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Association patterns between 2D:4D ratio and age at menarche: the impact of methodology

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ABSTRACT: The ratio of index finger to ring finger length (2D:4D) is a sexually dimorphic feature and widely used as an indicator of prenatal androgen-estrogen exposure. Several studies have investigated the relationship between the 2D:4D ratio and the onset of the first menstrual period (menarche) in women.
The present study tested the association patterns between 2D:4D ratio and age at menarche. Furthermore, the impact of methods of measuring finger lengths was considered.
Two samples were used to conduct the study. One sample consisted of 110 women who self-measured their finger lengths. The finger length measurements in the second sample (88 women) were taken from one trained observer using the caliper-based technique. Age at menarche was determined using a retrospective method.
Women from the first sample reported an average age for the onset of the first menstrual bleeding at 12.9 (SD = 1.4) years of age. There was no significant association between the left and right 2D:4D ratio and the age at menarche. The second sample showed a mean age at menarche at 12.8 (SD = 1.3) years of age. Here, the right hand 2D:4D ratio and age at menarche were significantly correlated ($p<0.001$).
A more feminine 2D:4D ratio was significantly associated with an earlier menarcheal age only in the sample with direct finger length measurements by a trained investigator. The sample using self-measurements yielded no significant associations between menarcheal age and 2D:4D. The use of a reliable and well-founded methodology is essential for obtaining meaningful results.

KEY WORDS: Digit ratio, age at menarche, female reproduction, prenatal hormone exposure

Introduction

According to the *"fetal origin hypothesis"*, environmental stress factors affect prenatal development and may influence health and disease patterns throughout the postnatal life span (Gluckman and Hanson 2005). Nutritional conditions apparently "program" the fetus for impaired growth, but also for the development of chronic diseases in adulthood (Rasmussen 2001). Beyond chronic stress factors and malnutrition, the exposure to androgens and estrogens during the early prenatal phase seems to be associated with an individual´s subsequent physiology, as well as with behavioral parameters, phenotype, sexuality and life history

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traits such as reaching reproductive maturity or reproductive success (Lummaa and Clutton-Brock 2002; Li et al. 2019). A widely used proxy of intrauterine sex hormone levels is the so called digit ratio. Since the 19th century it has been known that males and females differ not only in absolute finger lengths, but also in the ratio of index to ring finger length (Baker 1888). Consequently, the index to ring finger ratio (2D:4D) is interpreted as a sexually dimorphic feature. From the second prenatal trimester onwards, the index finger (2D) in males is shorter than the ring finger (4D), whereas in females these two fingers either have roughly the same length or the index finger is longer (Malas et al. 2006; Manning 2002). Accordingly, a low digit ratio (<1.0) is mainly interpreted as being typical of males, while a higher ratio (>1.0) is viewed as a female characteristic. Zheng and Cohn (2011) demonstrated the physiological basis of this sexual dimorphism using an experimental mouse model: sex hormones modulated digit development during a narrow developmental phase via androgen and estrogen receptors, which are expressed in fetal cartilaginous tissues. Moreover, the differentiation of the urogenital system and the development of the digits is controlled by the same homeobox genes (Kondo et al. 1997; Lutchmaya et al. 2004). Currently, the 2D:4D ratio is proposed as a biomarker for intrauterine testosterone and estrogen exposure by the 13th or 14th gestational week (Manning et al. 1998; Putz et al. 2004; Zheng and Cohn 2011; Gooding and Chambers 2018).

The available evidence suggests that intrauterine sex hormone levels, indicated by the 2D.4D ratio, are associated with several somatic parameters such as body composition (Pruszkowska-Przybylska et al. 2018), pigmentation (Sitek et al. 2018) and occupational choice (Koziel et al. 2018). Furthermore, several disorders such as congenital adrenal hyperplasia (Brown et al. 2002), cancer (Bunevicius 2018), lung cancer (Kasielska-Trojan et al. 2020), carpal tunnel syndrome (Kasielska-Trojan et al. 2019), autism (Schieve et al. 2018) sensation seeking behavior (Fink et al. 2006), personality as well as sociosexuality (Manning and Fink 2008) also showed relationship with 2D:4D ratio.

