Anthropometric biomarkers for abnormal prenatal reproductive hormone exposure in women with Mayer-Rokitansky-Küster-Hauser syndrome, polycystic ovary syndrome, and endometriosis

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Objective: To study whether markers of prenatal exposure to reproductive hormones are related to Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, polycystic ovary syndrome (PCOS), and endometriosis.

Design: Case-control study. Comparison of sex hormone-related external genital and digital characteristics in cases and controls.

Setting: University hospital.

Patient(s): We enrolled 172 women in four groups—women with MKRH, women with PCOS, women with endometriosis, and controls (43 in each group).

Intervention(s): Measurement of two anthropometric biomarkers: anogenital distance and digit ratio.

Main Outcome Measure(s): Anogenital distance was measured from the anus to the anterior clitoral surface (AGDac) and from the anus to the posterior fourchette (AGDaf). For the digit ratio we used a direct, as well as a computer-assisted graphic measurement to measure the length of the second and fourth digit.

Result(s): After adjustment for body mass index and age, AGDac was the shortest in endometriosis and the longest in PCOS groups, with a mean difference of 10 mm (95% confidence interval 3.1–16.8). AGDaf but not AGDac measures were found to be significantly larger in the MRKH group, with a mean difference compared with controls of 2.6 mm (95% confidence interval 0.1–5.2). The digit ratio was not significantly different between the groups.

Conclusion(s): In this study we did find limited evidence for androgen exposure during the development of MRKH. This is compatible with the hypothesis that the uterovaginal agenesis may have been the result of temporary prenatal exposure to altered gonadal hormone concentrations. For endometriosis and PCOS we confirm previously observed associations for anogenital distance reflecting possible estrogen-based and androgen-based intrauterine origins, respectively.

Dutch Trial Registration Number: NTR7492. (Fertil Steril® 2020; –: – –. © 2020 by American Society for Reproductive Medicine.)

Key Words: Mayer-Rokitansky-Küster-Hauser syndrome, anogenital distance, digit ratio, polycystic ovary syndrome, endometriosis

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The Mayer-Rokitansky–Küster-Hauser (MRKH) syndrome is characterized by congenital aplasia of the uterus and upper part of the vagina, resulting from embryonic underdevelopment of the Müllerian duct. The etiology of the MRKH syndrome is still largely unclear (1). In male embryos, regression of the Müllerian duct occurs in early embryonic development after exposure to antimüllerian hormone (AMH) produced by the fetal testes from the sixth week of development. In the present study we hypothesize that exposure to testes-derived hormones (e.g., AMH) may cause uterine and vaginal agenesis in MRKH syndrome. A method for the measurement of prenatal exposure to AMH is not available. However, there has been much interest in the measurement of biomarkers reflecting exposure to androgens in early gestation (2).

There is increasing evidence in animal models that the measurement of the anogenital distance (AGD) can be used as biomarker for intrauterine androgenic influence (3). Prenatal exposure to higher androgen levels results in a longer AGD, whereas lower androgen levels lead to a shorter AGD (4). In most mammals, including humans, AGD is approximately two times longer in males than in females (5). Cross-sectional studies in humans have reported an association between measures of AGD and reproductive function (6–9). In addition, the reports of a longer AGD in women with polycystic ovary syndrome (PCOS) have contributed to the idea that PCOS has an intrauterine origin and is influenced by prenatal exposure to androgens (10, 11). In women with severe endometriosis the opposite has been hypothesized, the presence of a shorter AGD possibly reflects prenatal estrogenic influence (12).

In addition, the ratio between the second and fourth digit (2D:4D ratio) is hypothesized to be an indicator of androgen exposure during fetal development (13–15). Generally, women have a higher 2D:4D ratio compared with men. A decreased 2D:4D ratio in women with PCOS reflects a possible prenatal androgenic milieu (16). However, this has not been confirmed (17).

