Maternal polycystic ovary syndrome and attention deficit hyperactivity disorder in offspring at 3 years of age: Odense Child Cohort

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Abstract

Introduction: Previous data suggested a link between maternal polycystic ovary syndrome (PCOS) and offspring attention deficit hyperactivity disorder (ADHD), which could be mediated by higher prenatal androgen exposure.

Material and methods: The study was part of the prospective Odense Child Cohort and included 1776 pregnant women, 165 (9%) with PCOS and 1607 (91%) controls. ADHD symptoms at 3 years of age were defined using the parent-reported questionnaire Child Behavior Checklist/1.5–5 (scores >90th centile of Danish national standard). Maternal blood samples were collected in the third trimester measuring total testosterone by mass spectrometry, sex hormone-binding globulin, and calculated free testosterone. Offspring anogenital distance was measured at 3 months of age. Regression models were performed with presence of ADHD symptoms as the dependent variable and adjusted for maternal age, body mass index, parity, smoking status, educational level, and parental psychiatric diagnoses.

Results: ADHD symptoms were present in 105/937 (11%) boys and 72/839 (9%) girls. In boys, maternal PCOS was positively associated with ADHD symptoms (unadjusted odds ratio [OR] 1.91, 95% CI 1.07–3.43, p = 0.03, adjusted OR 2.20, 95% CI...
Attention deficit hyperactivity disorder (ADHD) is a childhood-onset neurodevelopmental disorder defined by functional impairment due to persistent attention deficits, hyperactivity, and impulsive behavior. ADHD is the most common psychiatric childhood disorder and has a prevalence of 3.4% in the general population. The etiology of ADHD is complex and includes genetic, prenatal, and perinatal environmental factors.

Polycystic ovary syndrome (PCOS) is the most prevalent endocrinopathy in women of reproductive age, affecting 6%–20% of women. Previous studies found higher prevalence of ADHD in children born of mothers with PCOS compared with controls, but the mechanism for higher prevalence of offspring ADHD is undetermined. However, ADHD is most prevalent in boys and hyperandrogenism is an important part of the diagnostic criteria of PCOS. Testosterone influences sexual differentiation of the fetal brain and excess prenatal testosterone exposure could increase the risk of ADHD. In addition, women with PCOS are characterized by obesity and hyperinsulinemia, which can affect the fetal environment. Testosterone is lipophilic and passes from maternal to fetal circulation through the placenta; as a result maternal circulating testosterone reflects fetal testosterone exposure. However, fetal testosterone exposure from maternal circulation will also be dependent on placental function. Placental aromatase activity protects the fetus from androgen exposure, whereas placental steroidogenesis can increase fetal androgen exposure. Dysfunctional placental tissue could be associated with maternal testosterone levels, maternal insulin resistance, and blood glucose. In the Odense Child Cohort (OCC), we recently reported significantly higher third-trimester levels of total testosterone in a lean study cohort of women with PCOS compared with controls. We are not aware of studies investigating associations between maternal testosterone levels in pregnant women with PCOS and risk of ADHD in their offspring.

In the male fetus, onset of testicular biosynthesis of testosterone occurs around gestational week 9, whereas in the female fetus, the ovaries remain inactive until late in fetal development. As a result, the male fetus has a higher endogenous testosterone level after the first trimester compared with the female fetus. Anogenital distance (AGD) is a marker of prenatal androgenization. AGD is defined as the distance from the anus to the genital tubercle, and AGD is 50%–100% longer in boys compared with girls. In animal studies, testosterone injections during pregnancy were associated with longer AGD in females. Human studies showed that anti-androgens (eg, phthalates) shortened male AGD. We previously reported comparable AGD length in women with PCOS compared with controls; however, maternal testosterone was an independent positive predictor of offspring AGD in boys. These findings suggested that boys could be more susceptible to maternal testosterone exposure than girls. To our knowledge, associations between offspring AGD and risk of ADHD have not been investigated in human studies.

The aim of the present study was to determine if higher risk of ADHD in offspring born of mothers with PCOS was associated with higher third-trimester maternal testosterone concentration, and whether longer offspring AGD increased the risk of ADHD at 3 years of age.

2 | MATERIAL AND METHODS

2.1 | Study population

The present study was based on data from OCC, which is a population-based, prospective mother–child cohort. Pregnant women within the Municipality of Odense, Denmark, were recruited during 2010–2012 and included a total of 2874 pregnant women. In the present study, 56 twin pregnancies were excluded. Further, 74 women were pregnant more than once within the inclusion period, and only the first pregnancy was included in the data set. Maternal age, pre-pregnancy body mass index (BMI), and parity were collected...
from hospital charts. Maternal smoking status and educational levels were obtained from self-reported questionnaires. Parental psychiatric diagnoses before the birth of the index child were captured from national registers. Offspring sex was retrieved from birth files. Offspring AGD measurements were conducted at 3 months in 1169 children. In total, 1776 mother–child pairs were included in the study cohort. A flowchart of the study population is shown in Figure 1. Oral glucose tolerance test (OGTT) was performed according to Danish national guidelines, which included presence of risk factors for gestational diabetes mellitus (GDM): BMI ≥27 kg/m², family history of diabetes mellitus (DM), glucosuria during pregnancy, previous GDM, or previous delivery of a macrosomic child. Pregnant women with two or more risk factors or with previous GDM were offered an early diagnostic OGTT between gestational weeks 14 and 20 and again in weeks 28–30, whereas women with one risk factor were scheduled for the late OGTT only. According to Danish guidelines for antenatal care, GDM was defined by 2-h glucose ≥9.0 mmol/L and only these women received treatment for GDM. In the present cohort, 34 women were diagnosed with DM or GDM.

