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**No Relationship Between Prenatal Androgen Exposure and Autistic Traits:
Convergent Evidence From Studies of Children With Congenital Adrenal Hyperplasia
and of Amniotic Testosterone Concentrations in Typically-Developing Children**

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Abstract

Background: There is a marked male preponderance in autism spectrum conditions. The extreme male brain theory and the fetal androgen theory of autism suggest that elevated prenatal testosterone exposure is a key contributor to autistic traits. The current paper reports findings from two separate studies that test this hypothesis. **Methods:** A parent-report questionnaire, the Childhood Autism Spectrum Test (CAST), was employed to measure autistic traits in both studies. The first study examined autistic traits in young children with congenital adrenal hyperplasia (CAH), a condition causing unusually high concentrations of testosterone prenatally in girls. 81 children with CAH (43 girls) and 72 unaffected relatives (41 girls), aged 4 to 11 years, were assessed. The second study examined autistic traits in relation to amniotic testosterone in 92 typically-developing children (48 girls), aged 3 to 5 years. **Results:** Findings from neither study supported the association between prenatal androgen (testosterone) exposure and autistic traits. Specifically, young girls with and without CAH did not differ significantly in CAST scores and amniotic testosterone concentrations were not significantly associated with CAST scores in boys, girls, or the whole sample. **Conclusions:** These studies do not support a relationship between prenatal testosterone exposure and autistic traits. These findings augment prior research suggesting no consistent relationship between early androgen exposure and autistic traits. **Keywords:** Congenital adrenal hyperplasia, fetal testosterone, prenatal testosterone exposure, autism, autistic traits, extreme male brain.

**No Relationship Between Prenatal Androgen Exposure and Autistic Traits:
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Autism Spectrum Conditions (ASCs) are neurodevelopmental conditions characterized by deficits in social communication and interaction, and restricted, repetitive patterns of behavior or interests. Although ASCs can be conceptualized as clinical conditions distinct from the general population, it also has been suggested that autistic traits are continuously distributed across the general population, with ASCs representing the upper extreme of the distribution (Constantino & Todd, 2003).

There is a male preponderance in ASCs (Fombonne, 2005), with male-to-female ratios ranging from 4:1 for classic autism (Chakrabarti & Fombonne, 2001) to 11:1 for Asperger's Syndrome (Gillberg, Cederlund, Lamberg, & Zeijlon, 2006). In addition, males in the general population have been reported to exhibit more autistic traits than females (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). The extreme male brain (EMB) theory suggests that ASCs are an extreme manifestation of certain male-typical traits (Baron-Cohen, 2002). In non-human mammals, manipulations of prenatal or early postnatal androgen exposure permanently alter behavioral characteristics that show sex differences (Arnold, 2009). Similarly, androgens, such as testosterone, influence human sexual differentiation during fetal development and may exert enduring effects on subsequent gender-related behaviors (Hines, 2004). Based on this evidence, the fetal androgen theory of autism suggests that heightened exposure to testosterone during fetal development is a key mechanism underlying the EMB (Baron-Cohen, Lutchmaya, & Knickmeyer, 2004; Baron-Cohen, Knickmeyer, & Belmonte, 2005). These two related theories, the fetal androgen theory of autism and the EMB theory, predict that high concentrations of testosterone prenatally increase autistic traits.

