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No association between fluctuating asymmetry in highly stabilized traits and second to fourth digit ratio (2D:4D) in human fetuses

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ABSTRACT

Recent studies have suggested that the ratio of the length of the second and fourth digit (2D:4D) may be associated with developmental instability (DI) as measured by the left–right asymmetry of the same digits. Because the 2D:4D ratio is amongst others, determined prenatally as a result of exposure to sex hormones, such an association could indicate that the same prenatal developmental processes determine levels of DI. In this study we criticize these earlier findings and show by simulations that they are confounded by the fact that (non-) linear combinations of the digit lengths are used as both dependent (average asymmetry in digits 2 and 4) and independent (ratio of the lengths of digits 2 and 4) variable. We therefore studied associations between 2D:4D ratios and asymmetry not only in digits but also in several other skeletal elements in deceased human fetuses. In contrast to the earlier studies, we did not find an association between 2D:4D ratios and asymmetry in digits 2 and 4. We argue that this may be due to the low levels of DI in this study, which limits the confounding effects of DI. Also, no associations were detected with the asymmetry of all other trait either. Thus, there appears to be very little evidence of any link between DI and 2D:4D in this population for limb measurements. We conclude that highly stabilized and functionally important traits such as human limbs may in general show limited increases in asymmetry with prenatal stress.

1. Introduction

Fluctuating asymmetry (FA, directionally random deviation from perfect symmetry) is most commonly used as a measure of developmental instability (D.I., the sensitivity of a developing system to random perturbations). DI is assumed to reflect individual (genetic) quality and stress experienced during development because degrees of asymmetry correlate with a range of fitness correlates and types of stress. However, patterns reported in the literature are heterogeneous, and it is at present not clear what factors determine levels of DI, and during which periods of development levels of DI, and possibly individual quality as well, are most likely to be affected (e.g., [20,23]). Thus, in order to be able to understand why associations between FA and different types of stress and fitness correlates may or may not exist, there is a need for studies that identify if and when particular types of stress affect levels of DI.

It has recently been suggested that levels of FA increase with prenatal exposure to either high testosterone or high estrogen levels. Two studies found relatively strong U-shaped associations between the ratio of the length of the second digit over the length of the fourth digit (2D:4D ratio) and asymmetry in humans [17,18]. There is growing evidence that the 2D:4D ratio reflects sex steroid exposure during early development. Relatively low 2D:4D values in males have been interpreted as being related to exposure to higher prenatal testosterone concentrations, while the higher ratios in females would reflect higher exposure to estrogen [14]. There is also evidence of an influence of post natal development [21,7]. While, on average the differences between males and females are generally small, albeit statistically significant and consistently found in many studies, between-individual variation in 2D:4D can be very high. This variation, at least in part, appears to result from between-individual exposure to sex steroids [13]. High concentrations of testosterone lead to low 2D:4D whereas high estrogen concentrations to high 2D:4D, and the U-shaped association with asymmetry [17,18] would indicate that levels of DI may be strongly influenced by prenatal factors, and suggest a primary role for testosterone and estrogen during early development.

If these associations between 2D:4D ratios and asymmetries would reflect a general phenomenon, it could form the basis to formulate predictions about the existence associations between asymmetry and measures of stress and fitness in general. However, [24] recently showed that most of the observed association between 2D:4D ratios and degrees of asymmetry are likely to be a statistical artifact. Strongest associations

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were found with asymmetries of digits 2 and 4 in particular. Indeed, when regressing single hand 2D:4D ratios against asymmetries in these digits, (non-)linear combinations of the same measurements are used both as dependent and independent variables. Consequently, U-shaped associations arise even in the absence of any association between single hand 2D:4D and DI [24]; and see Fig. 1 for a simulated dataset illustrating this). In addition, [24] provided a review of the available literature and some new data to conclude that there is little evidence of an association between 2D:4D and DI. Nevertheless, some studies have shown an association between asymmetry and testosterone levels (e.g. [9]) warranting further research on the role of sex hormone exposure on DI and individual health and quality.

