Digit ratios do not serve as anatomical evidence of prenatal androgen exposure in clinical phenotypes of polycystic ovary syndrome

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BACKGROUND: Polycystic ovary syndrome (PCOS) is heterogeneous in its clinical presentation and four major phenotypes have been identified. The precise etiology of PCOS is unknown; however, variable exposure to prenatal androgens may be responsible for the spectrum of endocrine and metabolic disturbances characteristic of this syndrome. Since prenatal testosterone exposure is known to decrease the ratio of the second to fourth finger lengths (2D:4D), we characterized the left and right hand 2D:4D in women with clinical variants of PCOS. We hypothesized that if prenatal androgens were involved in the development of the phenotypic spectrum of PCOS, then lower 2D:4D would be differentially expressed among clinical variants of the syndrome.

METHODS: Digit ratios were determined in 98 women diagnosed with PCOS by the 2003 international consensus guidelines and in 51 women with regular menstrual cycles, no clinical or biochemical signs of hyperandrogenism and normal ovarian morphology. Women with PCOS were categorized into four clinical phenotypes (i.e. Frank, Non-PCO, Ovulatory and Mild) and 2D:4D among groups were compared by Tukey–Kramer multiple comparisons tests.

RESULTS: Left (P = 0.77) and right (P = 0.68) hand 2D:4D were similar among the four clinical phenotypes and no phenotype of PCOS demonstrated a 2D:4D that differed from controls (Left Hand, P = 0.44 and Right Hand, P = 0.75).

CONCLUSIONS: Women with PCOS do not demonstrate finger length patterns that are consistent with increased prenatal androgen exposure. These findings do not preclude a role for prenatal androgens in the development of PCOS; however, low 2D:4D are not a characteristic of PCOS.

Key words: digit ratios / polycystic ovary syndrome / prenatal androgen exposure

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder of unknown cause. As a syndrome, PCOS is defined as a collection of signs and symptoms where no single feature is considered diagnostic (Rotterdam-Group, 2004a, b). There is significant controversy over the exact clinical definition of the PCOS (Aziz et al., 2006) and the lack of a definitive clinical marker has hampered attempts to understand its precise etiology. In 2003, an international coalition of experts met to define the clinical spectrum of PCOS (Rotterdam-Group, 2004a, b). The result was a set of consensus criteria that defined PCOS as the presence of two of the following three symptoms: (i) oligo- or chronic-anovulation, (ii) clinical and/or biochemical hyperandrogenism and (iii) polycystic ovaries on ultrasonography. Assuming a broad spectrum, PCOS could therefore be manifest as four major clinical phenotypes: (i) Frank, (ii) Non-PCO, (iii) Ovulatory and (iv) Mild—based on four possible combinations of the three diagnostic markers (Norman et al., 2007). Although the validity of these phenotypes is currently being debated (Geisthovel, 2003; Aziz, 2006; Franks, 2006), in clinical research settings, careful stratification of subjects by phenotype is allowing potential variations in prevalence, etiology, degree of symptomology and health risks to be revealed in women with the variants of PCOS (Diamanti-Kandarakis and Panidis, 2007; Lam et al., 2007; Kauffman et al., 2008).
A heterogeneous clinical presentation that is strongly influenced by environmental factors suggests a variable etiology for PCOS (Diamanti-Kandarakis et al., 2006). However, a theory has emerged from studies investigating consequences of prenatal androgen exposure in primates that implicates a single etiologic factor for PCOS. Abbott et al. showed that pregnant rhesus monkeys receiving daily injections of testosterone propionate at various stages of gestation had androgenized female offspring that demonstrated variable levels of reproductive and metabolic disturbance, consistent with features of PCOS in humans [Reviewed in (Abbott et al., 2005)]. Namely, female monkeys that received androgen treatment in early gestation exhibited enlarged polycystic ovaries, hyperandrogenism, oligo-anovulation, increased basal luteinizing hormone (LH) secretion, insulin resistance, abdominal obesity and hyperlipidemia. In contrast, female monkeys that received treatment in late-gestation demonstrated many of the same reproductive and metabolic disturbances but not the changes in LH secretion or insulin sensitivity. The differential effects of androgens administered at different stages of fetal development were interpreted to mean that distinct "programming" windows existed for androgens to permanently alter future metabolic and endocrine function (Abbott et al., 2005). Although it is uncertain how mothers might expose their fetuses to increased androgens—or if increased androgens might be of fetal origin in women destined to develop PCOS—the notion of a single etiologic factor that gives rise to variable susceptibility to PCOS seems plausible.