Ethical considerations preclude experimental manipulation of testosterone during early human development. However, some information on the influences of testosterone has come from individuals exposed to unusual levels of testosterone and other androgens prenatally due to medical conditions, such as classical congenital adrenal hyperplasia (CAH). CAH¹ is an autosomal, recessive condition, occurring in approximately 1 in 16,000 to 1 in 20,000 births (White, 2009). It is caused by an enzyme deficiency (21-hydroxylase in >90% of cases) that impairs cortisol production (White, 2009). Consequently, cortisol precursors are shunted into the androgen pathway. Although prenatal androgen exposure varies among girls with CAH, partly depending on the specific genotype, androgen concentrations at mid-gestation in girls with CAH are generally elevated to levels more characteristic of boys (Wudy, Dörr, Solleder, Djalali, & Homoki, 1999). Girls with CAH are usually born with some degree of genital virilization, leading to rapid diagnosis. They are treated postnatally with hormones with the goal of normalizing their androgen and cortisol concentrations, are usually assigned and reared as girls, and often have surgery to feminize their external genitalia. Despite the early diagnosis and treatment, and female sex of rearing, they show more male-typical behavior in several areas, including childhood play preferences, sexual orientation, and gender identity (Hines, 2011). In contrast, gender-related behaviors of males with CAH are generally unaltered, and their androgen concentrations prenatally appear to be largely in the normal male range (Hines, 2011).

One study has evaluated the hypothesis that autistic traits are increased in females with CAH. Knickmeyer et al. (2006) assessed autistic traits, using the Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001), a self-report measure, in 12-to-45-year-old individuals with CAH (34 females, 26 males) and their unaffected relatives (24 females, 25 males). Unaffected males had higher AQ scores than unaffected females (Cohen's $d = 0.55$, p

¹ In this report, CAH refers to classical CAH, unless stated otherwise.

< .10) and females with CAH scored higher on the AQ than unaffected females (Cohen's $d = 0.50$, $p < .10$). These group differences were significant only with one-tailed tests, however. AQ scores of males with and without CAH did not significantly differ (Cohen's $d = 0.25$, $p > .10$, with higher scores in unaffected males). These findings have been interpreted to support the EMB theory and the fetal androgen theory of autism.

This prior study used the AQ, a measure that was originally developed in mostly adult samples. It may be less appropriate for the adolescent participants in the study. It also would be of interest to evaluate CAH-related differences in autistic traits in young children, before the possible impact of the disorder and its consequences for socio-cognitive processes have accumulated over many years.

A second type of investigation has related normal variability in testosterone prenatally to autistic traits in typically-developing children. One such approach involves measuring testosterone concentrations in amniotic fluid obtained during clinical amniocentesis. Amniocentesis is a procedure typically performed during the second trimester of pregnancy, coinciding with the presumed critical period for human sexual differentiation (Collaer & Hines, 1995). Three studies, all from one research group and all with participants drawn from a single pool of individuals for whom measures of amniotic testosterone were available, reported that amniotic testosterone correlated positively with parent-report autistic traits in boys and in girls (Auyeung et al., 2009, 2012; Auyeung, Taylor, Hackett, & Baron-Cohen, 2010; see Table 1). The current report seeks to replicate this finding in an independent sample.

-----*Insert Table 1*-----

Both current studies extend research testing the hypothesis that prenatal androgen exposure predicts autistic traits. Study 1 focused on children with CAH; Study 2 examined amniotic testosterone and autistic traits in a sample of typically-developing children. Two hypotheses were evaluated: (1) girls, but not boys, with CAH show more autistic traits than

unaffected same-sex relatives; and (2) amniotic testosterone concentrations correlate positively with autistic traits in typically-developing boys and girls.

Study 1: CAH and Autistic Traits

Method

Participants

Children with CAH were recruited through a national CAH support group and through 11 endocrine clinics in the UK. A total of 153 children, aged 4 to 11 years ($M = 7.40$, $SD = 2.30$), participated. Data on participation rate as a function of numbers of contacts are not available. All children with CAH had classical CAH with 21-hydroxylase enzyme deficiency. In girls, classical CAH is characterized by excessive adrenal androgen secretion during fetal development, causing genital virilization and ambiguity at birth, whereas girls with the milder non-classical form of CAH do not show signs of prenatal androgenization (White, 2009). Classical CAH has two subtypes, a more severe, salt wasting subtype, and a less severe, simple virilizing, subtype. The simple virilizing subtype is characterized by genital ambiguity in affected girls. In the salt-wasting subtype, in addition to genital ambiguity in girls, there are sodium-deficiency symptoms in both sexes, such as dehydration and weight loss, resulting from inadequate secretion of salt-retaining steroids. The current study included 43 girls with CAH (37 salt-wasting, 6 simple-virilizing), 38 boys with CAH (35 salt-wasting, 3 simple-virilizing), 41 unaffected female relatives (31 sisters, 9 first cousins, 1 adopted sister), and 31 unaffected male relatives (23 brothers, 8 first cousins). Diagnostic information was obtained from medical background interviews with parents and confirmed by information on medications being taken. None of the children had been treated prenatally with dexamethasone, a glucocorticoid that is sometimes used to treat pregnancies where CAH is suspected, and which can reduce physical virilization.