At present, no studies have examined these anthropometric biomarkers in women with MRKH syndrome. The objective of this article is to assess the measures of the AGD and 2D:4D ratio as biomarkers of the intrauterine hormonal milieu in MRKH syndrome. By comparing the measures to control women and to women with PCOS and endometriosis, we can verify our measurement method and determine whether there is evidence for prenatal exposure to hormones in MRKH syndrome. We hypothesize that exposure to androgens results in a longer AGD and a decreased 2D:4D ratio in women with PCOS and MRKH syndrome. Women with endometriosis are hypothesized to have a shorter AGD and an increased 2D:4D ratio. This article reports on the measurement of both biomarkers in these three groups of patients when compared with control women.

**MATERIALS AND METHODS**

The study protocol has been approved by the Institutional Review Board of the Vrije Universiteit Medical Center, Amsterdam, the Netherlands. The trial was registered in the Dutch National Trial Registry (trial registration number NTR7492).

**Study Subjects**

This observational case-control study compared the measurements of the biomarkers in four groups—women with MRKH syndrome, women with PCOS, women with endometriosis and control women. Participants were included at the hospital Amsterdam UMC, location VUmc, between October 2018 and June 2019. Through recruitment in the Dutch patients’ association (‘Stichting MRK-vrouwen’) we enrolled women with MRKH syndrome for a previous study (18). The women who gave consent to contact them for participation in follow-up studies were informed about this study. Eligible patients with PCOS or with endometriosis were women attending the outpatient clinic of reproductive medicine or the Endometriosis Center Amsterdam UMC and included prevalent and newly diagnosed cases. The PCOS was diagnosed using the Rotterdam criteria, when a minimum of two of three criteria were present, as follows: oligomenorrhea or amenorrhea; polycystic ovaries; and clinical or biochemical hyperandrogenism (19). As result of the standard clinical work-up, the endocrine pathologies, such as thyroid dysfunction, congenital adrenal hyperplasia, and hyperprolactinemia, were ruled out in the PCOS group. Endometriosis was diagnosed using pelvic ultrasound, surgery, and/or magnetic resonance imaging. Only women with deep infiltrating endometriosis (infiltrating the peritoneum by 0.5 mm) and/or American Society for Reproductive Medicine grade > 3 were included. Patients with PCOS or with endometriosis were allowed to continue hormonal therapies. The control group comprised women with regular cycles who attended the in vitro fertilization (IVF) clinic for intracytoplasmic sperm injection (ICSI) treatment and no history of PCOS or endometriosis. We selected this group as controls because these women undergo a fertility treatment in our center as a result of severe male factor subfertility (total motile sperm count [VCM] < 1 × 10⁸), for which the standard treatment is ICSI. We considered this to be a group of women without any fertility disturbing factors. Exclusion criteria in all groups were age < 18 years, pregnancy, and history of vaginal delivery. For the women with PCOS, diagnosis of endometriosis was an exclusion criterion and for the women with endometriosis, diagnosis of PCOS was an exclusion criterion. All participants provided written informed consent before starting measurement of the biomarkers.

**Measurement of Biomarkers**

A stainless steel digital calliper (Digi-Met, Helios Preisser) was used to measure the AGD. The AGD measurements were performed by three trained researchers (H.P., C.L., and C.T.). For measuring AGD, the women were asked to lay down on the gynecological chair in the lithotomy position. The AGDac (anus-clitoral hood) was measured from the center of the anus to the anterior labial commissure (or anterior clitoral surface). The AGDaf (anus-fourchette) was measured from the center of the anus to the posterior fourchette (or posterior labial commissure) (6, 10) (Fig. 1). Two researchers
performed the same measurements in a subsample of 63 patients. For the 2D:4D ratio, there is currently no consensus on how to measure the digit lengths and there has been some discussion on what is the best technique (20). Therefore we used a direct (d2D:4D), as well as a computer-assisted graphic measurement (indirect measurement, i2D:4D). For the direct measurement a digital calliper, similar to the one used for AGD measurement, was used to measure the length of the second and fourth finger. We also made digital scans (200 dpi) of the hands using a Hewlett Packard scanner (HP Color Laser Jet). The left and right hand were scanned separately. The digits were measured in the scanned image using Adobe Photoshop CS6. The digit lengths were measured on the ventral surface of the hand, from the basal crease of the digit to the tip of the finger in the midline. The digit ratio was calculated by dividing the length of the second finger by the length of the fourth finger. The computer-assisted graphic measurements were performed by two researchers (C.L. and C.T.). Two researchers performed the same measurement for i2D:4D in a subsample of 30 patients. Three measurements were taken per researcher for all the measures (AGD, d2D:4D, and i2D:4D) and the average was used in analysis.