Even if we assume that some of the observed associations between FA and 2D:4D ratios are biologically meaningful, it is difficult to make a priori predictions about its expected shape. On the one hand one, it has been argued that extreme exposures to sex hormones, reflected in extreme 2D:4D ratios, may not all be beneficial and are expected to increase DI (e.g., Martin et al., 1999; [17]). Under this scenario a U-shaped pattern is indeed expected. On the other hand, relatively low 2D:4D ratios in men and high 2D:4D ratios in women are related to increased attractiveness and fitness (e.g. [18]). More specifically, low 2D:4D ratios associate with more 'masculine' faces in men and high 2D:4D ratios with more 'feminine' faces in females [4]; but see [11]. Since, more masculine faces in men and more feminine faces in women are more symmetric [8,12], lower levels of asymmetry are expected for individuals with more extreme 2D:4D ratios. On the basis of these patterns the inverse of a U-shaped association would be expected contrary to the patterns in [17] and [18]. In addition, relatively low 2D:4D ratios in men relate positively with semen quality [14]; but see [5], and men with high quality semen are more symmetric [15,5], again predicting lower asymmetry in men with low 2D:4D ratios. Consequently, predictions could be formulated contrary to observed patterns [3,17,18].

These paradoxical results emphasize the need for more studies on the association between 2D:4D ratios and FA. We studied this association in over 300 deceased human fetuses. Many fetuses experienced major congenital abnormalities leading to their early death [6]. However, digit ratios did not relate to these abnormalities [9] and asymmetry was only increased to a small extent [26]. Asymmetry was measured for a range of skeletal elements of the limbs from X-rays. If prenatal hormone exposure affects levels of DI, we predicted that associations between FA and 2D:4D ratios will already emerge during early development when the relative lengths of digits 2 and 4 are formed under the influence of hormone levels. The advantage of studying such a young population is that there is only a short time elapsed between the determination of the relative length of digits 2 and 4 and the assessment of asymmetry. This implies that FA and 2D:4D rations will be minimally affected by other factors such as handedness [2]; Auerbach and Ruff, 2005) or other forms of stress experienced later in life. If indeed any associations exist between FA and 2D:4D ratios, they are likely to be strongest at that age.

2. Materials and methods

2.1. Study population and measurements

Since 1980 deceased fetuses which arrive for examination at the VU Medical Centre in Amsterdam are standard radiographed ventrally and laterally (23 mA, 70–90 kV, 4–12 s, Agfa Gevaert D7DW Structurix films). This work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and subjects gave informed consent to the work. Data on ethnicity were not available, but the patient population of the VU Medical Centre is predominantly Caucasian. This research is carried out on the ventrally taken radiographs of 643 deceased fetuses taken between 1992 and 1999. 316 fetuses were excluded for measurements, because the fingers could not be properly positioned for the radiographs. In total, 169 male and 158 female (aged 14–42 weeks, mean 28 weeks, ± 11 weeks) fetuses were examined for the left and right hand 2D:4D. In addition, next to digits 2 and 4, the length of the left and right ulna, radius, femur, tibia and fibula were obtained. Measurements were made from the midpoint of the proximal end of the bones to the midpoint of the distal end with a transparent ruler with a resolution of 0.01 cm. Digits were measured from the proximal end of the proximal phalanx to the distal end of the distal phalanx (Fig. 2).

