Kent Academic Repository
Full text document (pdf)

Citation for published version

Kung, Karson T.F. and Thankamony, Ajay and Ong, Ken K.L. and Acerini, Carlo L. and Dunger, David B. and Hughes, Ieuan A. and Hines, Melissa (2020) No relationship between prenatal or early postnatal androgen exposure and autistic traits: evidence using anogenital distance and penile length measurements at birth and 3 months of age. Journal of Child Psychology and Psychiatry

DOI

https://doi.org/10.1111/jcpp.13335

Link to record in KAR

https://kar.kent.ac.uk/85305/

Document Version

Publisher pdf

No relationship between prenatal or early postnatal androgen exposure and autistic traits: evidence using anogenital distance and penile length measurements at birth and 3 months of age

Karson T.F. Kung,[1,2,3] Ajay Thankamony,[4] Ken K.L. Ong,[4] Carlo L. Acerini,[4] David B. Dunger,[4] Ieuan A. Hughes,[4] and Melissa Hines[3]

[1]Department of Psychology, University of Hong Kong, Pokfulam, Hong Kong; [2]School of Psychology, University of Kent, Canterbury, UK; [3]Department of Psychology, University of Cambridge, Cambridge, UK; [4]Department of Paediatrics, University of Cambridge, Cambridge, UK

Background: Autism is more prevalent in males than in females. Hypotheses related to the extreme male brain theory of autism suggest that heightened androgen exposure during early development contributes to autistic traits. Whilst prior research focused mostly on the prenatal period, the current study tests the influences of androgen exposure during both the prenatal and the early postnatal periods on autistic traits during childhood. **Methods:** Anthropometric measures that are putative biomarkers of early androgen exposure were employed. Anogenital distance (AGD) was measured at birth and 3 months of age in boys and girls. Penile length at birth and 3 months of age was also measured in boys. When the children were 9–13 years old, a parent-reported questionnaire (the 10-item children's version of the Autism Spectrum Quotient; AQ-10 Child) was used to assess autistic traits in 97 boys and 110 girls. **Results:** There were no significant associations between any of the AGD or penile length measures and scores on the AQ-10 Child in boys, girls or the entire sample. **Conclusions:** The current study provides the first test of whether early measurements of AGD and/or penile length predict subsequent autistic traits. The current findings do not support a relationship between prenatal or early postnatal androgen exposure and autistic traits. The current study augments prior research showing no consistent relationship between early androgen exposure and autistic traits. **Keywords:** Anogenital distance; penile length; autism; autistic traits; gender; extreme male brain.

Introduction

It has been well-documented that males are more likely to receive a diagnosis of autism than are females, with 4:1 being the most frequently stated ratio (Halladay et al., 2015). It has also been reported that boys and men in the general population tend to score higher on measures of autistic traits than do girls and women (Allison et al., 2008; Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). By investigating mechanisms underlying the male preponderance, research may provide insights into aetiological processes of autism.

Theoretical background

A potential contributing factor is early androgen exposure. Experiments in nonhuman mammals show that androgen exposure during early critical windows exerts enduring masculinising and defeminising effects on neural and behavioural development (Arnold, 2009). The early critical windows correspond to times when androgen levels are higher in males than females, and the affected behavioural characteristics are those that show average sex differences (Arnold, 2009; Hines, 2020). In humans,

androgenic hormones, such as testosterone, are higher in male than female foetuses from about 8 to 24 weeks of gestation (Reyes, Boroditsky, Winter, & Faiman, 1974). Testosterone is also higher in males than females during early infancy, with the sex difference peaking at 1 to 3 months postnatal before testosterone declines to typical low childhood levels by 6 months of age (Forest, Sizonenko, Cathiard, & Bertrand, 1974; Winter, Hughes, Reyes, & Faiman, 1976). These prenatal and early postnatal periods are the presumed critical windows for human neurobehavioural sexual differentiation (Hines, 2020). Studies have related androgen exposure during these presumed critical windows to aspects of gender-related behaviour in humans, although research findings vary depending on the study populations, measures and outcomes (Hines, 1982, 2020).