2.2 | PCOS status

Maternal PCOS status was defined according to the Rotterdam criteria. The diagnosis of PCOS was obtained by retrospective information, as PCOS cannot be diagnosed during pregnancy. Data on PCOS diagnosis before pregnancy were collected from an electronic questionnaire during the second trimester. As previously reported,
66% of women participating in OCC answered the questionnaire. Women were classified with PCOS if they either agreed with “has a doctor ever told you that you have PCOS?” or reported both facial hair and oligomenorrhea (menstrual cycles of ≥35 days). An ultrasound diagnosis of polycystic ovary morphology did not qualify for the diagnosis of PCOS. In questionnaire non-responders, data on PCOS status were extracted from medical records.

As previously reported, diagnosis of PCOS was obtained from questionnaires in 201/241 women with PCOS in the full data set of OCC and from medical records in the remaining 40/241 women with PCOS. Cohort staff were blinded regarding PCOS diagnosis, and PCOS status did not affect follow up during pregnancy. In the present study cohort, PCOS was diagnosed in 165 (9%) women.

### 2.3 Offspring AGD

Measurements of AGD were conducted in accordance with standardized methods. The child was placed on a horizontal surface and positioned in the frog position with legs held back and apart. The legs were placed in a 45–60° angle from the torso at the hip. A mark was made near the center of the anus. In boys, AGD was measured from the mark to the posterior base of the scrotum (AGDas) and to the cephalad insertion of the penis (AGDap). The penile width was measured at the base of the penis. In girls, AGD was measured from the mark to the posterior fourchette (AGDaf) and to the top of the clitoris (AGDac). AGD measurements were conducted with a Vernier caliper with numbers facing away from the examiner and were repeated three times without repositioning the child, and the arithmetic mean of AGD measurements was calculated. The examiners were specially trained to ensure high accuracy. A recent study published results concerning method, quality assessment, and reproducibility of AGD measurements in OCC.

### 2.4 Child Behavior Checklist for ages 1.5–5

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), defines the ADHD diagnosis. The parent-reported Child Behavior Checklist (CBCL) is a valid measure of general behavior problems, emotional aspects, and social problems in children. The preschool version of the CBCL includes 100 items and is intended for children aged 1.5–5 years. Each item describes a specific behavioral or emotional problem and is rated on a three-point Likert scale: 0 (not true), 1 (somewhat/sometimes true), and 2 (very often/often true). All item scores are summed up to a total problem score ranging from zero to 200. Further, scores are calculated for five diagnostic-oriented subscales. Using CBCL/1.5–5 it is possible to assess early signs of psychopathology, including ADHD symptoms. A DSM-oriented ADHD subscale consists of six items. The normal distribution of CBCL/1.5–5 ADHD scores from Danish National normal scores determines cut-off to the 90th centile consistent to a validity of six or more points. An ADHD score above the 90th centile is associated with later ADHD diagnosis. ADHD is recognized as a spectrum disorder and children rarely fulfill complete diagnostic criteria at this early age. Therefore, diagnostic ADHD symptoms from the 3 years questionnaire data were used in the present study. CBCL/1.5–5 assessments were available in 1779 children at the age of 3 years, which excluded 965 children with missing CBCL/1.5–5 data.

### 2.5 Serum testosterone

Maternal testosterone levels were measured in the morning, fasting venous serum samples taken around weeks 27–28 of pregnancy. Extracted full blood was permitted to clot for at least 30 min at room temperature and was subsequently centrifuged for 10 min at 2000g at 20°C within 2 h after sampling. Serum samples were stored at −80°C until analysis. Serum samples were analyzed for total testosterone using liquid chromatography-tandem mass spectrometry calibrated by intern-prepared calibrators and the relative standard deviation (SD) was less than 10%. Quality was ensured by monthly involvement in the external quality control program for steroid hormones from The United Kingdom National External Quality Assessment Service. Sex hormone-binding globulin was established on serum samples by a Roche assay on Cobas e602 (Roche) with an accuracy of 1.8%–4.0% (14.9–21.9 nmol/L). Free testosterone levels were calculated using the Vermeulen equation assuming a plasma albumin concentration of 42 g/L. Following enrollment, third-trimester blood samples for androgen status were available in 1084 women. Three women had high total testosterone levels (14.6, 14.9, and 25.7 nmol/L) and were excluded from the study cohort, because they were considered outliers. The cause of their high testosterone was not found by review of the analytical method or patient records.