2.2. Statistical analyses

2.2.1. Reliability of measurements

Measurements of all traits were carried out by one of two investigators without prior knowledge of the autopsy reports (however, several congenital anomalies can be seen in radiographs). To compare the accuracy of the measurements of the two investigators, 38 fetuses were independently measured by each investigator. In

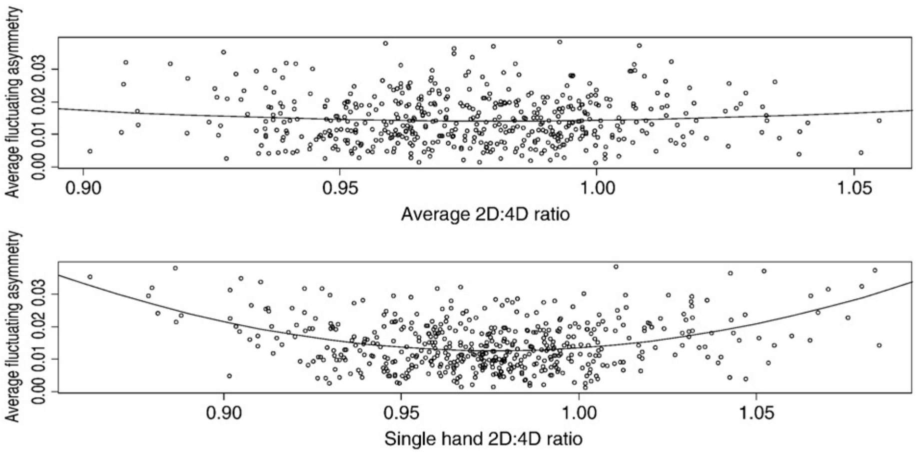

Fig. 1. Associations between the ratio of the length of the second to fourth digit (2D:4D ratio) and average fluctuating asymmetry in digits 2 and 4 for a simulated dataset where no association was expected. In the upper graph the average 2D:4D ratio was used as independent variable, resulting in a non-significant association ($r^2 = 0.4\%$). In the bottom graph, single hand 2D:4D ratios were used (as in [17] and [18]) yielding a highly significant ($p < 0.0001$) U-shaped pattern ($r^2 = 16.0\%$) which must have resulted from a statistical artifact because no such association was involved in the simulation of these data (see note). Note: Data were simulated assuming a common 2D:4D ratio of 0.975 (length digits 2 = 7.7 and of digit 4 = 7.9) and level of DI (variance = 0.04) for each individual ($N = 500$). Lengths of digits 2 and 4 were simulated from a normal distribution with mean equal to 7.7 or 7.9 resp. and variance equal to 0.04. In this way, the average degree of asymmetry was about 1.5%, and the maximum about 4% of trait size respectively. This dataset was generated without any between-individual variation in 2D:4D ratio and DI such that association between 2D:4D and FA could be expected.