In autism research, there are two related hypotheses about the role of gender and hormones. The first argues that autism is an extreme version of the male-typical cognitive profile and brain type (Baron-Cohen, 2002). According to this hypothesis, brain types are defined psychometrically by two constructs, systemising and empathising, and an 'extreme male brain' is characterised by increased systemising and decreased empathising (Baron-Cohen, 2002). The second hypothesis argues that

---

Conflict of interest statement: No conflicts declared.

heightened prenatal androgen exposure is the key neurobiological mechanism underlying the development of an 'extreme male brain' (Baron-Cohen, Knickmeyer, & Belmonte, 2005). The terminology, the extreme male brain theory of autism (EMB), has been used to refer to both the first hypothesis (Baron-Cohen, 2002) and the second hypothesis (McCarthy, 2019; Mottron et al., 2015; Whitehouse, 2016). The second hypothesis has also been referred to as the foetal testosterone theory of autism (Baron-Cohen, Auyeung, Ashwin, & Knickmeyer, 2009) and the prenatal steroid theory of autism (Baron-Cohen et al., 2019).

Prior studies on early androgen exposure and autistic traits

One way to test the hypothesis that prenatal androgen exposure relates to autism is to examine autistic traits in individuals with classic congenital adrenal hyperplasia (CAH). CAH is a rare genetic condition that causes unusually high levels of androgens prenatally in females but not in males (Wudy, Dörr, Solleder, Djalali, & Homoki, 1999). Females with CAH are usually born with ambiguous external genitalia due to the masculinising effects of androgens prenatally, whereas there is no genital ambiguity in males with CAH. If prenatal androgen exposure contributes to autistic traits, one would expect to find elevated autistic traits in females with CAH compared with unaffected females but unaltered autistic traits in males with CAH compared with unaffected males. Knickmeyer et al. (2006) and Kung, Spencer, et al. (2016) assessed autistic traits in individuals with CAH and their unaffected relatives (see Table S1 in Appendix S1 for details of the studies). Knickmeyer et al. (2006) found the expected pattern in a sample of adolescents and adults aged 12 to 45 years, supporting the hypothesis, although the elevation in autistic traits in females with CAH was only significant with a one-tailed statistical test at $\alpha = .05$. Also, in a sample of children aged 4 to 11 years, Kung, Spencer, et al. (2016) found no statistically significant increase in autistic traits in either girls or boys with CAH, suggesting no influences of prenatal androgen exposure. The substantially younger sample in Kung, Spencer, et al. (2016) may offer a more direct test of the influences of prenatal androgen exposure because there would have been less time for postnatal influences. In addition, it is possible that social challenges facing females with CAH may become more salient to them as they grow older, which might explain the slightly elevated autistic traits observed in female adolescents and adults with CAH in Knickmeyer et al. (2006). Whilst these studies offered some useful evaluation of the hypothesis, their focus on extreme prenatal androgen exposure in females with a genetic condition may limit the generalisability of the findings.

In an attempt to relate normal variability in prenatal androgen exposure to autistic traits in both males and females, some researchers have examined testosterone in amniotic fluid. Amniocentesis is typically performed during mid-gestation, which overlaps with the presumed prenatal critical window. Four studies have investigated the relationship between amniotic testosterone and autistic traits in children (Auyeung et al., 2009, 2012; Auyeung, Taylor, Hackett, & Baron-Cohen, 2010; Kung, Spencer, et al., 2016; see Table S2 in Appendix S1 for details of the studies). The first three studies (Allison, Auyeung, & Baron-Cohen, 2012; Auyeung et al., 2009, 2010, 2012) reported correlations in boys and in girls in the expected direction. Nevertheless, using highly similar methods, Kung, Spencer, et al. (2016) found no correlation[1] in either boys or girls and the nonsignificant correlations were not in the expected direction. These inconsistent findings may reflect limitations of amniotic testosterone as a measure of prenatal androgen exposure. Although amniotic testosterone has been described as a measure of foetal testosterone and is often assumed to be a proxy of prenatal androgen exposure (Baron-Cohen et al., 2005), one study found no correlation between testosterone in amniotic fluid and testosterone in foetal blood (Rodeck, Gill, Rosenberg, & Collins, 1985). Also, amniocentesis is typically only performed on pregnant women at increased risk of having a baby with a genetic condition and only a single amniotic fluid sample is collected. The measurement may reflect androgen exposure at only one time point during pregnancy, but not the entire critical window, and it is hard to assess generalisability or reliability. Hence, research measuring amniotic testosterone may struggle to establish robust and consistent effects.