Furthermore, the 2D:4D ratio is related to several female life history characteristics, such as age at menarche (Helle 2010; Manning and Fink 2011; Matchock 2008; Osberg and Villamor 2012; Li et al. 2019), timing of reproduction (Velez et al. 2016), reproductive fitness (Helle 2010) and age at menopause (Kirchengast et al. 2020). A particular focus of earlier studies, was the associations between prenatal sex hormone exposure, indicated by 2D:4D, and age at menarche. However, the outcomes of these studies were inconstant. Matchock (2008), Reimers (2007), Manning and Fink (2011) and Kalichman et al. (2011) found a negative association between the 2D:4D ratio and the age at menarche, indicating that a more masculine digit ratio is associated with a delayed onset of menarche. In contrast, Osberg and Villamor (2013) stated that a low digit ratio predicts an early age at menarche among Colombian schoolgirls. Helle (2010) found no relationship between the ratio of either the right or left hands and menarcheal age, among post-reproductive Finnish women.

These inconsistent results are difficult to explain. A prevailing issue pertains to the different methods of measuring finger lengths in different studies. There are several ways to determine finger lengths.

The direct measurement using a caliper can show a high reliability and accuracy of 0.01mm, if well-trained investigators perform the task (Allaway et al. 2009; Manning et al. 1998). Some authors used hand scans, photos or even x-rays for such measurements. (Allaway et al. 2009; Berenbaum et al. 2009; Gillam et al. 2008; Hönekopp et al. 2007; Matchock 2008; Muller et al. 2012). Their results show that computer-based evaluation of scans is ideally suited for determining 2D:4D (Allaway et al. 2009). Nonetheless, Kemper and Schwerdtfeger (2009) noted that this is the most time-consuming method. In addition, the partially low resolution of the scans can lead to measurement errors. In the BBC study (Reimers 2007; Manning and Fink 2011) finger lengths were measured by the participants themselves, based on precise instruction.

The aim of the present study was to test association patterns between the digit ratio and age at menarche. In such a study protocol usually requires that finger length measurement is conducted by a trained investigator using an electronic caliper. Unfortunately, the COVID-19 pandemic and the national lockdown in Austria made the planned data collection procedure impossible. Therefore, the method of the BBC study was applied, i.e. self-measurement of finger lengths by the participants after detailed instruction. In order to compare the different methods, an available, but not yet analyzed data set of digit ratio and menarcheal age were used as a second test group.

The following hypotheses were tested:

– H1. Women with a low right 2D:4D ratio, have a late onset of menarche.
– H2. The method of measuring finger lengths influences the association patterns between the age at menarche and 2D:4D.

Material and methods

Study design

The COVID-19 pandemic and the national lockdown in Austria prohibited collecting data using face to face interviews and directly measuring body height, body weight and finger lengths by a trained investigator. Therefore, data collection of sample 1 took place via an online tool between April and May 2020. The online survey was created and conducted with the SoSci-Survey program (Leiner 2019). The online link was distributed via a snowball system using WhatsApp, an Instagram call and a notice requesting participation placed in the neighborhood. Sociodemographic data such as age, nationality, but also data concerning age at menarche, menstrual cycle patterns, body weight and body height were collected by means of an online questionnaire based on previously validated questions of the BBC study (Reimers 2007). Additionally, the anamnesis of diseases of the hands and fractures of the ring and index fingers was carried out using this questionnaire. Finger lengths were measured by the participants themselves according to the methods described in the BBC-internet study (Reimers 2007; Manning and Fink 2008). In this study, participants self-measured finger length from the fingertip to the most proximal crease using a ruler to the nearest millimeter. A diagram of the hand and detailed instructions on how to measure finger length were provided.

Finally, the unpublished data of a previous study focusing on the associations between 2D:4D and menarcheal age were analyzed. This data collection took place in 2016. Finger lengths were measured directly by a trained investigator and data

on sociodemography and menarche were collected by face to face interviews. Both data collections corresponded to the guidelines of the Helsinki declaration. Besides explaining the objectives of the study, the right to withdraw at any time was ensured.