Clinical Information

The MRKH diagnosis was confirmed by contacting the general practitioner or gynecologist to retrieve the detailed information about the diagnosis. With this information and the information retrieved from the questionnaire the patients were identified as having typical MRKH syndrome (also referred to as type 1) in case of no known other malformations, and as atypical (type 2) MRKH syndrome when renal and/or skeletal malformations were present (21). When vaginal reconstruction made it possible, transvaginal ultrasound was performed for antral follicle count.

The women with PCOS, women with endometriosis, and control women underwent complete gynecological examination in our hospital according to regular care, including transvaginal ultrasound. The mean duration of the menstrual cycle without the use of hormonal contraceptives was used for analyses. Antral follicle count ≥12 on transvaginal ultrasound in one or both ovaries was classified as polycystic ovary morphology (PCOM). During standard work-up in women with PCOS, blood serum levels of testosterone, androstenedione, and free androgen index (FAI) were determined using liquid chromatography mass spectrometry (LCMS). Biochemical hyperandrogenism was defined as testosterone >2 nmol/L, androstenedione >6 nmol/L, and/or FAI >4.4.

All participants completed a questionnaire comprising questions about demographic information, handedness (right or left), serious trauma to the digits, clinical signs of hyperandrogenism, and in the case of MRKH syndrome, about vaginal reconstruction. To assess clinical hyperandrogenism we asked two questions in our questionnaire. The first question was “Do you suffer from excessive hair growth?” If yes, a simplified Ferriman Gallwey score (22) was obtained by self-assessment. Hirsutism was defined as simplified Ferriman Gallwey score of >3. The second question was “Do you or have you ever suffered from severe acne?” We defined clinical hyperandrogenism as the self-reported presence of hirsutism or severe acne.

Statistical Analysis

The primary outcome measure was the AGDac. Group sizes of 43 were selected based on power calculations...
informed by previous work on AGD in women with PCOS (10).

Normally distributed variables were compared between groups using one-way analysis of variance and not normally distributed variables using the Kruskal-Wallis test. For categorical variables the $\chi^2$ test was used. Strength of association between biomarkers and baseline variables was quantified by means of Pearson’s correlation. Given the exploratory nature of this study, the small sample sizes, as well as the a priori hypothesis, we did not perform corrections for multiple comparisons. For the digit ratio the t2D:4D of the right hand was used in correlation analyses. The paired t-test was used to compare right versus left hand and direct versus indirect measurement. By linear regression (using general linear models) we estimated the mean differences in biomarkers in the four research groups and studied the association between the biomarkers and various reproductive characteristics. We included age and body mass index (BMI) as possible confounding factors in the linear regression analyses because earlier studies reported associations with AGD (10). A P value $<.05$ was considered statistically significant. A two-way random intraclass correlation coefficient (ICC) analysis, for absolute agreement, was performed to assess intraobserver reliability and interobserver reliability for measurement of all biomarkers. For calculating the interobserver reliability, the mean of the three measurements per observer was used. Coefficients of variation were used to assess intraexaminer and interexaminer variability in AGD and digit measurements. Statistical analyses were performed using SPSS 24.0 (SPSS Inc.).

