Endometriomas and deep infiltrating endometriosis in adulthood are strongly associated with anogenital distance, a biomarker for prenatal hormonal environment

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STUDY QUESTION: Is the length of the anogenital distance (AGD), a biomarker of the in-utero prenatal hormonal environment, associated with the presence of endometriomas and deep infiltrating endometriosis (DIE)?

SUMMARY ANSWER: Shorter AGD is associated with presence of endometriomas and DIE.

WHAT IS KNOWN ALREADY: It is debated whether hormonal exposure to estrogens in utero may be a risk factor for endometriosis in adulthood. AGD is a biomarker of prenatal hormonal environment and observational studies have shown an association between AGD and reproductive parameters in both sexes.

STUDY DESIGN, SIZE, DURATION: This case–control study of 114 women with endometriosis (endometriomas and/or DIE) and 105 controls was conducted between September 2014 and May 2015.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Cases were attending the Endometriosis Unit of the Hospital. Prevalent as well as incident cases, diagnosed by transvaginal ultrasound (TVUS), were included. Controls were women without endometriosis attending the gynecological outpatient clinic for routine gynecological exams. Participants completed health questionnaires, followed physical and gynecological examinations, including TVUS. Measurements from the anterior clitoral surface to the upper verge of the anus (AGD(AF)) were obtained in all subjects. Unconditional multiple logistic regression was used to estimate the association between AGD measurements and presence of endometriomas and/or DIE while accounting for important confounders and covariates, including age, body mass index, vaginal delivery or episiotomy.

MAIN RESULTS AND THE ROLE OF CHANCE: AGD(AF) was related to presence of endometriomas and/or DIE. For all cases of endometriosis (endometriomas and DIE), women in the lowest tertile of the AGD(AF) distribution, compared with the upper tertile, were 7.6-times (95% CI 2.8–21.0; P-trend < 0.001) more likely to have endometriosis. With regard to DIE, women with AGD(AF) below the median, compared with those with AGD(AF) above the median, were 41.6-times (95% CI 3.9–438; P-value = 0.002) more likely to have endometriosis.
Limitations, reasons for caution: In case—control studies, information and selection bias has to be ruled out. Physicians conducting the measurement were blind to the status of the patients. Controls came from the same population as the cases. We adjusted for known and suspected confounders and covariates, but the possibility of residual confounding or chance findings should always be considered. As with all observational studies, causal inference is limited.

Wider implications of the findings: This study suggests that endometriosis, especially the DIE, might have a prenatal origin that may be traced back to the hormonal milieu in which the fetus develops.

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Introduction

Endometriosis is a chronic estrogen-dependent disease characterized by endometrial glands and stroma outside the uterus, often in the peritoneal cavity (Vercellini et al., 2014), with an estimated prevalence of about 10%, and affecting 4–30% of women during their reproductive years (Leibson et al., 2004; Buck Louis et al., 2011b). Women present chronic pelvic pain, dysmenorrhea, dyspareunia or infertility (Mahmood et al., 1991; Denny and Mann, 2007), that can be incapacitating and can negatively affect their quality of life (Jones et al., 2004).

Intrauterine etiology and early-life influences are one of the etiological hypotheses being investigated for endometriosis (Missmer et al., 2004; Hediger et al., 2005; Buck Louis et al., 2007, 2013; Rizner, 2009; Somigliana et al., 2011; Wolff et al., 2013; Ferrero et al., 2014; Upson et al., 2015; Vannucchi et al., 2016). Anogenital distance (AGD) is a sexually dimorphic feature, which is almost twice as long in males than in females, and a biomarker of prenatal hormonal environment (Greenham and Greenham, 1977; Kurzrock et al., 2000). In observational studies, AGD is a biomarker of prenatal exposure to endocrine disruptors (Swan et al., 2005, 2015; Bornehag et al., 2015) and androgens during the formation of the reproductive system (Dean and Sharpe, 2013; Jain and Singal, 2013). Prenatal exposure to stressful life events result in a longer (more masculine) AGD in infant girls (Barrett et al., 2013), while exposure to anti-androgens results in a shorter (more feminine) AGD in infant males (Swan et al., 2005, 2015). In adult women, AGD length is associated with female reproductive function (Mendiola et al., 2012; Mira-Escolano et al., 2014a,b; Barrett et al., 2015). We hypothesize that a prenatal estrogenic environment resulting in relatively shorter AGD will be associated with higher endometriosis risk. The aim of this study was to assess the relationship between AGD measurements, as a biomarker of intrauterine hormonal milieu, and the presence of endometriomas and deep infiltrating endometriosis (DIE) in adulthood.