Participants

Sample 1

110 women between the ages 17 and 50years (x = 26.9; ±6.5.) were enrolled in sample 1. All participants met the following strict inclusion criterion: no fractures of the index and/or ring finger, no injuries to the fingers, no disease affecting hands or fingers such as arthritis, gout or rheumatism. As in other studies (Caswell and Manning 2009; Manning and Fink 2008, 2011) women reporting extreme values of the 2D:4D ratio of <0.80 and >1.29 were excluded from the sample. All in all, 7 women had to be excluded from the statistics. Sample 1, therefore, consisted of 103 participants. The main nationality in sample 1 was Austrian (83.5%). 9.7% had a German nationality and 6.8% had other nationalities (Belgium, France, Iran, Italy, Hungary and New Zealand). 2D:4D did not differ significantly between Austrian participants and participants of other nationalities. Therefore, all 103 participants were included in sample 1.

Sample 2

Sample 2 comprised the data of 88 women aged 18 to 30 years (x = 25.9; ± 3.5). None of the participants reported fractures of the index and/or ring finger, injuries of the fingers, or any kind of diseases affecting hands or fingers such as arthritis, gout or rheumatism. All participants were students at the University of Vienna. Most were Austrians or of Central European origin.

Digit Ratio

Sample 1

Finger lengths were measured by the participants themselves as in the BBC study (Reimers 2007; Manning and Fink 2008). Participants were told to open their hand, stretch out their fingers, and, if possible, place their hand on a surface. Measurements were taken with a ruler from the middle of the most proximal (closest to the palm) flexor crease to the tip of the finger (Manning and Fink 2008, 2011; Reimers 2007). Supported by a precise description and a schematic illustration, the participants could observe how the measurement should be carried out.

Sample 2

The lengths of the ring and index fingers of both hands were measured twice by a trained examiner using digital Vernier calipers measuring to 0.01 mm. This eliminated interobserver variability. The mean finger length of the two measurements was calculated. While measuring, the participant's hand was lying flat on a table, with the palm facing up. Finger length was measured from the interphalangeal crease to the top of the fingertip.

Digit ratio of both samples was calculated by dividing the length of the index finger by the length of the ring finger, i.e. 2D: 4D, whereby the mean value of the two successive measurements of each finger (right & left) was used. Following previously published studies (Klimek et al. 2014, 2016), the participants were divided into two groups: low or masculine 2D:4D <1 versus high or feminine 2D:4D ≥1.

Somatometric parameters

Body weight and Body height

In sample 1, body weight and body height were self-reported by the participants. In sample 2, body height and body weight were measured by a trained investigator, where height was measured to the nearest 0.5cm using a standard anthropometer, and body weight was calculated to the nearest 0.1kg on a balance beam scale. The BMI (Body Mass Index) was calculated according to the equation: weight (in kg) / height (in m^2).

Age at menarche

Age at menarche was determined using a retrospective method in both samples. This technique relies on self-reported age at menarche. Menarcheal age was reported as a continuous variable, i.e., chronological age when the first menstrual bleeding occurred. We are aware of the limitation of retrospective methods, especially in comparison with the status quo method. Nevertheless, this method was used since the status quo method was not applicable in both samples.

Statistical analyses

Statistical analysis was carried out using IBM SPSS Statistics Version 23.0 (Microsoft corp.). In the first step, descriptive statistics (means, SDs) and Kolmogorov-Smirnov tests were calculated. Since the KS-test yielded no normal distribution for most metric variables, non-parametric Spearman rank correlations and multiple regression analyses were used to test the association patterns between 2D:4D ratio and age at menarche. Mann Whitney U-tests were performed to test group differences between participants showing a more feminine 2D:4D ratio (≥1) and those showing a more masculine 2D:4D ratio (<1). P value < 0.05 was considered significant.