The hypothesis that in utero androgens are implicated in the genesis of PCOS is further supported by a recent report of anatomical evidence of prenatal androgen exposure in women with PCOS (Cattrall et al., 2005). Prenatal androgens are known to influence the ratio of the second (2D) to fourth (4D) finger lengths, with men typically demonstrating lower digit ratios (2D:4D) than women (Manning et al., 1998, 2003). Evidence to support this theory in humans is primarily derived from studies reporting lower 2D:4D in children that had higher amniotic testosterone to estradiol ratios (Lutchmaya et al., 2004) and in men with a genetic propensity to produce more sensitive androgen receptors (Manning et al., 2003). There is also evidence of lower 2D:4D in females with congenital adrenal hyperplasia—a condition that causes females to produce excess amounts of androgens in utero (Brown et al., 2005; Okten et al., 2002) although this is controversial (Buck et al. 2003).

Cattral et al. measured 2D:4D in 70 women with PCOS and detected a small, but significantly lower, difference in the right hand 2D:4D of women with PCOS compared with controls (Cattrall et al., 2005). Ratio asymmetry is a common finding in studies investigating 2D:4D as an index of prenatal androgen exposure and there is some experimental evidence for the right hand being more sensitive to prenatal androgens than the left [reviewed in (McIntyre, 2006)]. Lower right-hand 2D:4D were also recently noted in healthy women reporting delayed menarche (Matchock, 2008) and this is consistent with lower 2D:4D in women with PCOS, who as a group, also experience delayed menarche (Sadrzadeh et al., 2003). Cattral et al. limited their investigation of 2D:4D to women with severe forms of PCOS which was consistent with older diagnostic criteria for PCOS. The objective of the current study was to elaborate on these previous findings by characterizing 2D:4D of women in newly recognized clinical variants of PCOS. We hypothesized that if prenatal androgen exposure was involved in the development of the phenotypic spectrum of PCOS then lower 2D:4D would be differentially expressed among women with different clinical variants.

Materials and Methods

Study subjects

There were 98 women diagnosed with PCOS, based on agreement by two clinical evaluators, enrolled in the study. Evaluation for PCOS involved: (i) clinical history to determine the extent of menstrual cycle disturbance or duration of infertility, (ii) a physical examination to assess the degree of terminal hair growth on nine regions of the body, (iii) transvaginal ultrasonography to detect the presence or absence of polycystic ovaries and (iv) a series of blood tests to assess levels of total and free androgens as well as other hormones that would exclude a diagnosis of PCOS (e.g. prolactin, 17-OH progesterone, cortisol, TSH). A group of 51 women with regular menstrual cycles, no excess terminal body hair growth, standard endocrine values and normal ovarian morphology served as controls. Subjects ranged in age from 18 to 35 and had not used hormonal contraception, fertility medications or valproate in the 3 months prior to enrollment. The ability to visualize at least one ovary by transvaginal ultrasonography was necessary for inclusion in the study. Participants were excluded if there was a history of injury or illness affecting the hands or fingers.

