported that 2D:4D was not predicted by CAG<sub>n</sub> or by neonatal levels of testosterone, but that the interaction between CAG<sub>n</sub> and testosterone was predictive of 2D:4D. However, Hampson & Sankar (2012) were not able to replicate the findings of Knickmeyer *et al.* (2011). It is becoming clear that low 2D:4D is a powerful predictor of performance in sports that show a substantial male advantage (e.g. Bennett *et al.*, 2010). These reports may map on to our finding that testosterone supplementation was more likely in KS individuals, who had low 2D:4D (and presumably low CAG<sub>n</sub> and high sensitivity to testosterone). However, we emphasize that the decision of treatment with testosterone for KS men is based on a sum of clinical and behavioural arguments. Therefore, a simple measurement of 2D:4D is unlikely to be a major factor in such a decision.

In conclusion, we have found that KS individuals have shorter than expected fingers particularly when finger length is adjusted for height, and have higher 2D:4D than that of their fathers or control males. These findings are likely the consequence of low foetal testosterone levels and their effect on finger growth, and in particular growth of the fourth digit. In addition, KS individuals who are prescribed testosterone supplementation have low right–left 2D:4D compared with KS males who are not prescribed testosterone. This finding may arise because the former are more sensitive to testosterone than the latter.

## CONFLICT OF INTEREST STATEMENT

The Authors declare no conflicts of interest.

## REFERENCES

- Akslae L, Wikström AM, Raipert-De Meyts E, Dunkel L, Skakkebaek NE & Juul A. (2006) Natural history of seminiferous tubule degeneration in Klinefelter syndrome. *Hum Rep Update*, 12, 39–48.

- Auger J & Eustache F. (2011) Second to fourth digit ratios, male genital development and reproductive health: a clinical study among fertile men and testis cancer patients. *Int J Androl*, 34, e49–e58.
- Bennett M, Manning JT, Cook CJ & Kilduff LP. (2010) Digit ratio (2D:4D) and performance in elite rugby players. *J Sports Sci* 28, 1415–1421.
- Bojesen A, Hertz JM & Gravholt CH. (2011) Genotype and phenotype in Klinefelter syndrome – impact of androgen receptor polymorphism and skewed X inactivation. *Int J Androl*, 34, e642–648.
- Brown WM, Hines M, Fane BA & Breedlove SM. (2002) Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Horm Behav*, 42, 380–386.
- Butovskaya ML, Vasilyev VA, Lazebny OE, Burkova VN, Kulikov AM, Mabulla A, Shibalev DV & Ryskov AP. (2012) Aggression, digit ratio, and variation in the androgen receptor, serotonin transporter and dopamine D4 receptor genes in African foragers: the Hadza. *Behav Genet*, 42, 647–662.
- Forti G, Corona G, Vignozzi L, Krausz C & Maggi M. (2010) Klinefelter's syndrome: a clinical and therapeutic update. *Sex Dev*, 4, 249–258.
- Giltay JC & Maiburg MC. (2010) Klinefelter syndrome: clinical and molecular aspects. *Expert Rev Mol Diagn*, 10, 765–776.
- Hampson E & Sankar JS. (2012) Re-examining the Manning hypothesis: androgen receptor polymorphism and the 2D:4D ratio. *Evol & Hum Behav*, 33, 557–561.
- Hönekopp J & Watson S. (2010) Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. *Am J Hum Biol* 22, 619–630.
- Hurd PL, Vaillancourt KL & Dinsdale NL. (2011) Aggression, digit ratio and variation in androgen receptor and monoamine oxidase a genes in men. *Behav Genet*, 41, 543–556.
- Klinefelter HF, Reifenstein EC & Albright F. (1942) Syndrome characterized by gynecomastia, aspermatogenesis without Leydigism, increased excretion of follicle hormone stimulating hormone. *J Clin Endocrinol* 2, 615–627.
- Knickmeyer RC, Woolson S, Hamer RM, Konneker & Gilmore JH. (2011) 2D:4D ratios in the first 2 years of life: Stability and relation to testosterone exposure and sensitivity. *Horm Behav*, 60, 256–263.
- Künzig HJ, Meyer U, Schmitz-Roeckerath B & Broer KH. (1977) Influence of fetal sex on the concentration of amniotic fluid testosterone: antenatal sex determination? *Arch Gynakol*, 223, 75–84.
- La Spada AR, Wilson EM, Lubahn DB, Harding AE & Fischback KH. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, 352, 77–79.
- Lanfranco F, Kamischke A, Zitzmann M & Nieschlag E. (2004) Klinefelter's syndrome. *Lancet* 364, 273–283.
- Loehlin JC, Medland SE & Martin NG. (2011) Is CAG sequence length in the androgen receptor gene correlated with finger-length ratio? *Pers Indiv Diff* 52, 224–227.
- Manning JT. (2002) *Digit Ratio: A Pointer to Fertility, Behavior, and Health*. Rutgers University Press, New Brunswick.
- Manning JT. (2008) *The Finger Book*. Faber & Faber, London.
- Manning JT. (2011) Resolving the role of prenatal sex steroids in the development of digit ratio. *Proc Natl Acad Sci USA* 108, 16143–16144.
- Manning JT, Scutt D, Wilson J & Lewis-Jones DI. (1998) The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum Reprod* 13, 3000–3004.
- Manning JT, Baron-Cohen S, Wheelwright S & Sanders G. (2001) The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol*, 43, 160–164.
- Manning JT, Bundred PE, Newton DJ & Flanagan BF. (2003) The second to fourth digit ratio and variation in the androgen receptor gene. *Evol Hum Behav*, 24, 399–405.
- Nielsen J, Pelsen B & Sorensen K. (1988) Follow-up of 30 Klinefelter males treated with testosterone. *Clin Genet*, 33, 262–269.
- Okten A, Kalyoncu M & Yaris N. (2002) The ratio of second- and fourth-digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early Hum Dev*, 70, 47–54.
- Peters M, Mackenzie K & Bryden P. (2002) Finger length and distal finger extent patterns in humans. *Am J Phys Anthropol*, 117, 209–217.
- Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF & Zinn A. (2005) Early androgen deficiency in infants and young boys with 47, XXY Klinefelter syndrome. *Horm Res*, 64, 39–45.
- Sutcliffe AG, Manning JT, Katalanic A, Ludwig A, Mehta M, Lim J, Basatemur E & Ludwig M. (2010) Perturbations in finger length and digit ratio (2D:4D) in ICSI children. *Reprod Biomed Online*, 20, 138–143.
- Trivers R, Manning J & Jacobson A. (2006) A longitudinal study of digit ratio (2D:4D) and other finger ratios in Jamaican children. *Horm Behav*, 49, 150–156.
- Wood S, Vang E, Manning J, Walton J, Troup S, Kingsland C & Lewis-Jones ID. (2003) The ratio of second to fourth digit length in azoospermic males undergoing surgical sperm retrieval: predictive value for sperm retrieval and on subsequent fertilization and pregnancy rates in IVF/ICSI cycles. *J Andrology* 24, 871–877.
- Zheng Z & Cohn MJ. (2011) Developmental basis of the sexually dimorphic digit ratio. *Proc Natl Acad Sci USA*, 108, 16289–16294.
- Zitzmann M, Depenbusch M, Gromoll J & Nieschlag E. (2004) X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. *J Clin Endocr Metab*, 89, 6208–6217.