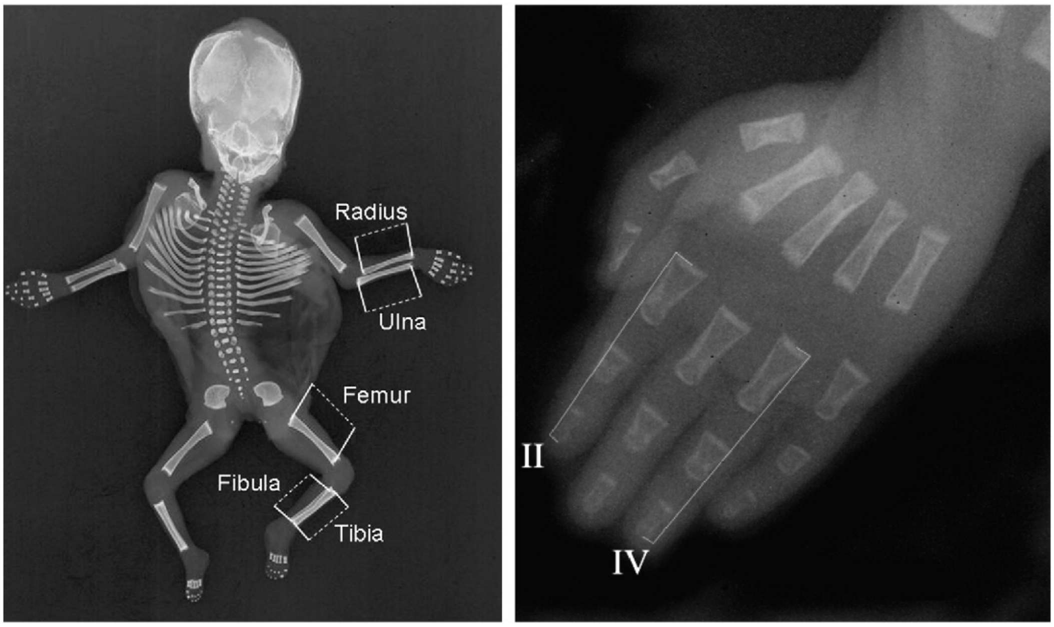

Fig. 2. Left: Radiograph of an entire fetus. Lines indicate measurements of limb bones from the midpoint of the proximal end of the bones to the midpoint of the distal end; Right: Radiograph of right hand. Lines indicate length of the midpoint of the proximal end of the proximal phalanx to the midpoint of the distal end of the distal phalanx.

addition, the entire procedure of positioning a fetus and making a radiograph was repeated for 32 fetuses. Degree of measurement error (ME) and levels of directional asymmetry were analyzed using a mixed regression approach [22]. As 32 fetuses were remounted and photographed a second time independently, measurement error could be determined at two levels. At the level of individual measurements, ME was determined on the basis of repeated measurements on the same photograph, and a comparison of asymmetry levels between photographs of the same bone and fetus allowed to evaluate the error resulting from mounting the fetus. Levels of directional asymmetry were tested by *F*-tests [22].

2.2.2. Associations between asymmetries and digit ratio's

We applied linear models with asymmetry as dependent variable and 2D:4D and the quadratic term $2D{:}4D^2$ as independent variables. Both single hand (left and right) and average 2D:4D ratios were tested separately. Because asymmetry decreased with age, was higher in fetuses with major congenital abnormalities and differed between males and females [26], we added these three factors to the linear model. Associations were investigated for both single trait and 3 groups of average asymmetries (all traits; digits 2 and 4 only; all traits excluding digits 2 and 4) obtained after standardization. In addition to the quadratic models, linear associations were tested for males and females separately. All analyses were performed in SAS (version 9) and graphs were produced in R (version 2.5.1).

3. Results

3.1. Reliability of measurements

For each trait, levels of ME were smaller than levels of asymmetry and there were no indications of directional asymmetry (except for radius, but the *p*-value of 0.05 was non-significant after Bonferroni correction; Table 1). Thus, our measurements allowed to measure levels of asymmetry relatively accurately and there was no need to correct for directional asymmetry which could potentially decouple the presumed association between asymmetry and DI [10,23]. Distributions of the signed asymmetries were all leptokurtic (kurtosis ranging between 5.6 and 68.9), as is often observed in studies of FA. Most likely, this leptocurtosis is a reflection of between-individual variation in the underlying levels of DI (e.g. [23]). This is a prerequisite to be able to detect any associations between levels of DI and other covariates (e.g., [23].

Mean digit ratio equaled 0.91 in male and 0.92 in female fetuses, a difference that was statistically significant. A more thorough analysis and interpretation of this sexual dimorphism is presented elsewhere [9]. The repeatability of the measurement of 2D:4D ratios within single radiographs was high ($r^2=0.99$). In addition, the entire procedure of positioning a fetus and making a radiograph was repeated for 32 fetuses. Again the repeatability was high ($r^2=0.95$).