Definition of PCOS phenotypes

PCOS was defined by the 2003 international consensus guidelines (Rotterdam-Group, 2004a, b) of having two of three characteristics: (i) oligo- or chronic-amenorrhea [menstrual cycles <21 or >38 days (Fraser et al., 2007)], (ii) clinical and/or biochemical evidence of hyperandrogenism [modified Ferriman–Gallwey score ≥8 (Ferriman and Gallwey, 1961) as a measure of hirsutism] and/or a free androgen index ≥10 (internally validated cut-off described below), (iii) polycystic ovaries at ultrasonography [≥20 small follicles measuring 2–9 mm throughout the entire ovary (Allemand et al., 2006)]. Other etiologies of androgen excess and anovulatory infertility such as hyperprolactinemia, hypercortisolism, thyroid dysfunction and 21-hydroxylase deficiency were excluded. Women meeting the criteria for PCOS were categorized into four major clinical phenotypes: (i) Frank, defined as the presence of oligo-anovulation, hyperandrogenism and polycystic ovaries; (ii) Non-PCO, defined as the presence of oligo-anovulation and hyperandrogenism but no polycystic ovaries; (iii) Ovulatory, defined as the presence of hyperandrogenism, polycystic ovaries and regular menstrual cycles; and (iv) Mild, defined as the presence of oligo-menorrhoea and polycystic ovaries but no hyperandrogenism.

Transvaginal ultrasonography

Transvaginal ultrasonography was performed at a random time in women reporting absent or irregular menstrual cycles or during Days 2–5 of the menstrual cycle in women reporting regular menstrual cycles. Scans were performed by a single ultrasonographer using an UltraSonic RP ultrasound scanner equipped with a 9-MHz transvaginal probe (UltraSonic, Version 2.3.5, Vancouver, BC, Canada). Each ovary was scanned from the inner to outer margins in both the transverse and sagittal planes. Ultrasound scans were recorded digitally and later analyzed by a single observer for: (i) ovarian volume using the equation of a prolate spheroid (Nardo et al., 2003) and (ii) the total number of follicles ≥2 mm in the entire ovary using custom-designed medical imaging software (FRAME© and SYNERGYNE 2©, Saskatoon, SK, Canada). All subjects were followed over a 4–8 week period with periodic ultrasound scans to confirm the presence or absence of ovulation. In instances where an ovulation site
(i.e. corpus luteum) was evident on ultrasonography, a blood sample was taken to confirm post-ovulatory progesterone production.

Androgen assays
Total testosterone was measured by isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) using deuterated testosterone as an internal standard based on the method of Thienpont et al. (2008) with minor modifications. A 1 ml serum sample was mixed with the internal standard and equilibrated at room temperature. Samples were centrifuged, mixed with 1 ml phosphate buffer (100 mM, pH 6.0) and 1 ml water and purified on SPE copolymeric columns (United Chemical Technology, Bristol, PA, USA). Columns were washed with 3 ml of 10% methanol and 1 ml of hexane and eluted with 3 ml (10:1:39) isopropanol:ammonium-hydroxide:methylene chloride. The eluate was dried under nitrogen, reconstituted in 100 μl of mobile phase (45% aqueous: 55% methanol) and 60 μl were injected onto a Zorbax XDB-C18 chromatographic column. Samples were separated by gradient elution chromatography. Mobile Phase A consisted of 2 mM ammonium acetate and 0.1% formic acid in water. Mobile Phase B contained 2 mM ammonium acetate and 0.1% formic acid in methanol. The gradient consisted of 55% B to 100% B over 7.5 min. The effluent from the column was injected directly into an API-4000 tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) in electrospray positive ionization mode. Testosterone was quantified using a five point calibration curve that spanned the range of concentrations normally found in serum. Precision was determined on 10 replicates on 20 different days. The total error of the assay at three different concentrations (0.5, 5.4 and 1 nmol/l) was 7.4, 2.8 and 2.3%, respectively.

Sex hormone binding globulin (SHBG) was measured by a commercially available two-site chemiluminescent immunogenic assay and the immunoassay analyzer Immulite 2500 (Siemens Medical Solutions Diagnostics). Intra- and inter-assay coefficients of variation were both <5%. The free androgen index was calculated by dividing the total testosterone level by the SHBG level and multiplying 100. A free androgen index >10 was used to define biochemical hyperandrogenism in our study cohort based on a receiver operating curve analysis that showed 62% sensitivity and 98% specificity for discriminating between women with frank PCOS and controls.