Relevant research has also used umbilical cord blood. Based on a single longitudinal pregnancy cohort, two studies found no correlations between androgens in umbilical cord blood at birth and self-reported autistic traits, assessed using the Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001), in around 400 individuals aged 19 to 20 years (Jamnadass et al., 2015; Whitehouse et al., 2012). Whilst using umbilical cord blood enables researchers to take a relatively direct measurement from developing individuals in a general population sample, it has been acknowledged that androgens in umbilical cord blood may reflect androgen exposure during late gestation and may not address specifically the presumed critical window during mid-gestation (Jamnadass et al., 2015; Whitehouse et al., 2012).

Although much autism research has focused on the prenatal period, research in nonhuman animals suggests that testosterone during the early postnatal period may also masculinise subsequent development. Three studies have investigated the association between early postnatal testosterone measured

in saliva and later autistic traits (Auyeung et al., 2012; Kung, Constantinescu, Browne, Noorderhaven, and Hines (2016); Saenz & Alexander, 2013; see Table S3 in Appendix S1 for details of the studies). Two studies found little to no association between testosterone and autistic traits (Auyeung et al., 2012; Saenz & Alexander, 2013). However, neither of those studies found a sex difference in testosterone (Auyeung et al., 2012; Saenz & Alexander, 2013), perhaps because they collected saliva samples at 3 to 4 months postnatal after the peak of the postnatal testosterone surge at 1 to 3 months postnatal. Kung, Constantinescu, et al., (2016) measured testosterone in saliva during the peak of the postnatal surge and detected a sex difference in testosterone, but they found no correlation between testosterone and autistic traits in boys or girls. However, all three studies collected only one saliva sample from each participant, and the single measurement may not be a sufficiently sensitive indicator of androgen exposure throughout the postnatal surge.

Current study

Whilst prior studies have produced mixed findings, the relevant hypotheses remain popular. The EMB has been referred to as the most pre-eminent theory for gender-related differences in autism (Mottron et al., 2015), although researchers continue to raise questions (McCarthy, 2019; Whitehouse, 2016). Also, whilst studies examining the early postnatal period have reported negative findings, only one of those studies (Kung, Constantinescu, et al., 2016) looked at the peak of the postnatal surge. There is a need for further research employing measures of early androgen exposure that can address both the prenatal and the early postnatal periods more effectively. The current study aims to do so, using measurements of anogenital distance (AGD) and penile length, which are putative biomarkers of early androgen exposure.

AGD has been shown to be sensitive to prenatal androgen exposure in nonhuman animals, as well as to prenatal exposure to chemicals associated with androgen disruption in human epidemiological studies (Thankamony, Pasterski, Ong, Acerini, & Hughes, 2016). In humans, AGD at birth is approximately 1.4 times to 2.2 times longer in males than females (Thankamony et al., 2016). The sex difference in AGD can already be observed in human foetuses at 11 to 13 weeks of gestation, and the sex difference increases to a magnitude similar to that at birth by 17 to 20 weeks of gestation (Thankamony et al., 2016). Similarly, in regard to androgen-dependent penile development, the external genitalia are fully differentiated by around 12 weeks of gestation and penile growth appears to be more intense during mid-gestation than late gestation (Gallo, Costa, Furriel, Bastos, & Sampaio, 2013; Johnson

& Maxwell, 2000). In addition, both AGD and penile length increase rapidly during the first 3 months of life, which corresponds to the early postnatal testosterone surge (Thankamony, Ong, Dunger, Acerini, & Hughes, 2009). Penile growth during this period also relates positively to testosterone concentrations at age 3 months (Boas et al., 2006). Therefore, AGD and penile length at birth are influenced by androgen exposure during the prenatal critical window, and penile growth during the first 3 months of life relates to androgen exposure during the postnatal surge.

The current study provides the first test of whether anogenital distance (AGD) at birth, at 3 months of age or its growth between these time points relates to autistic traits in boys and in girls. In boys, penile length at birth, at 3 months of age, its growth between these time points and their relations to autistic traits were also assessed. Autistic traits were measured using a parent-reported questionnaire when the children were 9 to 13 years old.