### 2.6 Statistical analyses

Descriptive data were presented as numbers (%), mean and SD for normally distributed continuous variables, median (95% CI) for non-normally distributed continuous variables, and percentage for categorical variables. Characteristics of included women were compared according to offspring ADHD-related behavior (yes/no) by using unpaired t tests for continuous variables and chi-squared tests for categorical variables.

Maternal testosterone was processed as continuous and categorical variables were divided into quartiles. Division of maternal testosterone into quartiles allowed for examination of a possible U-shaped association between maternal testosterone and offspring ADHD. The reference group for testosterone was chosen as the first quartile (lowest). Data for boys and girls were presented separately.

Logistic regression was performed with the presence of ADHD symptoms divided at the 90th centile as the dependent variable and maternal testosterone, PCOS diagnosis, or AGD as predicting variables. In continuous analyses, data of total and free testosterone were
normalized by natural logarithm (ln) transformation. In analyses, where total and free testosterone were entered as quartiles, results were expressed as odds ratios (OR) with 95% CI. Confounders and intermediate factors were identified based on a priori review of published literature and using a directed acyclic graph (Figure 2). Maternal age, parity, BMI, and parental psychiatric diagnoses were considered as confounders of the relation between maternal PCOS and offspring ADHD-related behavior. Accordingly, all regression models were adjusted for maternal age, BMI, parity, smoking status, educational level, and parental psychiatric diagnoses. Prenatal testosterone and AGD were considered as potential mediators of the associations between maternal PCOS and offspring ADHD-related behavior. As a result of binary outcome, the KHB method (developed by Karlson, Holm, and Breen) was applied to conduct formal mediation analyses for the following mediators: maternal third-trimester testosterone level and offspring AGD. Confounders of the mediator and outcome pathways were identified using directed acyclic graphs (Figures S1 and S2). Hence, fertility treatment was included as a confounder in the analyses investigating maternal third-trimester testosterone levels as a mediator, additionally maternal testosterone levels at third-trimester, pre-eclampsia, and eclampsia were included as confounders in the mediation analyses for AGD. As a result of the collinearity in the subsample of female offspring, maternal testosterone and fertility treatment were excluded in the mediation analyses regarding AGDaf and AGDas.

Three sensitivity analyses were conducted. First, as psychiatric diagnosis of the parents could increase the risk of offspring ADHD, we performed a sensitivity analysis in which we excluded offspring born of parents with psychiatric disorders. Second, as maternal DM could affect placental function and pregnancy outcome, we performed a sensitivity analysis in which we omitted women with early DM before the third trimester and GDM. Finally, because of a high percentage of missing values of the outcome variable (35%), sensitivity analyses of Tables 3–6 were conducted using inverse probability weighting. The weights were based on the following covariates associated with missing outcomes: maternal BMI, maternal age, maternal education, and parity.

FIGURE 2 Directed acyclic graph showing correlations between exposure (maternal polycystic ovary syndrome [PCOS] status) and outcome (offspring attention deficit hyperactivity disorder [ADHD] symptoms). BMI, body mass index. Model drawn according to www.dagity.net. Red lines: biasing path, green lines: causal path, red circles: ancestor of exposure and outcome, blue circles: ancestor of outcome.
Model assumptions were assessed using deviance residual plots. A two-sided \( p \) value less than 0.05 was considered statistically significant. All data were analyzed using STATA/IC, version 16.0 (StataCorp).

2.7 | Ethical approval

All participants gave written informed consent to participate. The study was carried out in accordance with the Helsinki Declaration II and approved by the Regional Ethical Review Committee on November 23, 2009 (Project ID S-20090130) and the Danish Data Protection Agency (j.no 18/15692). The present study was a separate sub-study of OCC, and permission rights were obtained from the Danish Data Protection Agency on April 22, 2020.

3 | RESULTS

3.1 | Baseline characteristics

Mothers had a mean age of 30 years (SD ± 4.4 years), 56% were nulliparous, and 53% were pregnant with a boy (Table 1). ADHD-related behavior was 11% (105/937) in boys and 9% (72/839) in girls. Presence of ADHD-related behavior was associated with low maternal age, nulliparity, maternal smoking, low maternal educational level, and parental psychiatric diagnoses. In boys, presence of ADHD symptoms was associated with a maternal diagnosis of PCOS (\( n = 16 \) [15%] vs \( n = 72 \) [9%], \( p = 0.028 \)). Characteristics of PCOS vs controls are presented in Table 2. Women with PCOS had higher levels of total testosterone and free testosterone compared with controls, whereas maternal pre-pregnancy BMI and age were comparable.

3.2 | Associations between ADHD-related behavior and maternal PCOS, maternal testosterone, and offspring AGD

Boys

The risk of ADHD symptoms was significantly higher in boys born of mothers with PCOS compared with mothers without PCOS (unadjusted OR = 1.91, \( p = 0.03 \), adjusted OR = 2.20, \( p = 0.01 \), Table 3). Maternal total testosterone, free testosterone levels or AGD were not associated with ADHD symptoms (all \( p > 0.2 \), Tables 4–6).