Finger length measurements and digit ratios
Volunteers were asked to remove any jewelry or rings that would interfere with obtaining finger length measurements. Lengths of the second and fourth fingers were measured by a single experienced observer. Physical measurements were made on the ventral side of the hand by placing the bottom tip of the Vernier caliper (accurate to 0.01 mm) midline of the basal crease of the finger and extending to the tip without exerting pressure. The left index finger was measured first, followed by the left ring, right index and then right ring finger. Finger length measurements of both hands were repeated 4–5 weeks later by the same researcher. Digit ratios (2D:4D) for each hand were calculated by dividing the length of the index finger (2D) by the length of the ring finger (4D). The researcher determining 2D:4D had significant experience taking the finger length measurements using Vernier calipers. The level of inter-rater reliability associated with this researcher’s 2D:4D measurements was previously determined to range from 0.81 to 0.82 when measurements were made in the left and right hands of 60 individuals by three different investigators (Allaway et al., 2009). On the basis of an assessment of the two measurements made in the left and right hands of all 149 subjects in the current study, the level of reliability in obtaining 2D:4D (as expressed by two-way random intra-class correlation coefficient with absolute agreement) was 0.86 for the left hand and 0.83 for the right hand. For the purposes of this study, the 2D:4D reported for the left and right hand of each participant represent the mean of two measurements taken 4–5 weeks apart.

Ethical considerations
This study was approved by the University of Saskatchewan Biomedical Research Ethics Review Board. All study procedures conformed to the Canadian Tri-Council Guidelines for Human Research and International Good Clinical Practice Guidelines. Informed consent was obtained from all volunteers.

Statistical analyses
Mean left and right hand 2D:4D (± SD) were calculated for all women with PCOS, for each clinical phenotype of PCOS and for all controls. Descriptive statistics (mean ± SD) of clinical, hormonal and ultrasonographic features in women with PCOS and controls were also tabulated. Differences in 2D:4D and clinical features among groups were analyzed by t-tests and Tukey–Kramer multiple comparisons tests. Linear regression analyses were used to examine associations between 2D:4D and clinical, hormonal and ultrasonographic features of PCOS. P < 0.050 was considered statistically significant.

Results
All PCOS versus controls
Clinical, hormonal and metabolic features of the study subjects are summarized in Table I. Collectively, women with PCOS tended to be slightly older (P = 0.068), of greater body mass index (BMI; P < 0.001), and had larger waist circumferences (P < 0.001) and reported longer menstrual cycles (P < 0.001) compared with controls. Age of menarche was similar among groups (P = 0.193). Women with PCOS demonstrated higher indices of clinical and biochemical androgen excess, including greater hirsutism scores (P < 0.001) and increased levels of total testosterone (P = 0.021) and the free androgen index (P < 0.001). Transvaginal ultrasonography revealed an increased number of follicles throughout the entire ovary (P < 0.001) as well as an increased ovarian volume (P < 0.001) in

Table I Clinical, hormonal and metabolic features of women with PCOS and controls

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 98)</th>
<th>Control (n = 51)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>28.3 ± 4.5</td>
<td>26.9 ± 4.2</td>
<td>0.068</td>
</tr>
<tr>
<td>Age of menarche (year)</td>
<td>12.5 ± 1.8</td>
<td>12.7 ± 1.3</td>
<td>0.193</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.2 ± 8.4</td>
<td>24.1 ± 3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>102.5 ± 19.5</td>
<td>83.3 ± 16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Menstrual cycle length (d)</td>
<td>141 ± 99</td>
<td>29 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Modified FG score*</td>
<td>10.7 ± 6.3</td>
<td>3.3 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>3.6 ± 1.8</td>
<td>2.9 ± 0.8</td>
<td>0.021</td>
</tr>
<tr>
<td>Free androgen index (%)</td>
<td>12.3 ± 9.4</td>
<td>5.4 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total follicle count</td>
<td>43.4 ± 23.3</td>
<td>17.5 ± 6.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>9.7 ± 3.2</td>
<td>6.3 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Modified Ferriman–Gallwey score.
Digit ratios in PCOS phenotypes

Figure 1: A comparison of left and right hand digit ratios (2D:4D) in women with PCOS and controls. Mean (± SD) 2D:4D measured by physical measurements in women with PCOS (n = 98) did not differ significantly from controls (n = 51).