RESULTS

Study Population

A total of 172 women (43 women with MRKH syndrome, 43 women with PCOS, 43 women with endometriosis, and 43 control women) were recruited for this study. Table 1 shows the general characteristics of the participants. The median age in the study population was 35 years, with the PCOS group being significantly younger compared with the women with endometriosis ($P = .004$) and MRKH ($P = .003$). Significantly more women in the MRKH group were white compared with the endometriosis group. Other groups did not differ in ethnicity.

In the MRKH group, 74.4% was identified as having the typical MRKH syndrome. The women with atypical MRKH syndrome had a renal malformation (16.3%), a skeletal malformation (4.7%), and combined malformations (4.7%). The most commonly used method for vaginal dilation was the Frank method (using a vaginal mold as a dilator; 44.2%). A surgical method for vaginal construction was used in 25.6%. In 9.3% a functional vagina was created by natural dilation by sexual intercourse. Women who did not use any therapy for creation of a vagina amounted to 20.9%.

The women with PCOS in our study were diagnosed according to the Rotterdam criteria; 69.8% were classified as having “frank” PCOS (having all three criteria). In the other women two criteria were present—oligomenorrhea and PCOM (14.0%), biochemical and/or clinical hyperandrogenism and PCOM (9.3%), hyperandrogenism and oligomenorrhea (7.0%). Of all women with PCOS, 41.9% had biochemical hyperandrogenism (elevated serum levels of testosterone, androstenedione, and/or FAI). All women in the endometriosis group were diagnosed with severe and/or deep endometriosis; 58.1% underwent “therapeutic” endometriosis surgery. In 67.4% the presence of ovarian endometrioma was reported in medical history or current imaging diagnosis. The control group consisted of women currently undergoing ICSI treatment for severe male factor subfertility. Mean number of oocytes obtained after ovarian stimulation was 12 ($\pm5.5$). Based on the number of oocytes, 18.6% was classified as high responders (>15 oocytes).

Measurement of Biomarkers

The ICC analysis showed $>0.97$ degree of intraobserver reliability for both AGD measurements. The interobserver reliability showed an ICC of 0.98 for AGDac and 0.94 for AGDaf. Intraexaminer and interexaminer coefficient of variation for AGD was, respectively, 1% and 5% and for AGDaf, respectively, 4% and 7%. Intraexaminer and interexaminer coefficient of variation were 1% for all digit measurements. The ICC analysis showed $>0.97$ degree of interobserver reliability for digit ratio in both hands and both measurement methods and $>0.92$ degree of interobserver reliability for both hands in the indirect measurement.

The AGDac and AGDaf were positively correlated (Pearson’s correlation ($r = 0.50; P = .001$). The AGDac and AGDaf were correlated with BMI ($r = 0.52; P = .001$ and $r = 0.32; P = .001$, respectively), but not with age ($r = 0.06; P = .43$ and $r = 0.11; P = 0.17$, respectively). The AGDac and AGDaf were not correlated with any 2D:4D ratio. The 2D:4D ratio was not correlated with age ($r = 0.07; P = .39$) and BMI ($r = 0.07; P = .34$). The $r = 0.07; P = .39$. The $d2D:4D$ and $d2D:4D$ were strongly correlated for both hands (left, $r = 0.85; P = .001$; right, $r = 0.79; P = .001$).

Table 2 shows the unadjusted analysis of the anthropometric biomarker measurements in the four groups. The AGDac was significantly different between groups, with an increased AGDac in women with PCOS and a decreased AGDac in women with endometriosis (mean difference, 9.97 mm; $P = .001$). After adjusting for BMI and age, AGDac measures were still found to be different in the groups, again with post-hoc tests showing PCOS and endometriosis to differ. Overall, AGDac was the longest in the women with PCOS and a decreased AGDac in women with endometriosis (mean difference, 9.97 mm; $P = .006$).