Girls

Maternal PCOS was not significantly associated with ADHD symptoms. Maternal total testosterone was negatively associated with ADHD symptoms, which reached significance when total testosterone was entered as a continuous variable (adjusted OR = 0.44, \( p = 0.03 \), Table 4) and for the two highest quartiles of maternal testosterone compared with the lowest quartile (adjusted third quartile OR = 0.22, \( p = 0.009 \), adjusted fourth quartile OR = 0.33, \( p = 0.03 \), Table 4). Additionally, maternal free testosterone in the third quartile was negatively associated with ADHD symptoms compared with the first quartile (unadjusted OR = 0.31, \( p = 0.026 \), adjusted OR = 0.21, \( p = 0.007 \), Table 5). AGD was not associated with ADHD-related behavior (Table 6).

3.3 | Mediation analyses

We found no indirect effect, and hence no mediation, of either maternal testosterone levels measured at third trimester or offspring AGD in the association between maternal PCOS and child ADHD symptoms (all \( p ≥ 0.15 \)).

3.4 | Sensitivity analyses

Psychiatric disorders of parents

Excluding the offspring born of parents with psychiatric disorders resulted in a non-significant association between maternal PCOS and ADHD-related behavior in boys (unadjusted OR = 1.76, \( p = 0.109 \), adjusted OR = 1.85, \( p = 0.088 \)). In girls, maternal PCOS tended to be negatively associated with ADHD symptoms (unadjusted OR = 0.16, \( p = 0.073 \), adjusted OR = 0.17, \( p = 0.083 \)). Removing psychiatrically disposed offspring from the study population did not change the results on the associations between maternal testosterone and offspring ADHD-related behavior.

Missing CBCL/1.5–5 data

The sensitivity analyses using inverse probability weighting to address missing CBCL/1.5–5 data gave similar results to the unweighted analyses and did not change any significant results.

Early DM and GDM

Excluding women with early diagnosed DM and GDM from the data set did not change significant results.

4 | DISCUSSION

Our main study finding was that maternal PCOS was associated with a higher risk of offspring ADHD in boys. The lack of association between maternal third-trimester testosterone levels, offspring AGD, and risk of ADHD suggested that factors other than these markers...
| TABLE 1 | Maternal clinical and biochemical characteristics, parental psychiatric diagnoses and CBCL/1.5–5 ADHD problem scores in the offspring |
|---------|----------------------------------------------------------------------------------|
| Maternal |                                                                                   |
| BMI (kg/m²) | 23.3 [21.2; 26.3] | 23.3 [21.2; 26.3] | 23.4 [21.0; 26.7] | 0.46 | 23.5 [21.3; 26.7] | 23.5 [21.3; 26.7] | 23.7 [20.8; 28.1] | 0.71 | 23.1 [21.1; 26.1] | 23.1 [21.1; 26.1] | 23.4 [21.3; 26.6] | 0.54 |
| Age (years) | 30.0 ± 4.4 | 31.0 ± 4.4 | 29.0 ± 4.4 | <0.001 | 30.5 ± 4.5 | 30.7 ± 4.5 | 28.5 ± 4.4 | <0.001 | 30.4 ± 4.3 | 30.5 ± 4.2 | 29.1 ± 4.5 | 0.008 |
| Parity |                                                                                   |
| Nulliparous | 994 (56%) | 882 (55%) | 112 (63%) | 0.039 | 448 (54%) | 67 (64%) | 0.05 | 479 (57%) | 434 (57%) | 45 (63%) | 0.33 |
| Primiparous + multiparous | 782 (44%) | 717 (45%) | 65 (37%) | 422 (45%) | 384 (46%) | 38 (36%) | 0.33 |
| Smoking (yes) | 81 (5%) | 67 (4%) | 14 (8%) | 0.02 | 43 (5%) | 36 (4%) | 7 (7%) | 0.27 | 38 (5%) | 31 (4%) | 7 (10%) | 0.024 |
| TT (nmol/L) | 2.0 [1.4; 2.8] | 2.0 [1.4; 2.8] | 1.9 [1.3; 2.8] | 0.73 | 2.0 [1.4; 2.7] | 2.1 [1.5; 3.0] | 0.36 | 1.9 [1.5; 2.9] | 2.0 [1.5; 2.9] | 1.7 [1.3; 2.8] | 0.099 |
| FT (nmol/L) | 0.004 [0.003; 0.006] | 0.004 [0.003; 0.006] | 0.004 [0.003; 0.006] | 0.68 | 0.004 [0.003; 0.006] | 0.004 [0.003; 0.006] | 0.59 | 0.004 [0.003; 0.006] | 0.004 [0.003; 0.006] | 0.003 [0.003; 0.006] | 0.18 |
| SHBG (nmol/L) | 474.9 ± 113.0 | 474.4 ± 111.7 | 479.6 ± 125.9 | 0.66 | 470.6 ± 114.0 | 469.2 ± 111.9 | 482.5 ± 131.2 | 0.40 | 479.5 ± 111.8 | 479.8 ± 111.3 | 475.3 ± 119.4 | 0.80 |
| PCOS (yes) | 165 (9%) | 146 (9%) | 19 (11%) | 0.48 | 88 (9.4%) | 72 (9%) | 16 (15%) | 0.028 | 77 (9.2%) | 74 (10%) | 3 (4%) | 0.12 |
| Maternal education level |                                                                                   |
| Lower | 478 (28%) | 416 (27%) | 62 (37%) | 0.01 | 260 (28.3%) | 221 (27%) | 39 (39%) | 0.039 | 218 (26.6%) | 195 (26%) | 23 (33%) | 0.32 |
| Intermediate | 888 (51%) | 809 (52%) | 79 (47%) | 266 (51.5%) | 429 (52%) | 45 (45%) | 414 (50.5%) | 380 (51%) | 34 (49%) |
| Higher | 373 (21%) | 345 (22%) | 28 (17%) | 186 (20.2%) | 170 (21%) | 16 (16%) | 187 (22.8%) | 175 (23%) | 12 (17%) |
| Parental psychiatric diagnoses |                                                                                   |
| None | 1491 (84%) | 1353 (85%) | 138 (78%) | 0.022 | 782 (83.4%) | 701 (84%) | 81 (77%) | 0.065 | 709 (84.5%) | 652 (85%) | 57 (79%) | 0.19 |
| Disposition from parents (≥1) | 285 (16%) | 246 (15%) | 39 (22%) | 155 (16.6%) | 131 (16%) | 24 (23%) | 130 (15.5%) | 115 (15%) | 15 (21%) |