Of the 153 children, 137 were Caucasian, 1 was Mid-Eastern, 5 were

Indian/Bangladeshi, 1 was African/Afro-Caribbean, 8 were mixed race, and 1 was of other descent. The majority (96.1%) of the children came from two-parent families; 3.9% lived with their mother only. Regarding parental education, 22.9% of the children had parents with no post-secondary education; 60.8% had at least one parent with vocational training/ university degree; 16.3% had at least one parent with postgraduate education. There were no significant differences among the four groups in ethnicity, family structure, or parental education ($\chi^2 = 3.59-10.29, p = .66-.80$).

Procedures and Measures

Assessment procedures were 3 to 4 hours long and included measures of a range of gender-related outcomes. Data for other measures have been (Browne et al., 2015; Hines et al., 2016; Pasterski et al., 2015), or will be, reported separately. The data reported here are the only data that were collected on autistic traits. The participants were blind to the study hypotheses. Parents provided informed consent for their own and their child's participation in the study. The study protocols were approved by national and institutional research ethics committees.

Autistic Traits. The Childhood Autism Spectrum Test (CAST; Scott, Baron-Cohen, Bolton, & Brayne, 2002) is a 37-item parent-report questionnaire developed to detect ASCs in children ages 4 to 11 years. CAST items require a binary response ('yes/no') to 37 questions, 31 of which are scored. For these 31 items, an ASC-positive response scores 1 and an ASC-negative response scores 0. Some items are reverse scored so that not all 'yes' responses score 1. The remaining 6 items are control questions and are not scored. The CAST has good test-retest reliability ($r = .83$) after 2 weeks (Williams et al., 2006). A score of 15 or above indicates risk for ASCs (Scott et al., 2002; Williams et al., 2005). At that cut-point, the CAST has high specificity (97%) and sensitivity (100%) for ASCs (Williams et al., 2005). For 96% of the children, mothers completed the CAST; for 4%, fathers completed it. A

CAST score was not available for 1 girl with CAH. Internal consistency of the items, as indicated by Cronbach's α coefficient, was good ($\alpha = .76$)

Control Variables. The age of the child at the time of testing was recorded. Also, the vocabulary subtest of the Wechsler Intelligence Scale for Children (4th ed.) (WISC-IV) (Wechsler, 2003) or the Wechsler Preschool and Primary Scale of Intelligence (3rd ed.) (WPPSI-III) (Wechsler, 2002), as age appropriate, provided estimates of general intelligence. Age-scaled vocabulary scores were used for analyses, and were available for all children.

Data Analyses

The distributions of CAST scores were positively skewed. Following Auyeung et al. (2009), CAST scores were transformed by adding one and taking the square root of each score. This transformation produced distributions that were not significantly skewed in the overall sample or within either sex. Analyses were based on the transformed scores unless otherwise stated. All analyses were two-tailed with alpha set at .05. Descriptive statistics for CAST scores, age, and vocabulary for individual groups and the overall sample are reported in Table 2.

-----Insert Table 2-----

Results

There were no significant group differences in age or vocabulary (age: $F(3, 149) = 0.79, p = .51$; vocabulary: $F(3, 149) = 1.11, p = .35$). CAST scores were not significantly associated with age, $r(150) = -.05, p = .58$, but were significantly associated with vocabulary, $r(150) = -.24, p < .01$.