Materials and Methods

Study population

This case—control study conducted from September 2014 to May 2015 at the Department of Obstetrics and Gynecology of the University Hospital ‘Virgen de la Arrixaca’ in the Murcia Region (Spain). Patients were excluded from the study if they were pregnant, or having with oncological treatment or genitourinary prolapse. The age range was 18–50 years of age. Cases (n = 114) were women attending the Endometriosis Unit of the Hospital, and included prevalent and newly diagnosed cases. Diagnoses were established by medical history, and confirmed by ultrasounds [e.g. endometrotic cyst (endometriomas) or DIE] (Eskenazi et al., 2001; Abrao et al., 2007; Hsu et al., 2010; Somigliana et al., 2010; Johnson and Hummelshoj, 2013; Vercellini et al., 2014). Deep endometriosis was defined as endometriosis infiltrating the peritoneum by >5 mm. Therefore, women with endometriosis were further classified as endometriomas (n = 82) and DIE (n = 32) using symptoms, signs and ultrasound findings. When a patient presented both types of endometriosis (DIE and endometriomas), she was classified as DIE. Controls were women without endometriosis attending the gynecological outpatient clinic for routine exams (n = 105). Nine controls reported clinical symptoms compatible with endometriosis but the diagnosis was not confirmed by transvaginal ultrasound (TVUS). Written informed consent was obtained from all subjects. This study was approved by the Ethics Research Committee of the University of Murcia.

Gynecological history and physical examination

Cases and controls were interviewed in-person by gynecologists. All participants completed health questionnaires, gynecological and obstetrical histories, and underwent a gynecological examination including TVUS at a scheduled clinical visit. A visual analog scale (0 to 10) was used to measure endometriosis-associated pelvic pain (dysmenorrhea, chronic pelvic pain, dyspareunia, dysuria and dyschezia) at the time of the exam (Vincent et al., 2010). Height and weight were measured using a digital scale (Tanita SC 330-S, London, UK). Uterine and ovarian morphology were evaluated with TVUS (Voluson E-8® and 4–9 MHz transducer; General Electric Healthcare, USA). All women having disease were diagnosed by 2D, but for DIE cases, 3D was also used (30% of cases). Two gynecologists using the same methodology performed all clinical evaluations.

Anogenital measurements

Women lay in the lithotomy position with thighs at 45° to the examination table. Using a digital caliper (Stainless Steel Digital Caliper, VWR International, LLC, West Chester, PA, USA), AGD AC was measured from the anterior clitoral surface to the upper verge of the anus, and AGD AF from the posterior fourchette to the upper verge of the anus (Fig. 1). Two gynecologists unaware of the patient’ gynecological status measured each distance three times. Average values of the six measurements were used in the analyses.
Statistical analyses

Descriptive statistics are presented using raw data. Continuous variables were compared using unpaired Student T tests, and categorical variables were compared with χ² tests. Unconditional multiple logistic regression was used to explore the association between presence of endometriosis (endometriomas and/or DIE) (yes/no) and AGD (in tertiles from the whole distribution of cases and controls, with the highest tertile as reference category) using odds ratios (OR). Age (years) and BMI (kg/m²) were included as potential confounders in the model, and vaginal delivery and episiotomy were included as covariates as they may affect AGD. Tests for trend in the ORs across exposure strata for AGD measures were calculated using logistic models that included categorical terms as continuous variables. Coefficients of variation (%CV) were used to assess intra- and inter-examiner variability in AGD measurements. All tests were two-tailed at 0.05 significance level. Analyses were conducted with IBM SPSS 20.0 (IBM Corporation, Armonk, New York, USA).