Data presented as mean ± standard deviation for normally distributed continuous variables, median [confidence intervals] for non-normally distributed continuous variables, and n (%) for categorical variables. p values for differences between offspring ADHD scores split by the 90th centile tested using t test for continuous variables and chi-squared test for categorical variables.

Abbreviations: ADHD, attention deficit hyperactivity disorder; BMI, body mass index; CBCL/1.5–5, Child Behavior Checklist/1.5–5; FT, free testosterone; higher, high school +>4 years; intermediate, high school +1–3 years; lower, high school or less; n, number of individuals; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; TT, total testosterone.
of prenatal androgenization mediated higher risk of ADHD in boys born of mothers with PCOS (Figure 3). In accordance, we found no evidence of mediation of either maternal testosterone levels measured at third trimester or offspring AGD in the association between maternal PCOS and child ADHD symptoms.

We are not aware of previous human studies on associations between maternal testosterone levels, offspring AGD, and offspring ADHD-related behavior. One animal study used spontaneously hypertensive rats as a model of ADHD, and reported that postnatal androgen treatment predisposed newborn male rats to develop ADHD-like behavior. Furthermore, injections of testosterone propionate in rats the first week after birth resulted in deterioration of neurodevelopment with impaired spatial abilities. In the present study, AGD was used as marker of prenatal androgenization. Bivariate associations and multiple regression analyses regarding maternal testosterone levels and offspring AGD in OCC have been presented previously. Maternal testosterone levels (total and free testosterone) were positively associated with AGDas and AGDap in boys, whereas AGD measures in girls were not associated with maternal testosterone levels.

In the present study, third-trimester testosterone levels were not associated with risk of offspring ADHD. Other human studies found that low offspring 2D:4D finger length ratio was associated with higher risk of ADHD, but this was not confirmed by other studies. The

| TABLE 2 | Maternal clinical and biochemical characteristics and parental psychiatric diagnoses in women with PCOS vs controls |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Total (n = 1776) | PCOS (n = 165) | Controls (n = 1607) | p value |
| Maternal BMI (kg/m²) | 23.3 [21.2; 26.3] | 23.7 [21.3; 27.5] | 23.3 [21.2; 26.3] | 0.16 |
| Age (years) | 30.0 ± 4.4 | 30.8 ± 4.1 | 30.4 ± 4.4 | 0.29 |
| Parity | | | | |
| Nulliparous | 994 (56%) | 90 (55%) | 903 (56%) | 0.68 |
| Primiparous + multiparous | 782 (44%) | 75 (45%) | 704 (44%) | 0.55 |
| Smoking (yes) | 81 (5%) | 6 (4%) | 75 (5%) | <0.001 |
| TT (nmol/L) | 2.0 [1.4; 2.8] | 2.4 [1.7; 3.4] | 1.9 [1.4; 2.7] | <0.001 |
| FT (nmol/L) | 0.004 [0.003; 0.006] | 0.005 [0.004; 0.008] | 0.004 [0.003; 0.006] | <0.001 |
| SHBG (nmol/L) | 474.9 ± 113.0 | 440.8 ± 106.1 | 478.5 ± 113.2 | 0.001 |
| Maternal education level | | | | |
| Lower | 478 (28%) | 42 (26%) | 434 (28%) | 0.88 |
| Intermediate | 888 (51%) | 85 (52%) | 801 (51%) | 0.05 |
| Higher | 373 (21%) | 36 (22%) | 337 (21%) | 0.55 |
| Parental psychiatric diagnoses | | | | |
| None | 1491 (84%) | 135 (82%) | 1352 (84%) | 0.44 |
| Disposition from parents (≥1) | 285 (16%) | 30 (18%) | 255 (16%) | 0.55 |