The regression model with sex, CAH status, interaction between sex and CAH status, and vocabulary scores entered as predictors of CAST scores was significant, $R^2 = .08, F(4, 147) = 3.19, p < .05$. Vocabulary was a significant negative predictor, $B = -0.05, SE = .02, p < .01$, but sex, CAH status, and the interaction between sex and CAH status were not

significant ($p = .29-.67$), and remained non-significant when vocabulary was removed from the model ($p = .15-.87$).

To further examine the association between CAST scores and CAH, and allow direct comparison to Knickmeyer et al. (2006), independent samples t -tests and Cohen's d s were computed. Positive values indicate higher scores in boys or individuals with CAH. There was no significant difference in CAST scores between boys and girls without CAH, $t(70) = -0.52$, $p = .61$, $d = -0.12$, girls with and without CAH, $t(81) = 1.36$, $p = .18$, $d = 0.30$, boys with and without CAH, $t(67) = 1.65$, $p = .103$, $d = 0.39$, or boys and girls with CAH, $t(78) = -0.34$, $p = .74$, $d = -0.08$. The above analyses were repeated using original CAST scores, with highly comparable results and similar effect sizes as in the initial analyses.

Three children with CAH (1 boy, 2 girls) and 3 unaffected relatives (1 boy, 2 girls) scored at or above the cut-off of 15 on the CAST. There were no significant group differences in having a CAST score of 15 or above (Fisher's exact $p = .46-1.00$).

Study 2: Amniotic Testosterone and Autistic Traits

Method

Participants

This study included 92 children (48 girls, 44 boys), between 3 and 5 years of age ($M = 4.27$, $SD = .33$), whose mothers underwent amniocentesis for clinical purposes at Imperial College, NHS Trust. Invitations to participate in this study were sent to 107 parents, and 92 (86%) participated. A pediatrician confirmed all children as healthy at birth. Of the 92 children, 62 were Caucasian, 4 were Mid-Eastern, 7 were Indian/Bangladeshi, 5 were African/Afro-Caribbean, 13 were mixed race, and 1 was of other descent. Most children (90.2%) came from two-parent families; 9.8% lived with their mother only. Regarding parental education, 7.6% of the children had parents with no post-secondary education; 59.8% had at least one parent with vocational training/ university degree; 32.6% had at least

one parent with postgraduate education. Boys and girls did not differ significantly in ethnicity, family structure, or parental education ($\chi^2 = 0.52-6.12, p = .26-.77$).

Procedures and Measures

Amniotic Testosterone. Amniocentesis was performed between 15 and 25 weeks of gestation ($M = 16.93, SD = 2.01$), coinciding with the presumed critical period for human sexual differentiation (Collaer & Hines, 1995; Hines, 2004). All amniotic fluid samples were taken in the morning between 9:30 am and 12:30 pm. An aliquot of up to 4 mL of amniotic fluid surplus to clinical requirement was drawn for the study and stored at -80°C until assay. Total testosterone was assayed by radioimmunoassay using the “Coat-A-Count” method (Coat-A-Count, DPC, Los Angeles, California), after solvent extraction and concentration. The intra- and inter-assay coefficients of variation of the amniotic fluid testosterone assay were 7.5% and 8.9%, respectively. Amniocentesis is typically performed with mothers whose children are at risk of genetic abnormalities associated with increased maternal age. Mothers had a mean age of 37.3 years ($SD = 4.05$) at their child’s birth. Boys and girls did not differ significantly in gestational age at amniocentesis or maternal age at birth ($t = 0.14-0.40, p = .69-.99$).

Other procedures and measures were identical to those for study 1 (CAH), with the following exceptions. In study 2, CAST scores were not available for 1 boy, Cronbach’s alpha for the CAST was 0.67, and vocabulary scores were not available for 3 boys and 1 girl.