3.2. Associations between asymmetry and digit ratio

Standardized regression coefficients of quadratic and linear associations between the different asymmetry measures and digit ratios analyzed for both sexes are provided in Table 2. Of all tests of the significance of the quadratic association between single trait asymmetries on the one hand and 2D:4D ratios (left, right and average) on the other hand, none were statistically significant neither for digits 2 or 4, nor for any other single trait (all $p>0.05$ in 21 tests). Similarly, quadratic associations were not significant for average asymmetry of digits 2 and 4 (left: $F_{1,187}=0.00$, $p=0.9$; right: $F_{1,190}=1.09$, $p=0.3$; average: $F_{1,182}=0.05$, $p=0.8$), average asymmetry across all traits (left: $F_{1,242}=0.60$, $p=0.4$; right: $F_{1,264}=0.24$, $p=0.6$; average: $F_{1,184}=0.06$,

Table 1
Overview of levels of measurement error as a result of mounting the fetus and taking a photograph on the one hand (ME-photograph) and of measuring on a single photograph (ME-measurement) relative to real fluctuating asymmetry (FA) and levels of directional asymmetry (DA) in different bones of deceased fetuses.

Trait	Real FA	ME-photograph	ME-measurement	DA
Femur	4.37	0.45	0.19	$F_{1,427}=0.56$ ($p=0.45$)
Fibula	14.2	0.00	0.28	$F_{1,303}=0.08$ ($p=0.77$)
Tibia	8.47	0.00	0.16	$F_{1,431}=0.14$ ($p=0.70$)
Radius	2.32	1.18	0.48	$F_{1,442}=3.96$ ($p=0.05$)
Ulna	12.6	0.63	0.18	$F_{1,444}=0.32$ ($p=0.57$)
Digit 2	1.90	0.00	0.15	$F_{1,229}=0.00$ ($p=0.99$)
Digit 4	2.41	1.65	0.12	$F_{1,210}=1.43$ ($p=0.23$)

Variance components were multiplied by 1000.

Table 2
Standardized regression coefficients (SE) of the quadratic and linear associations between single-hand or average digit ratios on the one hand and single-trait or average asymmetry on the other hand in deceased human fetuses.

	Linear			Quadratic		
	Left 2D:4D	Right 2D:4D	Average 2D:4D	Left 2D:4D	Right 2D:4D	Average 2D:4D
Single trait asymmetries						
Digit 2	0.03 (0.04)	−0.02 (0.04)	0.01 (0.04)	−1.01 (2.45)	−0.50 (0.82)	−1.02 (1.48)
Digit 4	0.07 (0.05)	0.10 (0.06)	0.09 (0.05)	0.07 (1.56)	2.76 (1.67)	0.86 (1.81)
Radius	−0.03 (0.02)	−0.01 (0.04)	−0.03 (0.05)	−0.12 (0.81)	−0.33 (0.56)	1.10 (1.02)
Ulna	−0.02 (0.08)	0.01 (0.07)	0.00 (0.10)	−2.38 (2.61)	−0.18 (1.69)	−1.01 (3.75)
Femur	0.04 (0.07)	−0.05 (0.05)	−0.04 (0.08)	−2.92 (2.34)	−0.00 (1.34)	−2.01 (2.92)
Fibula	−0.10 (0.13)	0.00 (0.07)	−0.08 (0.09)	−1.77 (2.25)	−1.80 (1.57)	−2.33 (3.15)
Tibia	0.00 (0.12)	−0.04 (0.06)	0.02 (0.07)	−4.03 (2.07)	1.46 (1.40)	−3.20 (2.76)
Average asymmetries						
Digits 2 and 4	0.05 (0.04)	0.03 (0.04)	0.04 (0.04)	−0.11 (0.77)	0.89 (0.75)	−0.11 (0.84)
All traits	0.00 (0.02)	0.03 (0.02)	0.01 (0.02)	−0.44 (0.30)	0.07 (0.19)	−0.10 (0.40)
All traits except digits 2 and 4	−0.00 (0.02)	0.00 (0.02)	−0.01 (0.02)	−0.39 (0.35)	0.03 (0.22)	−0.05 (0.47)

Data from males and females were pooled, and regression coefficients were obtained after correcting for sex, age and the presence of major congenital abnormalities. Uncorrected estimates were comparable (not shown) and none of the associations was statistically significant (see text for details).