Results

For the whole population, cases were older and had had more birth deliveries than controls, but were similar for other demographic and lifestyle factors (Table I). AGDₐₓ and AGDₐᶠ had normal distributions, were correlated [Pearson correlation (r) = 0.52, P < 0.001] and had a positive association with BMI (P-values < 0.01). Intra- and inter-examiner CV were 5% and 10% for AGDₐₓ and AGDₐᶠ, respectively. Intraclass correlation coefficients were above 0.95 for both AGD measurements. Cases showed significantly shorter AGDₐₓ compared with controls.

Table II presents AGD measurements in tertiles (or by median for DIE) and ORs. AGDₐₓ, but not AGDₐᶠ, was related to presence of endometriomas and/or DIE (P-values, < 0.001 – 0.04) in the adjusted models. For all cases of endometriosis (endometriomas and DIE), women with AGDₐₓ in the lowest compared with the upper tertile were 7.6-times (95% CI 2.8 – 21.0; P-trend < 0.001) more likely to have endometriosis (Fig. 2). The strength of the association decreased when the analysis was restricted to women with endometriomas (OR 3.5, 95% CI 1.3 – 9.4; P-trend < 0.05). Women with DEI and AGDₐₓ below the median were 41.6 times (95% CI 3.9 – 438; P-value = 0.002) more likely to have endometriosis than those above the median.

Discussion

We found a strong association between AGDₐₓ and presence of endometriomas and DIE, suggesting that the intrauterine hormonal milieu during prenatal life may play an important role in the development of endometriosis.

An increased rate of endometriosis was described in women prenatally exposed to diethylstilbestrol (Missmer et al., 2004), and women who were regularly fed soy formula as infants were shown to have more than twice the risk of endometriosis compared with unexposed women (Upson et al., 2015).

It has been long argued that endometriosis, an estrogen-dependent disease, may have an intrauterine etiology (Missmer et al., 2004; Hediger et al., 2005; Buck Louis et al., 2007, 2013; Rizner, 2009). The development of the uterine endometrial gland begins in utero and is completed in puberty (Gray et al., 2001). Early hormonal signaling disruptions may result in altered morphology and function from very early on. Evidence of endometriosis has been found in the autopsies of female human fetuses at various gestational ages (Signorile et al., 2010, 2012). In our study, we were not able to measure specific exposures in utero but prenatal exposure to monobutyl phthalate in amniotic fluid has been inversely correlated with the anogenital index in female infants (Huang et al., 2009).

Few studies have explored AGD in women. A longer AGD has been related to higher ovarian follicular number (Mendiola et al., 2012) and to higher testosterone levels (Mendiola et al., 2013a). Moreover, longer AGDₐₓ in young women is associated with irregularities in their mother’s menstrual cycle before pregnancy (Mendiola et al., 2014a), suggesting that the prenatal environment may exert a long-lasting influence in the reproductive tract of female offspring. An altered female AGD may also be an important biomarker of the human ovarian dysgenesis syndrome (Buck Louis et al., 2011a).

The current study showed significant associations with the presence of endometriomas and DIE for AGDₐₓ, but not for AGDₐᶠ. There is not a clear interpretation for this difference at the moment. Similarly, almost all studies have reported associations for the short (equivalent) measurement (anus-scrotum) in males (Eisenberg et al., 2011; Mendiola et al., 2011) and females (Mira-Escolano et al., 2014b). However, it might be interesting and useful to take both measures, until a significant body of normative data has been accumulated in adults.

Selection and measurement bias has to be considered. Controls were patients attending the public hospital in the same period, and they stem from the same population from which cases emerged. Although TVUS cannot replace surgery for the diagnosis of noninvasive endometriosis (Nisenblat et al., 2016), TVUS has a relatively high values for sensitivity (93%) and specificity (96%) for endometriomas. Nonetheless, misclassification of disease status may have occurred, but, if present, it would contribute to underestimating the true magnitude of the association. AGD measures were performed by two gynecologists that were not members of the endometriosis unit and were unaware of the patient’s status.
Table I  Comparison of the general characteristics of controls and cases of endometriomas and deep infiltrating endometriosis (DIE).