Data analyses

Distributions of CAST scores were positively skewed and were transformed as described for Study 1 with the same results. Distributions of amniotic testosterone concentrations were also positively skewed and were transformed by adding one and taking the log of each value. These transformations resulted in distributions that were not significantly skewed in the overall sample or within either sex. As in Study 1, reported

analyses are based on transformed scores unless otherwise stated, and all analyses were two-tailed, with α set at .05. Descriptive statistics for amniotic testosterone concentrations, CAST scores, age, and vocabulary within each sex and for the overall sample, and effect sizes (Cohen's d) for differences between boys and girls in these variables are reported in Table 3.

-----Insert Table 3-----

Results

There was no significant gender difference in age or vocabulary (age: $t(90) = 1.09$, $p = .28$; vocabulary: $t(86) = -1.66$, $p = .10$). CAST scores were not significantly associated with age, $r(89) = -.16$, $p = .14$, but were significantly associated with vocabulary, $r(86) = -.23$, $p < .05$.

Independent samples t-tests showed that boys had significantly higher concentrations of amniotic testosterone, $t(65.07) = 10.64$, $p < .001$, equal variances not assumed, and significantly higher CAST scores, $t(89) = 2.07$, $p < .05$, than girls. Amniotic testosterone was not significantly correlated with CAST scores in boys, $r(41) = -.05$, $p = .73$, or girls, $r(46) = -.10$, $p = .48$, or in boys and girls combined, $r(89) = .11$, $p = .28^2$. When vocabulary scores were taken into account in a multiple regression model, the association between amniotic testosterone and CAST scores remained non-significant within each sex and for the entire sample ($p = .54-.72$).

The above analyses were repeated after excluding children under the age of 4 years (4 boys and 4 girls aged 3.81 to 3.99 years) because the CAST was developed and validated in a sample of children aged 4 to 11 years (Scott et al., 2002). Analyses were also repeated using original CAST scores and amniotic testosterone concentrations. Highly comparable results were found, showing similar effect sizes for associations between amniotic testosterone and autistic traits as in the initial analyses.

² The direction of the correlation changed when data from boys and girls were analyzed together, probably because boys had higher CAST scores and higher amniotic testosterone concentrations than girls.

One boy and no girls scored at or above the cut-off score of 15 on the CAST. Within the male group, the amniotic testosterone concentration of that high-scoring participant was slightly above the mean, at the 54th percentile.

Discussion

The EMB theory and the fetal androgen theory of autism propose that exposure to high concentrations of testosterone prenatally contributes to elevated autistic traits and to ASCs (Auyeung et al., 2009, 2010, 2012; Baron-Cohen, 2002; Baron-Cohen et al., 2004, 2005). This hypothesized effect of testosterone on autistic traits was investigated in the two studies reported here. The first study included children with CAH and their unaffected relatives, and provided the first test of autistic traits in young children with CAH, a potentially valuable group for testing the hypothesis that prenatal androgen exposure influences autistic traits. In this study, there was no significant difference in autistic traits between children with and without CAH for either girls or boys. The second study focused on typically-developing children whose mothers underwent amniocentesis during pregnancy, which is generally thought to provide relevant data on testosterone for testing the study hypotheses. In this study, amniotic testosterone was not significantly related to autistic traits in either boys or girls. In both studies, having an autistic traits score that indicated a risk of an ASC was not significantly associated with heightened exposure to testosterone prenatally. These findings do not support the hypotheses that prenatal testosterone contributes to autistic traits or to ASCs.

Autistic Traits in Children With CAH

Although there was a trend for girls with CAH to score higher on the autistic traits measure than unaffected girls in the current study, the group difference in the current study (Cohen's $d = 0.30$; $M = 6.76$, $SD = 3.76$ in girls with CAH; $M = 5.76$, $SD = 4.31$ in unaffected girls) was smaller than that in the previous study of older females with CAH

(Cohen's $d = 0.50$; $M = 18.44$, $SD = 5.51$ in females with CAH; $M = 16.00$, $SD = 4.25$ in unaffected females). There was also a trend for boys with CAH to score higher than unaffected boys on the autistic traits measure in the current study (Cohen's $d = 0.39$; $M = 6.42$, $SD = 3.39$ in boys with CAH; $M = 5.16$, $SD = 3.28$ in unaffected boys). By contrast, in the previous study, the trend suggested higher scores in unaffected males than males with CAH (Cohen's $d = 0.25$; $M = 17.35$, $SD = 6.05$ in males with CAH; $M = 18.88$, $SD = 6.21$ in unaffected males). The slightly, and non-significantly, elevated scores in both boys and girls with CAH in the current study are unlikely to be attributable to prenatal androgen exposure, because only girls, and not boys, with CAH are exposed prenatally to higher than normal androgen concentrations (Wudy et al., 1999).