PCOS phenotypes versus controls

Of the 98 women diagnosed with PCOS, 65% were categorized as having Frank PCOS (n = 64), 4% as Non-PCO PCOS (n = 4), 20% as Ovulatory PCOS (n = 20) and 10% as Mild PCOS (n = 10). Clinical, hormonal and metabolic features of the study subjects in each clinical phenotype are presented in Table II. All PCOS subjects were similar in age (P = 0.310). Women with Non-PCO PCOS had a younger age at menarche (P < 0.001) whereas women with Mild PCOS had a delayed age of menarche compared with the other phenotypes (P < 0.001). Women with Mild PCOS were leaner (P = 0.001) and had smaller waist circumferences (P = 0.004) compared with the other clinical phenotypes. Women with Ovulatory PCOS had shorter menstrual cycles compared with women with Frank PCOS (P = 0.001); however, differences in menstrual cycle length compared with Non-PCO and Mild PCOS did not reach statistical significance. Lower indices of androgen excess were evident in women with Mild PCOS compared with Frank PCOS (Hirsutism scores, P < 0.001 and free androgen index P = 0.001); however, total testosterone levels did not differ among phenotypes. As expected, women with Non-PCO PCOS had lower follicle counts compared with the other clinical phenotypes (P < 0.001); however, ovarian volumes were similar among groups (P = 0.177).

Mean left and right hand 2D:4D of women with specific clinical phenotypes of PCOS and controls are shown (Fig. 2). Left hand 2D:4D were similar among the four clinical phenotypes of PCOS (Frank 0.992 ± 0.031 versus Non-PCO 0.976 ± 0.060 versus Ovulatory 0.992 ± 0.026 versus Mild 0.993 ± 0.023; P = 0.775). Right hand 2D:4D were also similar among women with Frank, Non-PCO, Ovulatory and Mild PCOS (0.984 ± 0.033 versus 0.964 ± 0.021 versus 0.984 ± 0.026 versus 0.984 ± 0.033, respectively; P = 0.685). No clinical subgroup of PCOS demonstrated a 2D:4D that differed from controls (Left Hand, P = 0.442; Right Hand, P = 0.751).

Table II: Clinical, hormonal and metabolic features of women with four clinical phenotypes of PCOS

<table>
<thead>
<tr>
<th></th>
<th>Frank (n = 64)</th>
<th>Non-PCO (n = 4)</th>
<th>Ovulatory (n = 20)</th>
<th>Mild (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>27.7 ± 4.5a</td>
<td>31.4 ± 4.4b</td>
<td>29.2 ± 4.7a</td>
<td>28.5 ± 3.9a</td>
</tr>
<tr>
<td>Age of menarche (ys)</td>
<td>12.7 ± 1.8a-c</td>
<td>10.3 ± 1.5b</td>
<td>11.7 ± 1.0b</td>
<td>13.7 ± 1.6b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.4 ± 8.3c</td>
<td>40.1 ± 5.3a</td>
<td>31.4 ± 7.1a</td>
<td>22.2 ± 4.6b</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>106.6 ± 19.6a</td>
<td>120.8 ± 13.8a</td>
<td>96.1 ± 15.1ab</td>
<td>84.2 ± 11.3b</td>
</tr>
<tr>
<td>Menstrual cycle length (d)</td>
<td>131 ± 103a</td>
<td>75 ± 12b</td>
<td>32 ± 4a</td>
<td>101 ± 11.7b</td>
</tr>
<tr>
<td>Modified FG score</td>
<td>11.8 ± 6.3a</td>
<td>12.0 ± 3.9a</td>
<td>11.2 ± 4.3a</td>
<td>2.3 ± 2.7a</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>3.8 ± 2.0a</td>
<td>2.9 ± 1.7b</td>
<td>3.0 ± 1.1a</td>
<td>3.3 ± 1.2a</td>
</tr>
<tr>
<td>Free androgen index (%)</td>
<td>15.1 ± 10.1a</td>
<td>9.9 ± 3.7b</td>
<td>7.1 ± 3.0b</td>
<td>4.3 ± 1.8b</td>
</tr>
<tr>
<td>Total follicle count</td>
<td>44.5 ± 14.6a</td>
<td>15.6 ± 3.1b</td>
<td>34.9 ± 10.1a</td>
<td>44.3 ± 12.1c</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>10.1 ± 3.4a</td>
<td>7.2 ± 3.0a</td>
<td>9.0 ± 2.7a</td>
<td>9.1 ± 2.1a</td>
</tr>
</tbody>
</table>