The direct and indirect measurements for the right and the left hand. Comparing the digit ratios of the dominant hand also did not reveal any differences. The digit ratio of the direct measurement was significantly higher than of the indirect measurement for both hands (mean difference right,
0.017 ± 0.02 mm [P<.001]; mean difference left, 0.014 ± 0.018 mm [P<.001]). In the PCOS group there was a significant difference for the i2D:4D between the right and left hand (right < left) (mean difference of ratio 0.02 [P<.001]). In the MRKH, endometriosis, and control groups no differences were found comparing the right with the left hand, in indirect and direct measurement.

The relationship between reproductive characteristics with biomarker measurements, examined by linear regression analyses, is presented in Supplemental Table 1, available online. In the total study population, presence of hirsutism was associated with an increased AGDac; with the presence of hirsutism resulting in an average AGDac of 5.6 mm more (P < .01). In the MRKH, endometriosis, and control groups no differences were found comparing the right with the left hand, in indirect and direct measurement.

Overall, prenatal androgen exposure in MRKH syndrome is unclear. Embryonic Müllerian duct development is influenced by AMH, a testes-secreted hormone. We generated the hypothesis that androgens—as other testes-secreted hormones—could be of influence during the development of MRKH. In this study, we explored our hypothesis by measuring the AGDac, AGDaf, and 2D:4D ratio as biomarkers of prenatal androgen exposure. We observed longer AGDaf in women with MRKH syndrome compared with controls. None of the other biomarkers differed between the two groups.

In women with MRKH syndrome the AGDaf was significantly larger compared with the other three groups. This reveals for the first time some evidence for prenatal androgen phenotypes. In the PCOS group a marginally significant positive association was found between the presence of biochemical hyperandrogenism and AGDac. No associations were found between patient characteristics and i2D:4D ratio.

**DISCUSSION**

**TABLE 1**

General characteristics of all participating women in four groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MRKH (n = 43)</th>
<th>PCOS (n = 43)</th>
<th>Endometriosis (n = 43)</th>
<th>Controls (n = 43)</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>40.0 (29–55)</td>
<td>32.0 (29–35)</td>
<td>36.0 (32–41)</td>
<td>35.0 (33–37)</td>
<td>.001b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 (21.6–28.7)</td>
<td>26.4 (21.8–29.2)</td>
<td>23.9 (21.1–30.0)</td>
<td>23.2 (21.0–27.2)</td>
<td>.71</td>
</tr>
<tr>
<td>White</td>
<td>42 (97.7)</td>
<td>35 (81.4)</td>
<td>34 (79.1)</td>
<td>40 (93.0)</td>
<td>.02c,d</td>
</tr>
<tr>
<td>Pregnancy in medical history (%)</td>
<td>0</td>
<td>8 (18.6)</td>
<td>11 (25.6)</td>
<td>17 (39.5)</td>
<td>&lt;.001f</td>
</tr>
<tr>
<td>Live birth (by cesarean section) (%)</td>
<td>3 (7.0)</td>
<td>2 (4.7)</td>
<td>2 (4.7)</td>
<td>10 (23.3)</td>
<td>.036g</td>
</tr>
<tr>
<td>Pregnancy &lt;12 wk</td>
<td>—</td>
<td>5 (11.6)</td>
<td>23 (53.5)</td>
<td>43 (100)</td>
<td>&lt;.001b</td>
</tr>
<tr>
<td>Polycystic ovarian morphology (%)</td>
<td>1 (2.3)</td>
<td>40 (93.0)</td>
<td>0 (0.0)</td>
<td>9 (14.0)</td>
<td>&lt;.001h</td>
</tr>
<tr>
<td>Regular menstrual cycle (%)</td>
<td>—</td>
<td>2 (4.7)</td>
<td>23 (53.5)</td>
<td>43 (100)</td>
<td>&lt;.001b</td>
</tr>
<tr>
<td>Clinical hyperandrogenism</td>
<td>7 (16.3)</td>
<td>28 (65.1)</td>
<td>9 (20.9%)</td>
<td>8 (18.6)</td>
<td>.004f</td>
</tr>
<tr>
<td>Acne</td>
<td>2 (4.7)</td>
<td>15 (34.9)</td>
<td>8 (18.6)</td>
<td>7 (16.3)</td>
<td>.004f</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>5 (11.6)</td>
<td>17 (39.5)</td>
<td>2 (4.7%)</td>
<td>1 (2.3)</td>
<td>&lt;.001i</td>
</tr>
</tbody>
</table>