Method
Participants

This study included 97 boys and 110 girls recruited from the Cambridge Baby Growth Study (CBGS), an ongoing prospective study designed to investigate different types of influences on infant growth and early male reproductive development (Prentice et al., 2016). In the larger CBGS, mothers were recruited from the maternity unit of Addenbrooke's Hospital in Cambridge, UK, at around 12 weeks of gestation when they attended for routine prenatal ultrasound scan, between 2006 and 2008. The research protocol was approved by the Cambridge Local Research Ethics Committee, and the study was conducted in accordance with the standards for Good Clinical Practice. Postnatal initial anthropometric measurements were taken either at the hospital or at the participant's home, and subsequent measurements were taken in a dedicated follow-up research clinic. Details of the larger CBGS, including findings of the anthropometric measures, were reported in Thankamony et al. (2009). When the children were aged between 9 and 13 years ($M = 11.23$, $SD = 0.80$), their mothers were invited to participate in the current study. Because this follow-up study focuses on the influences of early androgen exposure on behavioural development in boys and girls, only mothers of children with data of AGD at birth were invited. The mothers gave written informed consent for themselves and their children to participate in the larger CBGS and the current study. A parent-reported autistic traits questionnaire was completed for 207 of the 708 invitees. Of the 207 children, the majority were Caucasian ($n = 191$) and the rest were Black ($n = 2$), Indian ($n = 2$), in the 'Other' category ($n = 5$) or did not provide information about ethnicity ($n = 7$). Analyses comparing the 207 continuing and the 501 attrited participants suggest that the two groups were largely similar (see Appendix S2 for details).

Measures

Predictors. AGD and penile length at birth and 3 months of age were measured by a research nurse trained with standardised procedure using Vernier callipers. AGD was measured from the centre of the anus to the base of the scrotum in males and to the posterior fourchette in females. Penile length was measured from the lower edge of the pubic bone to the tip

of the flaccid penis. Growth (Δ) in AGD and penile length was calculated by subtracting the value at birth from the value at age 3 months. At each interval, three consecutive measurements were taken, and the mean was used for analysis. Interobserver variability was assessed by calculating technical error of measurement (TEM; Ulijaszek & Kerr, 1999) in the larger CBGS. The absolute and relative TEM values for AGD were 3 mm and 9.6% in males and 1 mm and 5.7% in females.

Outcome. Autistic traits were assessed by the 10-item children's version of the Autism Spectrum Quotient (AQ-10 Child; Allison, Auyeung, & Baron-Cohen, 2012). The AQ-10 Child is a parent-reported questionnaire developed to assess autistic traits and detect autism in children aged 4 to 11 years. The items are answered on a 4-point Likert scale (definitely agree, slightly agree, slightly disagree and definitely disagree). Of the 10 items, 6 are reverse-scored. All items are scored in a binary format, 1 for a response in the 'autistic' direction and 0 for a 'nonautistic' response (Allison et al., 2012). The score range is 0–10. The cut-off score (at or above 6) has a sensitivity of .95 and specificity of .97 (Allison et al., 2012). The AQ-10 Child correlates strongly ($r = .94$) with the 50-item long version of AQ Child (Auyeung, Baron-Cohen, Wheelwright, & Allison, 2008), which has a good test–retest reliability ($r = .85$; Auyeung et al., 2008). In the current sample, internal consistency was satisfactory (overall: Cronbach's α = .64; boys: Cronbach's α = .63; girls: Cronbach's α = .65).

Control variables. Gestational age at birth, maternal age at delivery and child's age at autistic traits assessment were included as control variables. In addition to AGD and penile length, children's weight and body length at birth and age 3 months and growth (Δ) in weight and body length between the two time points were measured, so that other aspects of general body growth could be taken into account.

Analytical approach

Following prior research on AGD in the CBGS (Thankamony et al., 2009), this study excluded preterm (<37 gestation weeks) and low-birthweight children (weight < 2.5 kg) from analysis. Four boys and 3 girls were excluded. Of the remaining 200 children, 197 had complete data for the AQ-10 Child, 2 children had missing data for 1 item, and 1 child had missing data for 2 items. The missing values were replaced using person-mean substitution based on the child's mean scores on the other items. All statistical analyses were two-tailed, with alpha set at .05.