Data presented as mean ± standard deviation for normally distributed continuous variables, median [confidence intervals] for non-normally distributed continuous variables, and n (%) for categorical variables. p values for differences between maternal PCOS vs controls tested using t-test for continuous variables and chi-squared test for categorical variables. Abbreviations: BMI, body mass index; FT, free testosterone; n, number of individuals; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; TT, total testosterone.

| TABLE 3 | Unadjusted and adjusted odds ratio and 95% confidence intervals from logistic regression of CBCL/1.5–5 ADHD symptoms above the 90th centile in boys and girls according to maternal PCOS status |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Boys | Girls | | |
| | Odds ratio unadjusted (n = 933) | p value | Odds ratio adjusted (n = 916) | p value | Odds ratio unadjusted (n = 839) | p value | Odds ratio adjusted (n = 818) | p value |
| PCOS (no) | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. |
| PCOS (yes) | 1.91 [1.07; 3.43] | 0.03 | 2.20 [1.20; 4.02] | 0.01 | 0.41 [0.13; 1.33] | 0.14 | 0.43 [0.13; 1.42] | 0.17 |

Odds ratios with 95% confidence intervals for the binary variable PCOS (yes vs. no). Adjusted for maternal age, body mass index, parity, smoking status, educational level, and parental psychiatric diagnoses. Abbreviations: ADHD, attention deficit hyperactivity disorder; CBCL/1.5–5, Child Behavior Checklist/1.5–5; n, number of individuals; PCOS, polycystic ovary syndrome; Ref., reference.
### TABLE 4 Associations between maternal total testosterone concentrations (nmol/L) and CBCL/1.5–5 ADHD problem scores in boys and girls

| TT (nmol/L) | Boys | | Girls |
|-------------|------|----------|-------|
|              | Odds ratio | Odds ratio | Odds ratio |
|              | unadjusted | adjusted | unadjusted | adjusted |
| Continuous   | 1.33 [0.79; 2.22] | 0.28 | 0.98 [0.54; 1.78] | 0.94 |
| 1st quartile (≤1.40) | Ref. | Ref. | Ref. | Ref. |
| 2nd quartile (1.40 < 1.99) | 1.29 [0.58; 2.85] | 0.54 | 0.80 [0.34; 1.89] | 0.61 |
| 3rd quartile (1.99 < 2.77) | 1.20 [0.55; 2.58] | 0.65 | 0.68 [0.29; 1.58] | 0.37 |
| 4th quartile (>2.77) | 1.44 [0.67; 3.13] | 0.35 | 0.84 [0.36; 1.96] | 0.68 |

TT entered as continuous variable (top panel): TT was ln-transformed, and results presented as geometric mean (mean − SD; mean + SD). TT entered as quartiles (lower panel). Data presented as odds ratio with 95% confidence intervals for ADHD symptoms in the 2nd, 3rd and 4th quartile compared to the 1st quartile. Adjusted for maternal age, body mass index, parity, smoking status, educational level, and parental psychiatric diagnoses. Abbreviations: ADHD, attention deficit hyperactivity disorder; CBCL/1.5–5, Child Behavior Checklist/1.5–5; n, number of individuals; Ref., reference; TT, total testosterone.

### TABLE 5 Associations between maternal free testosterone concentrations (nmol/L) and CBCL/1.5–5 ADHD problem scores in boys and girls

| FT (nmol/L) | Boys | | Girls |
|-------------|------|----------|-------|
|              | Odds ratio | Odds ratio | Odds ratio |
|              | unadjusted | adjusted | unadjusted | adjusted |
| Continuous   | 1.22 [0.75; 2.01] | 0.42 | 0.91 [0.51; 1.62] | 0.74 |
| 1st quartile (≤0.0029) | Ref. | Ref. | Ref. | Ref. |
| 2nd quartile (0.0029 < 0.0041) | 1.60 [0.73; 3.49] | 0.24 | 1.03 [0.44; 2.40] | 0.94 |
| 3rd quartile (0.0041 < 0.0057) | 1.35 [0.60; 3.06] | 0.47 | 0.73 [0.30; 1.79] | 0.49 |
| 4th quartile (>0.0057) | 1.22 [0.53; 2.80] | 0.64 | 0.72 [0.29; 1.76] | 0.47 |

FT entered as continuous variable (top panel): FT was ln-transformed, and results presented as geometric mean (mean − SD; mean + SD). FT entered as quartiles (lower panel). Data presented as odds ratio with 95% confidence intervals for ADHD symptoms in the 2nd, 3rd and 4th quartile compared to 1st quartile. Adjusted for maternal age, body mass index, parity, smoking status, educational level, and parental psychiatric diagnoses. Abbreviations: ADHD, attention deficit hyperactivity disorder; CBCL/1.5–5, Child Behavior Checklist/1.5–5; FT, free testosterone; n, number of individuals; Ref., reference.