These inconsistent findings may be explained by methodological differences between the studies. The study of Knickmeyer et al. (2006) used a self-report measure that may not have been sufficiently sensitive for adolescent participants, perhaps because an age-appropriate measure was not then available. The ages of participants in the two studies also differed in ways that could have produced different results. The current child sample is substantially younger than the adolescent and adult sample used by Knickmeyer et al. (2006). It is possible that autistic traits, particularly those related to social communication and interaction, may change as individuals with CAH become older; if so, the differing findings between the studies could result from the different ages studied. The younger age in the current study may provide a more direct assessment of the effects of prenatal androgen exposure, because there would have been less time for postnatal influences.

Amniotic Testosterone and Autistic Traits in Typically-developing Children

Prior studies (Auyeung et al., 2009, 2010, 2012) generally reported significant positive correlations between amniotic testosterone and autistic traits in both typically-developing boys ($r = .22-.51$) and girls ($r = .08-.57$). In contrast to prior studies and the

hypothesized direction of effect, the current study found a non-significant negative correlation in boys ($r = -.05$) and girls ($r = -.10$).

The one prior study that related amniotic testosterone to autistic traits assessed using the CAST, the same measure used in the current study, found a significant correlation in boys but not in girls (Auyeung et al., 2009). That same study found positive correlations for both boys and girls between testosterone and scores on a different measure, the AQ-Child. The convergent validity between the CAST and the AQ-Child has been reported to be weak ($r = .25$; Auyeung et al., 2009), despite both measures having been psychometrically validated for assessment of autistic traits, with high sensitivity, specificity, and test-retest reliability (Auyeung et al., 2008; Williams et al., 2005, 2006). The implication is that the association between amniotic testosterone and measures of autistic traits is not robust.

While the current findings contradict previous research on amniotic testosterone and autistic traits, and CAH and autistic traits, they are consistent with the recent finding of no difference in amniotic testosterone concentrations between 128 males diagnosed with ASCs and 217 matched male controls (Baron-Cohen et al., 2015). Although Baron-Cohen et al. (2015) also reported that scores of a latent factor representing shared variance of five amniotic hormones (progesterone, 17α -hydroxy-progesterone, androstenedione, testosterone and cortisol) were significantly higher in the ASC group than the comparison group, it is unclear whether this latent hormonal factor is related to processes of sexual differentiation, given no evidence that it differs for males and females.

Early Androgen Exposure and Autistic Traits

The findings from the two current studies also appear to concur with previous research examining the link between perinatal or early postnatal androgen exposure and autistic traits in non-clinical samples. Although the EMB theory and the fetal androgen theory of autism focus on the role of prenatal androgen exposure (Baron-Cohen, 2002; Baron-Cohen

et al., 2004, 2005), studies of non-human mammals suggest that both prenatal and early postnatal androgen exposure contribute to neurobehavioral sexual differentiation (Arnold, 2009). Two prior studies, based on a longitudinal pregnancy cohort of around 400 individuals, found no relationship in either sex between perinatal androgen exposure, assessed by concentrations of hormones in umbilical cord blood samples collected at birth, and autistic traits in early adulthood, assessed by a self-report questionnaire (Jamnadass et al., 2015; Whitehouse et al., 2012). However, androgen concentrations in umbilical cord blood at birth can be influenced by the type of delivery and by labor, and may reflect androgen exposure during late gestation (Keelan et al., 2012), after the prenatal testosterone surge in male fetuses during the presumed critical period for human sexual differentiation (Collaer & Hines, 1995).