Significant differences for within row comparisons are denoted by different letters (P < 0.05). *Modified Ferriman–Gallwey score.
women with PCOS. Moreover, our study did not corroborate previous findings that women with PCOS demonstrate delayed menarche (Sadatrad et al., 2003) or that age of menarche is associated with lower 2D:4D (Matchock, 2008).

There are several reasons why our results may not have corroborated findings of a previous study investigating finger length patterns in women with PCOS (Cattrall et al., 2005). We used different criteria to define PCOS. Cattrall et al. used the 1990 National Institutes of Health (NIH) criteria which defined PCOS as the combined presence of: (i) oligo- or chronic-amenorrhea and (ii) clinical and/or biochemical evidence of hyperandrogenism (Zawadzki et al., 1992). It also is possible that we used different criteria to define amenorrhea, clinical hyperandrogenism and biochemical hyperandrogenemia, as these details were not reported in the earlier study. Disclosure of the criteria and methods used to determine cut-offs for PCOS symptoms is important since each clinical criterion has been criticized for being fraught with limitations and inconsistencies (Franks, 2007; Lujan et al., 2008). Our group used the 2003 Rotterdam criteria to diagnose PCOS which differ from the NIH criteria in that they include ultrasonographic evidence of polycystic ovaries as a third diagnostic marker and allow for a diagnosis based on the presence of two of three criteria. Given the broader clinical spectrum represented by the 2003 criteria, our collective sample of 98 PCOS subjects represented a more heterogeneous study population. Namely, our study population included a significant proportion of participants classified as having either Ovulatory or Mild PCOS (20 and 10% of the study population, respectively), that would not have been defined as PCOS by the NIH guidelines. Despite using the NIH criteria, Cattrall et al. reported that their clinical assessment revealed that 95% of their study subjects had polycystic ovaries. This would imply that 67 of their subjects could be categorized as having Frank PCOS and the remaining three subjects as having Non-PCO PCOS. We recruited a similar number of subjects to represent these two PCOS phenotypes; however, when we combined data for Frank and Non-PCO and compared their 2D:4D to controls, no differences were observed among groups (data not shown). Although a comparison involving combined data for Frank and Non-PCO would seem the most direct means of contrasting studies, since the ultrasonographic criteria used to define polycystic ovarian morphology were not reported by Cattrall et al., it is difficult to ascertain whether this comparison is wholly appropriate.

There may also be significant hormonal and morphological differences among the control subjects investigated by both studies. Our control population was carefully screened for all PCOS features and volunteers were excluded if they demonstrated any feature of PCOS. Cattrall et al. control population consisted of local staff members and it is difficult to know what measures were taken to exclude the presence of biochemical hyperandrogenism and polycystic ovarian morphology since these details were not reported. Methods used to assess androgen levels in women have been heavily criticized and a recent plea by the Endocrine Society has urged clinical laboratories to revaluate their methods for measuring androgens in serum (Rosner et al., 2007). We used LC/MS/MS to measure total testosterone and based diagnostic cut-offs for biochemical hyperandrogenism from receiver operating curves performed on levels assessed in carefully selected female controls and women with Frank PCOS. In addition, all control subjects underwent a transvaginal ultrasound to rule out the presence of polycystic ovaries. This parameter is