### TABLE 6 Associations between AGD and CBCL/1.5–5 ADHD problem scores in boys and girls

| AGD | Boys | | Girls |
|-----|------|----------|-------|
|     | Odds ratio | Odds ratio | Odds ratio |
|     | unadjusted | adjusted | unadjusted | adjusted |
| AGDAs | 1.03 [0.98; 1.07] | 0.25 | 1.02 [0.97; 1.07] | 0.47 |
| AGDas | 1.02 [0.98; 1.06] | 0.26 | 1.02 [0.98; 1.06] | 0.36 |
| Penile width | 0.93 [0.76; 1.15] | 0.52 | 0.88 [0.71; 1.10] | 0.26 |

Odds ratios with 95% confidence intervals for the continuous variable AGD. Adjusted for maternal age, body mass index, parity, smoking status, educational level, and parental psychiatric diagnoses.

Abbreviations: ac, anus to clitoris; ADHD, attention deficit hyperactivity disorder; af, anus to posterior fourchette; AGD, anogenital distance; ap, anus to penis; as, anus to scrotum; CBCL/1.5–5, Child Behavior Checklist/1.5–5; n, number of individuals.
reliability of low offspring 2D:4D finger length ratio as a marker of higher prenatal testosterone exposure is questionable.\(^{36}\) During a normal pregnancy, maternal circulating levels of total testosterone increase during the first trimester and are further elevated in the second and third trimesters because of increased estrogen production leading to higher sex hormone-binding globulin levels, however concentrations of free testosterone are elevated during the third trimester.\(^{11}\) Maternal sites of androgen synthesis involve ovaries, adrenal glands, and placental tissue.\(^{11}\) As recently reported, third-trimester levels of testosterone were higher in mothers with PCOS, which could suggest persistent ovarian testosterone production during pregnancy.\(^{15}\) Aromatase within the placenta converts maternal androgens to estrogen and partially protects the fetus from androgen exposure.\(^{11}\) Maternal levels of testosterone are similar in women pregnant with boys and girls, which indicates that testosterone does not pass through the placenta from the fetus to the mother.\(^{11}\) Interestingly, placental tissue in women with PCOS showed increased steroidogenesis and higher capacity for androgen production.\(^{12}\) In human and animal studies, prenatal testosterone exposure\(^{13,17,37}\) resulted in placental insufficiency\(^{13,37}\) and intrauterine growth restriction\(^{17}\) of the offspring. The present study cohort in OCC was relatively lean, which could have affected study results. GDM and insulin resistance could have a further negative impact on placental function\(^{14}\) and could result in even higher risk of offspring ADHD. Further research is necessary to understand the importance of placental dysfunction in PCOS, but unfortunately, placental tissue is not available in OCC.

In boys, we found that maternal diagnosis of PCOS was positively associated with ADHD-related behavior, whereas in girls, this association was not significant. Maternal testosterone levels were not associated with ADHD symptoms in boys, whereas maternal testosterone was negatively associated with ADHD symptoms in girls. These findings suggest that boys were susceptible to develop ADHD-related behavior if their mother had a PCOS diagnosis, whereas the opposite was seen in the girls. In OCC, the prevalence of ADHD symptoms was 11% in boys and 9% in girls, which agreed with male-dominant presentation of ADHD.\(^{7}\) Our findings contrasted with two Swedish register-based studies\(^{4,5}\) where associations between maternal PCOS and offspring ADHD were stronger in girls than boys. A Finnish register-based study\(^{7}\) found similar risk estimates for ADHD in boys and girls. The opposite correlation between maternal PCOS and ADHD in girls could be due to assessment of manifest ADHD\(^{4,5}\) in contrast to ADHD symptoms in the present study. Further, differences in chromosomal complement between girls and boys could be an explanation of sex differences in ADHD susceptibility.\(^{38}\) Further studies focusing on different mechanisms related to development of ADHD in boys and girls are necessary.

In boys with a parental psychiatric diagnosis, associations between maternal PCOS and ADHD symptoms became non-significant. We previously reported higher psychiatric morbidity in Danish women with PCOS in a national register-based setting,\(^{27}\) which was confirmed in a Swedish national register-based study.\(^{28}\) It is possible that associations between maternal PCOS and offspring ADHD are confounded by shared genetic factors. Our findings could indicate that mechanisms other than testosterone exposure mediate the risk of ADHD in boys born of mothers with PCOS. Women with PCOS are characterized by obesity and hyperinsulinemia, which affect the fetal environment.\(^{3}\) Prenatal exposure to maternal overweight, hyperinsulinemia,\(^{39}\) and increased levels of inflammatory factors\(^{40}\) was associated with higher risk of offspring ADHD in healthy study cohorts\(^{39}\) and in PCOS.\(^{40}\) Importantly, associations between maternal obesity and offspring ADHD became non-significant when a sibling comparison design was applied.\(^{39}\) This suggested that the association between maternal pre-pregnancy overweight/obesity and offspring ADHD could to a large extent be ascribed to unmeasured familial confounding\(^{39}\) and further support shared genetic factors in ADHD and obesity. In OCC, women were lean and maternal BMI was not associated with
offspring ADHD-related behavior. Studies regarding maternal inflammatory markers and offspring risk of ADHD are planned within the context of OCC.