Results

Descriptive statistics and inferential statistics regarding differences between boys and girls are summarised in Table 1. Independent *t*-tests show that, compared with girls, boys had significantly higher AGD at birth, AGD at 3 months, Δ AGD, weight at 3 months, Δ Weight, body length at birth, body length at 3 months and Δ body length. Although boys scored somewhat higher on the AQ-10 Child than did girls, the difference was not statistically significant. Pearson's correlations were conducted to relate predictor and control variables to the AQ-10 Child. The correlational statistics are summarised in Table 2. None of the correlations were significant in boys, girls or the entire sample; there were no significant associations between any of the early androgen predictor variables or control

variables and the AQ-10 Child. To address the possibility that the two periods act together to shape autistic traits (i.e. high levels of androgen exposure during both periods, but not just one period, contribute to autistic traits), regression models with interaction terms entered were conducted and these regression analyses yielded nonsignificant results (see Section 1 of Appendix S3 for details).

Seven boys and 7 girls scored at or above the cut-off (high scoring). Binomial logistic regressions were conducted to see whether the predictor variables were associated with high scoring. No significant bivariate or interaction effects were found in boys, girls or the entire sample (see Section 2 of Appendix S3 for details). Inspection of descriptive statistics shows that approximately half of these high-scoring children had values on predictor variables that were below the mean, suggesting no consistent patterns in the levels of early androgen exposure in these high-scoring children.

Children in the current sample were between 9 and 13 years of age. Since the AQ-10 Child was developed in children aged 4 to 11 years, all the above analyses were repeated after excluding children aged 12 years or above. Analyses based on the remaining sample, 78 boys and 91 girls aged between 9 and 11 years, yielded highly consistent results suggesting no significant association between early androgen exposure and autistic traits. Analyses were also repeated using AGD and penile length values adjusted for maternal age, gestational age, weight and body length, and these analyses also yielded highly consistent results suggesting no significant association between early androgen exposure and autistic traits.

In a set of additional analyses, the AQ-10 Child was scored using an alternative method and showed improved internal consistency and a significant gender difference in the expected direction. However, these additional analyses also suggest no relationship between early androgen exposure and autistic traits (see Section 3 of Appendix S3 for details).

Discussion

The current study tested whether androgen exposure during the presumed prenatal and early postnatal critical windows contributes to the development of autistic traits. AGD and penile length at birth and age 3 months and their growth between birth and age 3 months were used as measures of early androgen exposure. The current study is the first to relate these measures of early androgen exposure to autistic traits. There were no associations between measures of AGD and autistic traits in boys or girls, or between measures of penile length and autistic traits in boys. These findings augment prior research showing no relationship between autistic traits and other measures of early androgen exposure including testosterone in amniotic fluid, umbilical cord

Table 1 Descriptive statistics for boys, girls and the overall sample and inferential statistics for differences between boys and girls

	Boys (B)		Girls (G)		Overall		B vs. G	
	n	M (SD)	n	M (SD)	n	M (SD)	t	d
AQ-10 Child	93	2.44 (1.94)	107	2.07 (1.87)	200	2.24 (1.91)	1.34	0.20[ns]
AGD at birth (mm)	93	18.99 (5.43)	107	9.04 (2.77)	200	13.67 (6.52)	16.62	2.36***
AGD at 3 months (mm)	93	28.05 (7.35)	103	12.71 (2.84)	196	19.99 (9.41)	19.63	2.83***
Δ AGD (mm)	93	9.06 (7.72)	103	3.60 (3.33)	196	6.19 (6.43)	6.53	0.94***
Penile length at birth (cm)	92	3.05 (0.44)	–	–	–	–	–	–
Penile length at 3 months (cm)	93	3.41 (0.52)	–		–	–	–	–
Δ Penile length (cm)	92	0.34 (0.50)	–	–	–	–	–	–
Weight at birth (kg)	93	3.61 (0.46)	107	3.52 (0.43)	200	3.56 (0.44)	1.44	0.20[ns]
Weight at 3 months (kg)	93	6.36 (0.75)	105	5.82 (0.65)	198	6.08 (0.74)	5.39	0.77***
Δ Weight (kg)	93	2.75 (0.60)	105	2.30 (0.54)	198	2.51 (0.61)	5.51	0.79***
Body length at birth (cm)	93	51.80 (2.31)	105	50.89 (2.13)	198	51.32 (2.26)	2.88	0.41**
Body length at 3 months (cm)	93	61.96 (2.27)	103	60.42 (2.09)	196	61.15 (2.30)	4.92	0.71***
Δ Body length (cm)	93	10.16 (1.96)	101	9.47 (1.93)	194	9.80 (1.97)	2.45	0.35*
Gestation age (weeks)	93	40.25 (1.24)	107	40.03 (1.12)	200	40.13 (1.18)	1.27	0.19[ns]
Maternal age at birth (years)	93	33.73 (3.64)	106	33.76 (4.32)	199	33.74 (4.05)	−0.05	−0.01[ns]
Child's age at follow-up (years)	93	11.26 (0.78)	107	11.20 (0.82)	200	11.23 (0.80)	0.47	0.07[ns]