Results

Sample characteristics

Sample characteristics are presented in Table 1. Samples 1 and 2 did not differ significantly at menarcheal age or body height. In sample 1 the average age at onset of the first menstrual bleeding was

Table 1. Sample description

Parameters	Sample 1 (n = 103)			Sample 2 (n = 88)			
	Mean (SD)	Median	Range	Mean (SD)	Median	Range	Sign
Age	26.2 (6.1)	25	17–50	25.9 (3.5)	25	18-30	n.s.
Age at menarche	12.9 (1.4)	13	10–18	12.8 (1.3)	12.8	9–17	n.s.
Digit ratio left	0.98 (0.04)	0.98	0.87–1.25	0.98 (0.03)	0.97	0.92–1.08	n.s.
Digit ratio right	0.98 (0.04)	0.98	0.88–1.28	0.98 (0.03)	0.98	0.92–1.06	n.s.
Body height (cm)	167.3 (6.1)	167.5	150–183	167.4 (6.1)	168	152–184	n.s.
Body weight (kg)	63.4 (10.3)	61	42–96	60.8 (7.9)	60	50–92	0.048
Body mass index (kg/m^2)	22.66 (3.40)	21.69	15.43–34.48	21.69 (2.63)	21.26	17.56–35.06	0.049

12.9 years of age (*SD* = 1.4), ranging from 10 to 18 years of age. In sample 2 the mean age at menarche was 12.8 years of age (*SD* = 1.3), ranging from 9 to 17 years of age. Body weight, body mass index and weight status differed significantly between the two samples. The participants of sample 2 were younger, and were lighter in weight.

2D:4D ratio

2D:4D ratio did not correlate significantly with chronological age. This was true of both samples. Furthermore, the 2D:4D ratio did not differ significantly between the two samples. The average 2D:4D ratio in both samples was 0.98, which can be classified as slightly masculine. In sample 1, 43.6% of the participants showed a value (right hand) ≥ 1, which can be interpreted as being typically female. Based on the left hand ratio, only 35.5% corresponded to the definition of a female digit ratio. In sample 2, only 30.7% of the right hand ratios and 17.0% of the left hand ratios could be classified as female.

2D:4D and age at menarche

As presented in Table 2, age at menarche in sample 1 did not correlate significantly with 2D:4D ratios in either the left or right hands and age at menarche. In contrast, sample 2 showed that age at menarche significantly correlated ($p<0.001$) with the 2D:4D ratios of both hands. These associations have been shown to be negative. For example,the higher or the more feminine the digit ratio, the earlier onset of menarche. These findings were corroborated by those of the multiple regression analyses. While the digit ratio was not associated significantly with age at menarche in sample 1, the 2D:4D ratio of the right hand was significantly negatively associated with menarcheal age in sample 2. Body height was not associated with menarcheal age in either sample (Table 3)

Table 2. Age at menarche and digit ratio (Spearman correlations)

	Sample 1		Sample 2	
	r	*p*-value	r	*p*-value
2D:4D right	−0.05	0.623	−0.53	<0.001
2D:4D left	0.10	0.310	−0.38	<0.001

Discussion

According to Manning et al. (2014), the ratio of the 2nd to the 4th digit (2D:4D) is fixed in a relatively narrow developmental window at the end of the first trimester of pregnancy and can be used, as well as a biomarker for the balance between fetal testosterone and fetal estrogen. Several studies have reported a significant association between this ratio and female life history parameters such as menarcheal age, number of offspring, or age at menopause (Helle 2010; Manning and Fink 2008, 2011; Matchock 2008, Muller et al. 2012; Kalichman et al. 2013; Li et al. 2019; Kirchengast et al. 2020). The results of previous studies are inconsistent regarding the association patterns

Table 3. Age at menarche and digit ratio multiple regression analyses

	Sample 1				Sample 2			
	R^2	Coeff B	Sign.	95% CI	R^2	Coeff B	Sign	95% CI
2D:4D right		2.14	0.645	−7.06–11.34		−15.49	0.014*	−27.73–3.25
2D:4D left	0.006	−3.70	0.447	−13.29–5.91	0.205	−8.52	0.117	−20.98–3.94
Body height		0.01	0.631	−0.03–0.06		0.01	0.636	−0.03–0.05

between menarcheal age and 2D:4D ratio. Some yielded a negative association, indicating that a more female digit ratio is associated with an earlier onset of reproductive capability (Reimers 2007; Matchock 2008; Manning and Fink 2011, Kalichman et al. 2011; Li et al. 2019). In contrast, Osberg and Villamor (2013) and Tabachnik et al. (2020) reported a positive association. These latter studies concluded that a more feminine digit ratio is associated with later onset menarche. Our study predicted that a more masculine digit ratio should be associated with an older age at menarche. In testing this hypothesis, we analyzed two different samples that differed markedly in their finger length measurement methodology.