**Table III** Associations between digit ratios (2D:4D) and diagnostic signs and symptoms of PCOS

<table>
<thead>
<tr>
<th></th>
<th>Left</th>
<th>P</th>
<th>Right</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left HandR</td>
<td>0.028</td>
<td>NS</td>
<td>0.036</td>
<td>NS</td>
</tr>
<tr>
<td>Modified FG scorea</td>
<td>0.285</td>
<td>&lt;0.001</td>
<td>0.166</td>
<td>0.045</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>0.128</td>
<td>NS</td>
<td>0.150</td>
<td>NS</td>
</tr>
<tr>
<td>Free androgen index (%)</td>
<td>0.240</td>
<td>&lt;0.001</td>
<td>0.188</td>
<td>0.033</td>
</tr>
<tr>
<td>Total follicle count</td>
<td>0.109</td>
<td>NS</td>
<td>0.025</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>0.225</td>
<td>0.006</td>
<td>0.144</td>
<td>0.080</td>
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</tbody>
</table>

*aModified Ferriman–Gallwey score.*

**Discussion**

The present study was designed to evaluate potential variations in the level of in utero androgen exposure in women exhibiting four clinical phenotypes of PCOS. We hypothesized that women with severe forms of PCOS, such as Frank PCOS, would demonstrate finger length patterns that were consistent with increased prenatal androgen exposure based on a report of lower 2D:4D in the right hands of women with PCOS (Cattrall et al., 2005). In addition, we hypothesized that women with milder variants of PCOS would show 2D:4D that differed from women with more severe PCOS suggesting differential effects of prenatal androgens in women with less susceptibility for developing the full syndrome. Our findings were inconsistent with a previous report of lower 2D:4D in women with PCOS and did not support the hypothesis of increased in utero androgen exposure in
Digit ratios in PCOS phenotypes

particularly important since polycystic ovarian morphology on ultrasound has been reported to occur in up to 22% of the general population (Polson et al., 1988; Clayton et al., 1992; Farquhar et al., 1994; Botis et al., 1995; Cresswell et al., 1997; Michelmore et al., 2001) and the presence of this single feature might represent the mildest form of the syndrome (Balen and Michelmore, 2002).

Our hypothesis of lower 2D:4D in women with Frank PCOS was not supported regardless of our efforts to generate a genuine control population. In fact, control subjects tended to have lower 2D:4D than women with most PCOS phenotypes. The tendency for lower 2D:4D among controls contributed to the paradoxical positive correlations noted among 2D:4D and measures of androgen excess (i.e. hirsutism scores, free androgen index and ovarian volume). Despite our concern that previous studies might have included women with sub-clinical variants of PCOS, it is interesting to note that the mean 2D:4D and variance for our control data set for lower 2D:4D among controls contributed to the paradoxical positive correlations noted among 2D:4D and measures of androgen excess (i.e. hirsutism scores, free androgen index and ovarian volume). Despite our concern that previous studies might have included women with sub-clinical variants of PCOS, it is interesting to note that the mean 2D:4D and variance for our control data set were consistent with previous studies that did not take extensive measures to rule out subtle reproductive and metabolic dysfunction in their controls (Brown et al., 2002; Coolican and Peters, 2003; Allaway et al., 2009). The present study did use uneven sample sizes; however, similar to Cattrall et al. (2005), we ensured that we had 80% power to detect a difference of 0.019 in 2D:4D at an alpha level of 0.05 assuming a standard deviation of 0.040.