Note: Data are presented as median (interquartile range) or number (%), unless otherwise specified. Statistical analyses: Kruskal Wallis or χ² test. BMI = body mass index; MRKH = Mayer-Rokitansky-Kuster-Hauser syndrome; PCOS = polycystic ovary syndrome.

b Significant differences were found when comparing; bPCOS with other three groups; cPCOS with MRKH; dMRKH with endometriosis; eMRKH with other three groups; fPCOS with controls; gendometriosis with controls.


**TABLE 2**

Overview of anthropometric biomarker measurements in four patient groups.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>MRKH (n = 43)</th>
<th>PCOS (n = 43)</th>
<th>Endometriosis (n = 43)</th>
<th>Control (n = 43)</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anogenital distance (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGDac (mm)</td>
<td>108.2 ± 11.3</td>
<td>113.8 ± 16.9</td>
<td>103.9 ± 12.6</td>
<td>111.4 ± 13.7</td>
<td>.007c</td>
</tr>
<tr>
<td>AGDaf (mm)</td>
<td>24.6 ± 6.2</td>
<td>22.0 ± 5.8</td>
<td>21.9 ± 6.2</td>
<td>21.7 ± 6.2</td>
<td>.10</td>
</tr>
<tr>
<td>Digit ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d2D:4D-right</td>
<td>0.981 ± 0.027</td>
<td>0.983 ± 0.032</td>
<td>0.986 ± 0.030</td>
<td>0.980 ± 0.029</td>
<td>.76</td>
</tr>
<tr>
<td>d2D:4D-left</td>
<td>0.987 ± 0.029</td>
<td>0.987 ± 0.032</td>
<td>0.986 ± 0.035</td>
<td>0.977 ± 0.033</td>
<td>.44</td>
</tr>
<tr>
<td>i2D:4D-right</td>
<td>0.964 ± 0.025</td>
<td>0.963 ± 0.031</td>
<td>0.971 ± 0.033</td>
<td>0.965 ± 0.029</td>
<td>.59</td>
</tr>
<tr>
<td>i2D:4D-left</td>
<td>0.969 ± 0.032</td>
<td>0.979 ± 0.032</td>
<td>0.971 ± 0.032</td>
<td>0.964 ± 0.028</td>
<td>.19</td>
</tr>
</tbody>
</table>

Note: Data are presented as mean ± standard deviation. Anogenital distance (AGD) was measured from center of anus to anterior clitoral surface (AGDac) and from center of anus to posterior fourchette (AGDaf). Digit ratio was measured using direct measurement (d2D:4D) and computer assisted indirect measurement (i2D:4D). MRKH = Mayer-Rokitansky-Kuster-Hauser syndrome; PCOS = polycystic ovary syndrome.

c One woman refused AGD measurement.

d Due to severe digit trauma, data of two control women are missing for the right hand.

e One-way analysis of variance test. Post-hoc testing shows significant differences for: PCOS group with endometriosis (P = .006).

exposure in MRKH syndrome, reflecting a possible intrauterine origin related to reproductive hormonal environment. Our results suggest possible inadvertent androgen exposure during a critical window of embryonic development and by proxy could be considered as first evidence for exposure to the full range of male gonadal hormones of which AMH is the known suppressor of uterine development.