| Characteristics                          | Controls (n = 105) | All endometriosis (n = 114) |  | P-value<sup>c</sup> DIE (n = 32) |  | P-value<sup>c</sup> Endometriomas (n = 82) |  | P-value<sup>c</sup> |
|------------------------------------------|-------------------|----------------------------|---|-------------------------------|---|----------------------------|---|---------------------|
|                                          | Mean              | SD                      | Median | 5–95                    | Mean | SD | Median | 5–95 | Mean | SD | Median | 5–95 | Mean | SD | Median | 5–95 |
| Age (years)                              | 29.7              | 6.0                     | 31.0   | 20.0–39.0         | 36.2 | 7.5 | 37.0   | 22.9–48.0 | 0.001 | 37.6 | 6.1 | 37.5   | 25.3–47.7 | 0.001 | 35.8 | 7.8 | 36.5   | 21.9–48.5 | 0.001 |
| Body mass index (BMI) (kg/m²)            | 23.8              | 5.4                     | 22.2   | 17.6–36.2         | 23.5 | 3.8 | 22.5   | 18.5–30.8 | 0.47  | 23.2 | 3.1 | 22.6   | 18.5–29.7 | 0.58  | 23.6 | 4.0 | 22.5   | 18.4–31.2 | 0.53  |
| Age at menarche (years)                  | 11.9              | 1.3                     | 12.0   | 9.2–14.0         | 12.2 | 1.4 | 12.0   | 10.0–14.0 | 0.16  | 12.1 | 1.9 | 12.0   | 9.0–14.0 | 0.16  | 12.2 | 1.3 | 12.0   | 10.6–14.0 | 0.16  |
| Anogenital distance (AGDAF) (mm)         | 27.3              | 5.7                     | 26.2   | 18.9–38.9        | 23.5 | 5.8 | 22.3   | 15.3–33.8 | <0.001 | 19.1 | 3.5 | 18.3   | 14.4–27.9 | <0.001 | 25.3 | 5.6 | 24.6   | 18.2–35.2 | 0.02  |
| Anogenital distance (AGDAC) (mm)         | 75.7              | 11.7                    | 73.6   | 58.1–99.4        | 73.8 | 12.1 | 72.9   | 55.6–95.6 | 0.24  | 68.9 | 12.8 | 71.3   | 44.1–90.8 | <0.01  | 75.7 | 11.4 | 75.6   | 58.3–97.5 | 0.99  |
| Percentage (%)                           |                   |                         |        |                  | 74.5 | 75.3 | 0.90   | 75.0   | 0.96  | 75.3 | 0.90             |                   |        |                   |        |                         |
| Alcohol consumption<sup>a</sup>          |                   |                         |        |                  | 39.2 | 51.5 | 0.09   | 57.1   | 0.13  | 50.0 | 0.15             |                   |        |                   |        |                         |
| Tobacco consumption<sup>b</sup>          |                   |                         |        |                  | 21.0 | 41.2 | 0.001  | 28.1   | 0.47  | 46.3 | 0.001            |                   |        |                   |        |                         |
| Have had:                                |                   |                         |        |                  | 16.2 | 10.5 | 0.22   | 3.1    | 0.07  | 13.4 | 0.60             |                   |        |                   |        |                         |
| Endometriosis surgery                    |                   |                         |        |                  | –    | 36.0 | –      | 40.6   | –     | 34.1 | –                |                   |        |                   |        |                         |
| Vaginal delivery                         |                   |                         |        |                  | 21.0 | 41.2 | 0.001  | 28.1   | 0.47  | 46.3 | 0.001            |                   |        |                   |        |                         |
| Episiotomy                               |                   |                         |        |                  | 16.2 | 10.5 | 0.22   | 3.1    | 0.07  | 13.4 | 0.60             |                   |        |                   |        |                         |
| Parity                                   |                   |                         |        |                  | 0    | 77.1 | 62.3   | 76.7   | 57.3  |            |                   |        |                   |        |                         |
|                                          |                   |                         |        |                  | 1    | 16.9 | 17.9   | 6.7    | 22.7  |            |                   |        |                   |        |                         |
|                                          |                   |                         |        |                  | 2    | 3.6  | 18.9   | 16.7   | 18.7  |            |                   |        |                   |        |                         |
|                                          |                   |                         |        |                  | 3+   | 2.4  | 0.9    | –      | 1.3   |            |                   |        |                   |        |                         |