It is difficult to compare mean 2D:4D obtained in the current study with those from previous studies involving females with PCOS or congenital adrenal hyperplasia. Cattrall et al. did not report the precise mean for 2D:4D in women with PCOS. Rather, they presented their data by frequency distribution plot and showed a left-hand shift in the distribution curve for right 2D:4D ratios in women with PCOS compared with controls (Cattrall et al., 2005). Of the studies investigating 2D:4D in females with congenital adrenal hyperplasia, no study used direct measurements with Vernier calipers to measure finger lengths (Brown et al., 2002; Okten et al., 2002; Buck et al., 2003). Comparing absolute 2D:4D obtained from radiographs and photocopies to those obtained by direct measurements would be inappropriate since several studies have confirmed that ratios obtained from radiographs and photocopies are lower than those obtained from direct measurements (Buck et al., 2003; Manning et al., 2005; Burriss et al., 2007; Allaway et al., 2009; Kemper and Schwerdtfeger, 2009). To that end, the use of Vernier calipers for measurement of 2D:4D by our group and Cattrall et al. has other important ramifications for comparing findings among studies. Direct measurements with Vernier calipers are associated with lower levels of reliability when obtaining 2D:4D compared with computer-based techniques (Allaway et al., 2009; Kemper and Schwerdtfeger, 2009). Although we both reported high levels of intra-observer variability when obtaining ratios, it is important to acknowledge that the difference in right hand 2D:4D detected by Cattrall et al. was very small (i.e. 0.016 representing 98.3% of the measurement made in controls) and therefore, fell within the 1.5–2% margin of technical error associated with using Vernier calipers by experts (Voracek et al., 2007). Digital hand scans and computer-based calipers were recently associated with technical error margins approximating 1%, indicating that this method represents the most reliable option for detecting differences in 2D:4D among groups (Kemper and Schwerdtfeger, 2009). Our recent study investigating the reliability of obtaining 2D:4D using multiple techniques in men and women also suggested that less reliable measurements in women versus men might have interfered with the accuracy of the measurement (Allaway et al., 2009).

Our observation that reliability was improved when image quality was optimized using computer software—an opportunity provided by image analysis but not by direct measurements—was interpreted to mean that future studies involving 2D:4D in women would have a higher likelihood of replication if computer-assisted analyses were used over alternate methods (Allaway et al., 2009). The current controversy over anatomical evidence of prenatal androgen exposure in women with PCOS would benefit from a future reassessment of finger length patterns using digital imaging and computer-based calipers.

Although not a major objective, this study did afford us the opportunity to explore the recently reported association between 2D:4D and age of menarche (Matchock, 2008). Women with PCOS were previously reported to demonstrate a delayed age of menarche when compared with a reference cohort of women with tubal ligation (Sadrazadeh et al., 2003). We did not detect a delayed age of menarche in women with PCOS as a group, but did note a delayed age of menarche in Mild PCOS when subjects were stratified by phenotype. Again, it is important to acknowledge that criteria for PCOS and controls were likely different among studies and this may have contributed to the disparity among findings. That we detected an earlier age of menarche in obese women with Non-PCO PCOS and a delayed age of menarche in lean women with Mild PCOS, may reflect a tendency for females to progress to puberty earlier depending on adiposity rather than an effect of prenatal androgen programming. Lastly, unlike Matchock (2008) we did not detect a significant association between age of menarche and lower right 2D:4D. A major difference between our studies is that Matchock used photocopies to measured finger lengths whereas we performed direct measurements with Vernier calipers. Photocopies are associated with higher levels of reliability compared with direct measurements (Allaway et al., 2009; Kemper and Schwerdtfeger, 2009) and this may have affected our ability to detect an association between 2D:4D and age of menarche.

In summary, women with clinical variants of PCOS did not demonstrate 2D:4D consistent with increased prenatal androgen exposure. Our findings do not preclude a role for in utero androgens in the development of PCOS; however, low 2D:4D as a proxy for anatomical evidence of prenatal androgen exposure, is not a characteristic of PCOS.

Authors’ Roles

M.E.L. conceived, designed and coordinated the study, analyzed and interpreted the data, and drafted the final manuscript. T.G.B. participated in data collection and assisted in the statistical analyses and interpretation of the data. D.R.C. clinically evaluated the study volunteers and provided resources to complete the study. D.C.L. performed and provided resources to complete the endocrine assays. R.A.P. participated in the conception and design of the study and provided resources and equipment to complete the study. All authors critically evaluated the manuscript and have read and approved the final version.

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