In addition to the prenatal surge, testosterone is elevated in male infants during the early postnatal period called “mini-puberty”, with testosterone concentrations peaking at around 1 to 3 months postnatal before declining to low childhood values by around 6 months of age (Winter et al., 1976). Two prior studies found little to no association between salivary testosterone at 3 to 4 months postnatal and subsequent parent-report autistic traits in toddlers ($N = 35$ in Auyeung et al., 2012; $N = 84$ in Saenz & Alexander, 2013). A more recent study related salivary testosterone during the peak of mini-puberty at 1 to 3 months of age to subsequent parent-report autistic traits and also found no relationship in either sex in a sample of 86 toddlers (Kung, Constantinescu, Browne, Noorderhaven, & Hines, 2016). Although these studies had relatively small samples, they converge to suggest that there is little to no association between testosterone exposure during mini-puberty and autistic traits.

Limitations

Although boys and girls in the current amniotic testosterone study differed as expected in CAST scores, unaffected boys and girls in the current CAH study did not. This lack of a consistent gender difference on the measure of autistic traits might reduce the

theoretical basis for predicting a relationship to testosterone, because effects of testosterone are generally limited to outcomes that show gender differences. Nevertheless, the CAST has been validated as a measure of autistic traits and of risk for ASCs, and so both the lack of a gender difference and the lack of a relationship to testosterone argue against androgens causing autistic traits. More generally, not many studies have reported on gender differences in autistic traits measured by the CAST or by other parent-report autistic traits measures for children (e.g., Allison et al., 2008; Auyeung et al., 2009; Williams et al., 2008), and thus information regarding the magnitude and the reliability of these gender differences is limited.

As with previous research, the current research is limited by using amniotic testosterone concentrations as a measure of normal variability in prenatal androgen exposure. Participants undergoing amniocentesis may not be representative of the general population, although we only followed up individuals without clinical findings or abnormal birth outcomes. Also, the reliability of amniotic testosterone as an index of prenatal androgen exposure may be limited, because only a single amniotic fluid sample is collected, usually uncontrolled for time of day or gestational age, although in the current study time of day was controlled to some extent by taking all amniotic fluid samples in the morning. While it is often assumed that testosterone concentrations in amniotic fluid are a proxy for testosterone concentrations in fetal blood (Baron-Cohen et al., 2004), the one study that has investigated their relationship found no correlation between the two (Rodeck, Gill, Rosenberg, & Collins, 1985).

Finally, we conducted two studies on relatively small groups of children. Nevertheless, the sample in our CAH study is larger than that in the previous CAH study and the sample in our amniotic testosterone study is larger than that in the previous amniotic testosterone study showing the biggest effects. More importantly, the directions of the current effects do not support the hypothesized relationship.

Conclusion

Previous research relating prenatal testosterone exposure to autistic traits has been interpreted to support the EMB theory and the fetal androgen theory of autism, but convergent findings from the two current studies of children with CAH and amniotic testosterone concentrations in typically-developing children suggest that the hypothesized relationship may not be reliable or robust, especially in young children. On a broader level, the current studies augment information from studies using different measures and study populations to assess the influences of prenatal, perinatal, or early postnatal androgen exposure on autistic traits. This body of evidence suggests there is no consistent relationship between early androgen exposure and autistic traits.

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Key Points

1. Some prior research suggests that prenatal androgen exposure contributes to autistic traits.
2. First study on autistic traits in young children with congenital adrenal hyperplasia.
3. Autistic traits were not elevated in girls or boys with congenital adrenal hyperplasia.
4. Amniotic testosterone did not relate to autistic traits in typically-developing boys or girls.
5. Results from two studies converge to argue against testosterone causing ASCs.