$p=0.8$), and average asymmetry of all traits excluding digits 2 and 4 (left: $F_{1,240}=0.28$, $p=0.6$; right: $F_{1,262}=0.05$, $p=0.83$; average: $F_{1,182}=0.20$, $p=0.7$). Patterns were comparable if analyzed without correction for age, sex and the presence of major congenital abnormalities (not shown). In total, 7 of the above 30 analyses revealed positive and 23 negative slopes for the quadratic effect. For the linear associations combining data from males and females, 18 were positive and 12 negative, non being statistically significant (Table 2).

Standardized regression coefficients of linear association between asymmetry and 2D:4D ratios in males and females separately are provided in Table 3. A statistically significant positive relationship was found for digit 4 in females only (left: $F_{1,95}=4.29$, $p=0.04$; right: $F_{1,88}=4.45$, $p=0.04$; average: $F_{1,95}=5.97$, $p=0.02$). Thus, out of 30 tests in total, only these 3 were statistically significant, but not so after correction for multiple testing. In total, 22 out of 30 slopes were negative for males, and 11 out of 30 were negative for females (Table 3).

A graphic exploration indeed confirmed that our data did not support any association between asymmetry in skeletal traits or digits 2 and 4 and 2D:4D (Fig. 3).

4. Discussion

4.1. Digit ratio and asymmetry in digits 2 and 4

We anticipated U-shaped associations between single-hand digit ratios and asymmetries in digits 2 and 4 as a result of the fact that the same measurements are used as both dependent and independent variable (Fig. 1; [24]). However, these predictions were not confirmed by our data, neither for single digit or average asymmetry over digits 2 and 4. We argue that this is due to the overall low levels of asymmetry in these traits. The U-shaped associations are the result of the fact that high DI results in both asymmetric digits and extreme digit ratios. However, when DI is low on average, this confounding effect can be expected to be minimal, and in this case not resulting in a U-shaped pattern. Nevertheless, the use of the same measurements as dependent and independent variable should be avoided regardless of the levels of DI.

4.2. Digit ratio and asymmetry in other limb bones

We did not detect any association between levels of asymmetry and 2D:4D in any other single-trait or average FA . This is unlikely to be due to a lack of power because sample sizes were sufficiently large, measurement error was low and kurtosis of the signed asymmetry was high which indicates high between-individual variation in DI. These conditions form a prerequisite to be able to detect associations with other covariates (see [23] for review). Our 2D:4D measurements were performed on X-ray photographs and involve only the skeletal elements and did not include the tip of the fingers. However, we could show that excluding the finger tips had only marginal effects on the digit ratio ([9]; see also e.g. [19]). [16,17] have pointed out that differences in the measurement of digits may be at the basis of heterogeneous results on digit ratios in the literature. Indeed, [16], by comparing direct measurements and measurements from photocopies, suggested that fat pads in the finger tips may be an important

Table 3
Standardized regression coefficients (SE) of linear associations between single-hand or average digit ratios on the one hand and single-trait or average asymmetry on the other hand in deceased human fetuses.