Despite potential limitations (small samples sizes – 103 and 88 women, age at menarche was determined by a retrospective method (comparison of two subsamples), we achieved useful results and were also able to discuss methodological issues. The results clearly underline the importance of using a well founded method.

An interesting study result was that in both samples the majority of participants had a more masculine digit ratio (<1.00). In sample 1, only 43.6% (right hand) and 35.5% (left hand) of the participants showed a digit ratio that could be interpreted as being feminine. In sample 2, the percentage of feminine digit ratios was even lower: 30.7% for the right hand and only 17% for the left hand. A likely explanation for this tendency towards masculine digit ratios was probably due to most participants (especially in sample 2), being students of life sciences and natural sciences. Lippa (2006) reported significant associations between 2D:4D and occupational preferences in women, i.e. a masculine tendency towards a digit ratio among female natural scientists. Kainz et al (2018) found comparable masculine digit ratios among students of natural sciences.

Despite the limitations mentioned above, in sample 2 (professional measurements) we found a significant association between age at menarche and 2D:4D independent of body height. A high digit ratio, indicating higher estrogen levels during the intrauterine phase was significantly associated with an earlier age at menarche . Alternatively, a low digit ratio, indicating high prenatal androgen exposurewas significantly associated with a delayed age at menarche. Consequently, hypothesis 1 could be verified for sample 2. These findings are in accordance with those found in Reimers (2007), Matchock (2008), Manning and Fink (2011), Kalichman et al. (2011), and Li et al. (2019), but differ from those of Osberg and Villamor (2013) and Tabachnik et al. (2020).

Sample 1 (self-measurement), in contrast, yielded no significant associations between 2D.4D and menarcheal age. Hypothesis 1 could not be verified for sample 1. Consequently, in comparison to other studies (Manning and Fink 2008, 2011), that prediction could not be proved for this group. Interestingly, in studies conducted by Muller et al. (2012), Gooding et al. (2018) and Helle (2010), which had 9044, 202, and 282 participants respectively, also failed to find such an association. In their studies, finger lengths were obtained from scans and photography versus self-measurement in accordance with the BBC Internet study (Reimers 2007). We, therefore, assume an interobserver error for sample 1 in line with Caswell and Manning (2009) who noted that 2D:4D ratio calculated

from self-measured finger lengths may contain a high degree of random error. This result of sample 1, however, verifies hypothesis 2.

We are aware, that a comparison of different methods for finger length assessment should be carried out using only one sample. As explained earlier, this was not possible in the present study, as we could not take any direct measurement of the finger length due to the strict COVID-19 distance rules.. Consequently, two different samples had to be used. By comparing the two data sets and the underlying methodology, we concluded thatfinger length measurements conducted by a trained observer have much lower random errors. This finding has already been noted in Caswell and Manning (2009). Apparently, overcoming suboptimal methodologies requires a considerably larger sample size, as found in Reimers (2007) which had 255136 participants. Although, our sample 2 (professional measurements) had fewer participants (n = 88) it showed a significant association between right hand 2D:4D ratio and age at menarche.

Conclusion

This study has addressed various uncertainties related to whether the 2D:4D ratio is negatively related to age at menarche. We have showed that age at menarche was significantly negatively related to 2D:4D ratio in a sample when finger length is measured directly by a trained observer, while we found no significant association between age at menarche and 2D:4D ratio using self-measurements. We conclude that the methods of determining digit ratio may affect the results of a study.

The Authors' contribution

SS and RE designed the study, collected data, carried out statistical analyses, wrote the manuscript, SK designed the study, wrote the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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