However, there are some points to be considered. AGDac is generally considered to be the strongest measure for prenatal exposure to androgens (10), although previous studies (23, 24) have also shown strong associations for AGDaf. It has been suggested that the AGDac measurement is more difficult, showing high variability in different centers (25). This measurement is likely to be more subject to inconsistency because of uncertainty for the accurate point to measure the posterior fourchette. In addition it could be possible that the AGDac is influenced by a priori changed anatomy related to MRKH or subjective to the applied vaginal dilation techniques, as this measurement includes the posterior fourchette of the vagina. In addition, in women with MRKH, the presence of hirsutism was positively associated with AGDac. In recent years, a number of cases have been presented describing failure of Müllerian duct formation combined with hyperandrogenism (26, 27). This may represent a clinical disorder distinct from the MRKH syndrome, and is associated with mutations in the WNT4 gene. Possibly some of the hirsute women in our MRKH cohort fit this profile. We do not have data on biochemical hyperandrogenism in this group. Possibly WNT4 mutations lead to a prenatal androgen environment influencing the AGD. By excluding the small proportion of the women in our MRKH cohort with clinical signs of hyperandrogenism, the difference in AGDac becomes insignificant. Therefore it may be too premature to conclude that differences in AGDac in MRKH were definitively the result of altered prenatal androgen exposure.

This study provides limited support for our general hypothesis that the Müllerian duct abnormalities with the syndrome may have originated from overexposure to AMH, along with androgens, early in pregnancy. Potentially, with the origin of MRKH, there was only minimal androgen exposure but strong AMH exposure, and/or minimal effects of androgen exposure, but strong AMH effect. This could be related to placental aromatase activity. It must also be considered that the methodology has been insufficient to trace evidence of elevated androgen exposure, for instance, as a result of very subtle hormonal influence.

By measuring the biomarkers in four patient groups, our goal was to provide a perspective for assessment of these anthropometric biomarkers. Between the four groups, the AGDac was significantly different. We showed that the AGDac was the longest in women with PCOS and the shortest in women with endometriosis. This is consistent with the concept of the AGD measurement and follows existing literature on this subject, suggesting a prenatal androgenic environment in PCOS and an estrogenic prenatal environment in endometriosis. Although the cause of endometriosis remains not yet elucidated, an intrauterine origin due to prenatal exposure to estrogens has been described as a risk factor for endometriosis (28). By confirming earlier findings that endometriosis is associated with a shorter AGD (23), we provide additional evidence with respect to intrauterine hormonal influence in the early onset development of endometriosis.

In our study cohort the AGDac was longer in women with PCOS compared with the other groups; however, we could not confirm earlier studies that show significant differences. In retrospect a power issue could be present. We performed a sample size calculation assuming a relatively large difference in AGD of 7 mm (using data from a recent study (10) comparing PCOS with controls) however more recent studies (11, 29) revealed smaller differences in AGDac. If this difference had been used in our calculations, a much larger sample size would have been necessary to detect a significant difference. Also, considering the overall study population, our control group had a relatively long AGDac. Note the difference in AGDac between women with endometriosis and controls, which is larger than presented in earlier studies (30). The AGD has recently been reported to be positively associated with ovarian response in ovarian stimulation (6). Our control group comprises 20% hyper-responders, possibly this influenced the outcome as well. Furthermore, it has been demonstrated that the AGDac was associated with the severity of the phenotypic subtypes of PCOS (11). In our cohort the

### TABLE 3

Mean differences on anogenital distance and digit ratio between various patient groups after adjustment for body mass index and age as possible confounding factors.

<table>
<thead>
<tr>
<th>Group</th>
<th>AGDac</th>
<th></th>
<th>2D:4D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean diff</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>MRKH vs. controls</td>
<td>-4.8</td>
<td>-10.0, 0.4</td>
<td>.07</td>
</tr>
<tr>
<td>PCOS vs. controls</td>
<td>0.8</td>
<td>-4.3, 5.8</td>
<td>.77</td>
</tr>
<tr>
<td>Endometriosis vs. controls</td>
<td>-9.2</td>
<td>-14.2, -4.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PCOS vs. endometriosis</td>
<td>10.0</td>
<td>4.9, 15.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MRKH vs. PCOS</td>
<td>-5.6</td>
<td>-10.9, -0.2</td>
<td>.04</td>
</tr>
<tr>
<td>MRKH vs. endometriosis</td>
<td>4.4</td>
<td>-0.7, 9.4</td>
<td>.09</td>
</tr>
</tbody>
</table>