	Males			Females		
	Left 2D:4D	Right 2D:4D	Average 2D:4D	Left 2D:4D	Right 2D:4D	Average 2D:4D
Single trait asymmetries						
Digit 2	−0.00 (0.10)	−0.11 (0.08)	−0.11 (0.12)	0.10 (0.10)	0.03 (0.08)	0.10 (0.10)
Digit 4	−0.05 (0.12)	0.07 (0.11)	−0.01 (0.14)	**0.26 (0.12)**	**0.24 (0.12)**	**0.31 (0.13)**
Radius	−0.06 (0.07)	−0.01 (0.06)	−0.03 (0.09)	−0.04 (0.06)	−0.01 (0.05)	−0.05 (0.07)
Ulna	−0.16 (0.17)	0.14 (0.24)	0.26 (0.44)	0.15 (0.16)	−0.05 (0.09)	−0.12 (0.14)
Femur	0.11 (0.30)	−0.15 (0.10)	−0.16 (0.18)	−0.11 (2.34)	−0.06 (0.14)	−0.08 (0.24)
Fibula	−0.36 (0.18)	−0.15 (0.07)	−0.50 (0.26)	−0.01 (0.17)	0.11 (0.16)	0.09 (0.22)
Tibia	0.07 (0.14)	0.06 (0.15)	−0.13 (0.24)	0.06 (0.19)	−0.03 (0.13)	0.15 (0.19)
Average asymmetries						
Digits 2 and 4	−0.03 (0.10)	−0.06 (0.10)	−0.06 (0.10)	0.16 (0.10)	0.14 (0.10)	0.16 (0.09)
All traits	−0.00 (0.05)	−0.01 (0.04)	−0.01 (0.05)	0.04 (0.04)	0.02 (0.04)	−0.02 (0.05)
All traits except digits 2 and 4	0.00 (0.05)	−0.01 (0.04)	0.01 (0.05)	0.05 (0.35)	0.02 (0.24)	0.04 (0.05)

Data from males and females were analyzed separately, and regression coefficients were obtained after correcting for age and the presence of major congenital abnormalities. Uncorrected estimates were comparable (not shown) and statistically significant associations are indicated in bold (see text for details).

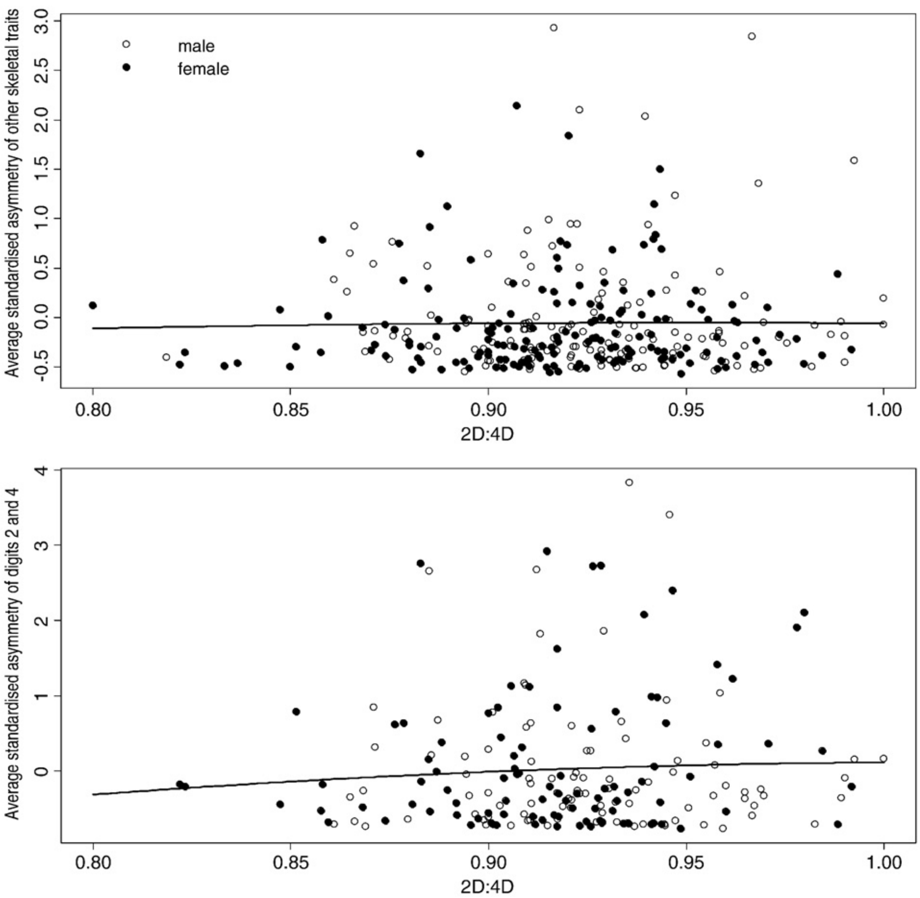

Fig. 3. Associations between average asymmetries and average digit ratio across both hands (2D:4D) in skeletal straits (excluding digits: top graph; for digits 2 and 4: bottom graph). Estimated quadratic curves, albeit not statistically significant (see text), are also provided to make results comparable to those in the literature.