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Table 1

Previous Studies on Amniotic Testosterone and Autistic Traits in Typically-Developing Children

Study	Sample size	Age	Measure	Cohen's <i>d</i> for gender differences in autistic traits ¹	Correlation between amniotic testosterone and autistic traits
Auyeung et al. (2009)	118 boys, 117 girls	<i>M</i> = 8.91 years <i>SD</i> = 0.95 year Range = 6–10 years	The Childhood Autism Spectrum Quotient (AQ-Child; Auyeung, Baron-Cohen, Wheelwright, & Allison, 2008)	AQ: <i>d</i> = 0.87***	AQ: <i>r</i> = .22*** in boys <i>r</i> = .27*** in girls <i>r</i> = .41*** in all children
			The Childhood Autism Spectrum Test (CAST; Scot, Baron-Cohen, Bolton, & Brayne, 2002)	CAST: <i>d</i> = 0.30*	CAST: <i>r</i> = .28*** in boys ² <i>r</i> = .08 ^{ns} in girls ² <i>r</i> = .25*** in all children
Auyeung, Taylor, Hackett, & Baron-Cohen, (2010)	66 boys, 63 girls	<i>M</i> = 19.25 months <i>SD</i> = 1.52 months Range = 18–24 months	The Quantitative Checklist for Autism in Toddlers (Q-CHAT; Allison et al., 2008)	<i>d</i> = 0.46*	<i>r</i> = .36* in boys <i>r</i> = .31* in girls <i>r</i> = .40** in all children
Auyeung et al. (2012)	15 boys, 20 girls	<i>M</i> = 19.34 months <i>SD</i> = 2.93 months Range = 18–35 months	The Quantitative Checklist for Autism in Toddlers (Q-CHAT; Allison et al., 2008)	<i>d</i> = 0.92**	<i>r</i> = .51* in boys <i>r</i> = .57* in girls <i>r</i> = .56** in all children

¹Positive *d* values indicate higher scores in males.

²Sizes of the correlation coefficients were not reported in Auyeung et al. (2009) but were provided in Auyeung (2008).

* $p < .05$. ** $p < .01$. *** $p < .001$. ^{ns}non-significant ($p > .05$). All tests were two-tailed.

Table 2

Study 1 Statistics: Means (Ms) and Standard Deviations (SDs) of Original Childhood Autism Spectrum Test (CAST) Scores, Age and Age-Scaled Vocabulary Scores in Boys and Girls With and Without Congenital Adrenal Hyperplasia (CAH) and in the Overall Sample

	CG		CB		CAHG		CAHB		Overall	
	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>
CAST	41	5.76 (4.31)	31	5.16 (3.28)	42	6.76 (3.76)	38	6.42 (3.39)	152	6.07 (3.75)
Age (years)	41	7.59 (2.51)	31	7.81 (2.36)	43	7.13 (2.28)	38	7.15 (2.04)	153	7.40 (2.30)
Vocabulary	41	11.32 (2.90)	31	10.84 (2.56)	43	10.16 (3.32)	38	10.82 (2.72)	153	10.77 (2.92)

Note. CG, control girls; CB, control boys; CAHG, girls with CAH; CAHB, boys with CAH.

Table 3

Study 2 Statistics: Means (Ms) and Standard Deviations (SDs) of Original Amniotic Testosterone Concentrations, Original Childhood Autism Spectrum (CAST) Scores, Age and Age-Scaled Vocabulary Scores Within Each Sex and in the Overall sample, and Cohen's ds for Differences Between Boys and Girls

	Boys (B)		Girls (G)		Overall		B vs. G
	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>	<i>n</i>	<i>M (SD)</i>	<i>d</i>
Amniotic testosterone (nmol/L)	44	0.83 (0.41)	48	0.23 (0.15)	92	0.52 (0.43)	1.98
CAST	43	6.49 (3.59)	48	5.14 (2.47)	91	5.78 (3.11)	0.44
Age (years)	44	4.31 (0.37)	48	4.23 (0.29)	92	4.27 (0.33)	0.24
Vocabulary	41	10.46 (2.70)	47	11.34 (2.27)	88	10.93 (2.50)	-0.35

Note. Positive *d* values indicate higher values in males.