AGDAF: Anogenital distance from the upper verge of the anus to the posterior fourchette.
AGDAC: Anogenital distance from the upper verge of the anus to the anterior clitoral surface.
SD: standard deviation; 5–95: 5th–95th percentile.
<sup>a</sup>Did you ever drink alcoholic beverages with a frequency of at least one a month?
<sup>b</sup>Have you ever smoked?
<sup>c</sup>T-student/Mann–Whitney U-test or χ² test, compared with control subjects.
AGD is an anthropometric measurement that for what is known (Thankamony et al., 2009) may be stable across the lifespan of an individual. Therefore, the status of the case, prevalent versus incident, should not affect the relationship between AGD and presence of endometriomas and/or DIE. Recently, it has been reported that AGD in adult rats displays a certain degree of plasticity, which may be mediated by modulation of local androgen/estrogen activities (Mitchell et al., 2015). However, it has also been shown that AGD is stable across the women’s menstrual cycle (Barrett et al., 2015).

In conclusion, our results provide the first evidence of a strong association between a biomarker of hormonal prenatal environment in women and the presence of endometriomas and DIE. Nevertheless, we are cautious in interpreting the results of the associations between AGD\textsubscript{AF} and DIE, due to the unstable estimates of these relationships. Our results, if

Table II  Odds ratio (OR) for cases of endometriomas and deep infiltrating endometriosis (DIE) versus controls according to tertiles of AGD measures, taking the third tertile as a reference or divided by the median (for DIE).

| AGD in tertiles (median for each tertile) | All endometriosis (n = 114) versus controls (n = 105) | Endometriomas (n = 82) versus controls (n = 105) | DIE (n = 32) versus controls (n = 105) |
|------------------------------------------|---------------------------------|--------------------------------|--------------------------------|
| **AGD\textsubscript{AF}**                |                                 |                                 |                                 |
| 3rd (31.1 mm)                            | 26 (95\% CI 1.0–3.2)            | 34 (95\% CI 0.9–3.2)            | 54 (95\% CI 2.6–10.7)           |
| 2nd (24.8 mm)                            | 34 (95\% CI 2.3–5.2)            | 38 (95\% CI 1.1–5.2)            | 19 (95\% CI <0.001)            |
| 1st (19.7 mm)                            | 54 (95\% CI 1.0–16.9)           | 19 (95\% CI 6.7–16.9)           | 19 (95\% CI <0.001)            |
| **AGD\textsubscript{AC}**                |                                 |                                 |                                 |
| 3rd (86.6 mm)                            | 37 (95\% CI 1.0–3.2)            | 36 (95\% CI 0.9–3.2)            | 41 (95\% CI 1.2–5.2)           |
| 2nd (73.6 mm)                            | 27 (95\% CI 2.1–5.2)            | 37 (95\% CI 1.3–5.2)            | 37 (95\% CI 1.8–4.0)           |
| 1st (63.3 mm)                            | 36 (95\% CI 3.7–5.1)            | 32 (95\% CI 1.6–3.8)            | 36 (95\% CI 0.9–3.2)           |

AGD\textsubscript{AF}: Anogenital distance from the upper verge of the anus to the anterior clitoral surface.

AGD\textsubscript{AC}: Anogenital distance from the upper verge of the anus to the posterior fourchette.

\( ^{a} \)Unadjusted OR.

\( ^{b} \)OR adjusted by age and BMI.

\( ^{c} \)OR adjusted by age, BMI, vaginal delivery and episiotomy.
confirmed, have important implications for endometriosis in terms of prevention, clinical practice and future research.

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Authors’ roles

A.M.T.-C., L.C.-L., J.M., M.L.S.-F. and A.N. were involved in the study conception and design. L.C.-L., R.J.-V., A.I.H.-P., S.C.-B., A.C.-B., M.T.P.-S. and M.L.S.-F. were involved in study execution and acquisition of data. L.C.-L., M.L.S.-F., M.T.P.-S., J.M., A.N. and A.M.T.-C. contributed to data analysis and interpretation. J.M., M.L.S.-F. and A.M.T.-C. drafted the manuscript. All authors provided substantial intellectual contributions and approved the final version of the manuscript.

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Conflict of interest

The authors have no competing interests to declare.

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