factor in determining biologically relevant variation in 2D:4D ratios. Nevertheless, [13] did detect relatively strong association between 2D:4D ratios taken from photocopies and sex hormone levels, and [3] reported high reliability of measurements from photocopies compared to direct measurements. Furthermore, [3] is the only study to report significant associations between asymmetry and 2D:4D ratios without measurements in digits [24]. It thus remains difficult to assess the validity of different measurement techniques and how they may confound patterns. However, the fact that we detected a sexual dimorphism in this population (Summary statistics presented here, more details in [9]) and showed a strong correlation between the ratios on the basis of skeletal elements only and the total finger lengths [9] suggests that our measurement protocol did not confound patterns.

One potentially confounding factor that we cannot completely rule out is ethnicity. Although the majority of the patient population at the medical center is Caucasian, we do not have data on individual ethnicity. Since digit ratios may differ among races, this may have obscured patterns. However, probably more importantly as we discuss below, the lack of any association may be attributable to the fact that the development of limb bones is highly stabilized and robust against developmental perturbations.

We studied deceased fetuses and infants of which the majority experienced major congenital abnormalities. Thus, the serious abnormal development could have led to spuriously high levels of asymmetry that might have obscured associations with digit ratios. However, we showed elsewhere that contrary to a priori expectations, levels of asymmetry in this population were surprisingly low and even lower than what has been observed in children. While relative asymmetry equaled about 1–1.5% in this study population [26], it decreased from about 6% to 2.5% in children and adolescents from 2 to 18 years old in a study of [25]. Our lower estimates could also be the result of our use of radiographic assessments herewith excluding soft tissues. Indeed, asymmetry measurements on fetal and adult human skeletal remains were more in the order of magnitude of our observations, yet still somewhat higher (Schultz, 1926; [2,1]). Thus, it seems that the amount of asymmetry is lower prenatally, even in a population that had zero fitness and died at a very young age. We also found little evidence of large increases of FA with congenital abnormalities in spite of the fact that they were often major and even lethal. Furthermore, only a few types of abnormalities appear to affect levels of DI [26]. Interestingly, 2D:4D ratios also did not relate to the severity of congenital abnormalities [9]. Thus, these patterns suggest that the fact that we studied a population of early deceased fetuses and infants did not appear to confound patterns either. However, we do argue that human limb development may be highly stabilized because of its functional importance. Consequently, these traits may show low sensitivity to the effects of even severe developmental perturbations. Future studies should therefore also study traits with lower degrees of developmental buffering.

4.3. Concluding remarks

In conclusion, we show that there was no association between limb asymmetry and 2D:4D in prenatal fetuses and young children. In spite of the fact that a sexual dimorphism in digit ratio was observed in this group [9], the exposure to different levels of sex hormones did apparently not affect levels of DI. We suggest that this lack of association might also be attributable to the low overall levels of FA and DI in this population. On the other hand, as indicated above, different a priori expectations could have been put forward with regard to the shape of the predicted association between asymmetry and 2D:4D ratios. The patterns observed here confirm earlier conclusions that the observed associations between asymmetry and digit ratios in the literature, probably resulted from a statistical artifact and were not supported when considering other traits [24]. A literature survey in [24] concluded that if associations based on digits 2 and 4 were excluded, little evidence for an association between 2D:4D ratios and FA exists, also in traits other than limb bones. Our study confirms this conclusion that exposure to either high testosterone or estrogen levels during early development does not appear to affect levels of DI, at least not in human limb bones during early developmental stages.

Acknowledgements

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