Note: 2D:4D = digit ratio; AGD = anogenital distance; AGDac = anogenital distance from center of anus to anterior clitoral surface; AGDaf = anogenital distance from center of anus to posterior fourchette; CI = confidence interval; diff = difference.

non-PCOM phenotype showed the largest AGDac; however, this phenotype had a low prevalence (n = 3).

Another important question may be asked. Does our technique allow the measurement of hyperandrogenism? Our results show that current hyperandrogenism, represented by AGDac, is positively associated with testosterone levels in the overall study cohort. In addition, in women with PCOS, biochemical hyperandrogenism was also associated with AGDac. A positive association of testosterone levels with AGDac suggests that the biomarker AGDac could be used as a marker for prenatal androgen exposure during a specific window, so-called masculinization programming window, between 8 and 14 weeks of gestation (4).

For the digit ratio, we did not find any differences between groups and we did not observe an association between digit ratio and hyperandrogenism. Our study is unique in measuring both anthropometric biomarkers, AGD and 2D:4D ratio, in a human study population consisting of three patient groups and one control group. Two indicators for the same, namely prenatal hormonal environment, should reasonably be correlated. However, this is not the case. One report in mice studying both biomarkers, similarly did not find a correlation between AGD and digit ratios (32). Conversely, Abbot et al. (33) have reported a positive association between right hand 2D:4D ratio and anogenital distance in a non-human primate study. The current body of research of the 2D:4D ratio as biomarker for prenatal androgen exposure is controversial (34). The present study also shows that digit ratio may represent an insufficient or weak measure reflecting prenatal androgen exposure. In consideration of this, interestingly, comparing right and left hand we found a significant difference only in the PCOS group, with the right hand being shorter and thus more “androgenized.” Because it has been suggested that the right hand is more sensitive to androgens, this might be an indication for exposure to fetal androgens in PCOS (16).

There are limitations to our study. Measurement bias could have occurred and should be taken into account. For practical reasons it was not possible to blind the researchers for the gynecological status of the participant. To reduce the variability, only three researchers did all measurements. Both biomarkers show a good reliability index. It is unknown whether ovarian stimulation or other hormonal treatment affect the AGD. However, AGD has been reported to be stable during the menstrual cycle (35). There are some data that suggest a decrease in AGD in women with age (36), whereas in our study age was not associated with AGD measurements. Furthermore, information on family history, obstetric complications, and specific details on intrauterine life were unknown. Also, we may have chosen a nonoptimal control group. The presence of low-grade endometriosis is not excluded in our control group, as laparoscopic visualization, the gold standard for diagnosing endometriosis, has not been performed in these women before starting IVF and ICSI treatment (37). However, the impact of possible occult endometriosis seems to be minimal, as women in the control group seem to show a somewhat androgenized profile that could be reflected by the relatively large proportion of hyper- responders during ovarian stimulation (6). In addition, it is important to note that there are two ways of measuring the AGD, from the center of the anus—as we did—or from the upper verge of the anus, resulting in a shorter distance. When also taking into account different statures due to ethnicity, and possibly different measurement techniques, it is difficult to compare AGD results between studies.

In conclusion, the present study does suggest for the first time some evidence for prenatal exposure to androgens in MRKH syndrome. Our results together with earlier studies suggest that the prenatal reproductive hormonal environment contributes to the development of PCOS and endometriosis. In addition, we propose not to use the 2D:4D ratio as a reliable marker for prenatal androgen exposure. Further research is needed to clarify the etiology of the MRKH syndrome and study the possible prenatal influence of male gonadal hormones.

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REFERENCES