Higher risk of ADHD-related behavior in offspring born to mothers with PCOS compared with controls was in agreement with findings predominantly from register-based studies. In OCC, PCOS was diagnosed in 10% of women, which corresponded to the estimated prevalence in the general population. In contrast, the prevalence of PCOS ranged from 0.4% to 2.2% in register-based studies, which would reflect a more severe PCOS phenotype. Furthermore, we categorized ADHD-related behavior according to the >90th centile of Danish national standard in children. International Classification of Diseases, 10 revision, diagnosis codes and/or prescription of ADHD medications were used to diagnose manifest ADHD in register-based studies, which resulted in prevalences of ADHD of 2.1% and 0.9%. Our findings therefore supported higher risk of ADHD-related behavior, which was also seen in children born of mothers with milder PCOS phenotype. Our results contrasted with one American prospective birth cohort (n = 1915), reporting no association between maternal PCOS and offspring ADHD diagnosis. The study resembled OCC regarding prevalence of maternal PCOS (12%), mean maternal age (31 years), and inclusion of ADHD-related behavior (ADHD prevalence 10%). However, the subset of women with PCOS was more obese than the total study cohort (30.4 vs. 26.9 kg/m²) and offspring age was 7 years by the time of evaluation. In OCC, children are followed until the age of 18 years, so the impact of maternal PCOS and offspring ADHD can be re-evaluated as the children grow older.

Strengths and limitations apply to the present study. Strengths include the large sample size, the prospective cohort design, inclusion of high-quality assays to measure maternal testosterone levels during pregnancy, and that the study staff were trained to measure AGD. Women with PCOS and controls were followed equally during pregnancy to avoid surveillance bias according to PCOS diagnosis. The CBCL/1-5 ADHD problem scale has been evaluated in several studies. In spite of the moderate sensitivity and specificity, the CBCL serves as a rapid and useful screening instrument. Limitations include the observational study design, allowing no causal conclusions, plus the use of only parents as informers of the child’s ADHD symptomatology. The method for obtaining maternal PCOS diagnosis differed between questionnaire responders and questionnaire non-responders. We did not compare the different methods for obtaining PCOS diagnosis, which is a study limitation. Women with PCOS could be misclassified as controls in questionnaire non-responders without a hospital diagnosis of PCOS, which could lead to underestimation of the risk of ADHD in offspring born of mothers with PCOS. The prevalence of PCOS was 9.4% in OCC, which resembled the estimated prevalence of PCOS in the background population. We grouped women according to quartiles of maternal testosterone to allow for the possibility of a U-shaped association between maternal testosterone and offspring ADHD. Women in the lowest quartile might differ with pathologically low levels of testosterone compared with women in the higher quartiles.

We have chosen the lowest quartile as reference group to retain the natural increasing order of maternal testosterone compared with the expected increased risk of offspring ADHD. Women in the lowest quartile of testosterone could differ pathologically from women in the higher quartiles, however, it is not clear how potential maternal pathological differences may affect offspring ADHD. In OCC, women with PCOS were relatively lean and our study may not apply to more obese populations. The women participating were more ethnically homogeneous, older, predominantly nulliparous, more educated, and less likely to smoke compared with the general population. This issue is often a problem in cohort studies, as the persons who drop out often represent ethnic minorities and persons of lower socioeconomic status. Offspring testosterone levels were not available and were not included in mediation analyses.

5 | CONCLUSION

Boys born of women with PCOS were more likely to develop ADHD-related behavior. Maternal testosterone and offspring AGD were not associated with ADHD-related behavior in the offspring. Further studies are needed to investigate the role of placental function for the risk of ADHD in the offspring.

ACKNOWLEDGMENTS

The technicians at Hans Christian Andersen’s Hospital for Children and Adolescents are acknowledged for their careful examination of the children.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTIONS

MSA, NB, and DG established the study idea. HB and TKJ contributed with funding, design, and running of the Odense Child Cohort. CMD, LGF, DG, RCJ, and PVL performed data analysis. CMD, MSA, NB, and DG took part in the interpretation of results, and revising and writing the paper. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Dalgaard CM, Andersen MS, Jensen RC, et al. Maternal polycystic ovary syndrome and attention deficit hyperactivity disorder in offspring at 3 years of age: Odense Child Cohort. Acta Obstet Gynecol Scand. 2021;100:2053-2065. https://doi.org/10.1111/aogs.14259