Independent-samples t-tests were conducted. Positive ds indicate higher values in males. *$p < .05$; **$p < .01$; ***$p < .001$. [ns] indicates nonsignificant results, with p-values ranging from .15 to .96.

Table 2 Pearson's correlations relating predictor and control variables to AQ-10 Child

	Correlations with AQ-10 Child					
	Boys (B)		Girls (G)		Overall	
	n	r	n	r	n	r
AGD at birth	93	.09[ns]	107	.01[ns]	200	.11[ns]
AGD at 3 months	93	.06[ns]	103	.08[ns]	196	.12[ns]
Δ AGD	93	.00[ns]	103	.07[ns]	196	.06[ns]
Penile length at birth	92	−.10[ns]	–	–	–	–
Penile length at 3 months	93	−.04[ns]	–	–	–	–
Δ Penile length	92	.02[ns]	–	–	–	–
Weight at birth	93	.04[ns]	107	.11[ns]	200	.08[ns]
Weight at 3 months	93	.09[ns]	105	.09[ns]	198	.11[ns]
Δ Weight	93	.08[ns]	105	.02[ns]	198	.08[ns]
Body length at birth	93	.11[ns]	105	.04[ns]	198	.09[ns]
Body length at 3 months	93	.09[ns]	103	.14[ns]	196	.14[†]
Δ Body length	93	−.03[ns]	101	.13[ns]	194	.06[ns]
Gestation age	93	.11[ns]	107	.12[ns]	200	.12[†]
Maternal age at birth	93	−.04[ns]	106	−.12[ns]	199	−.09[ns]
Child's age at follow-up	93	.19[†]	107	−.11[ns]	200	.04[ns]

[†]$p < .10$. [ns] indicates nonsignificant results, with p-values ranging from .11 to .99.

blood and saliva during infancy (Jamnadass et al., 2015; Kung, Constantinescu, et al., 2016; Kung, Spencer, et al., 2016; Whitehouse et al., 2012). The current study is also consistent with a prior report that girls exposed to unusually high levels of prenatal androgen exposure because of CAH did not score higher on a measure of autistic traits (Kung, Spencer, et al., 2016). Taken together, these findings suggest that it is unlikely that early androgen exposure plays a major role in shaping the development of autistic traits.

Although boys scored somewhat higher on the autistic traits measure than did girls in the current study, the difference was not statistically significant when the original scoring method was employed. Some prior research also found no gender difference in autistic traits in children (e.g. Kung, Spencer, et al., 2016; Saenz & Alexander, 2013). In addition, prior studies measuring autistic traits in children have not consistently reported information related to gender differences. It is therefore hard to evaluate the consistency or magnitude of gender differences shown by the relevant autistic traits measures. According to research in nonhuman animals (Arnold, 2009; Hines, 1982, 2020), the behavioural influences of early androgen exposure appear to be limited to characteristics that differ between males and females. The lack of a consistent gender difference in autistic traits in children challenges the theoretical foundation of the proposed link between early androgen exposure and autistic traits, as well as the approach for using gender

differences in autistic traits to study gender differences in autism.

It has been suggested that other hormones might contribute to the male preponderance in autism. One study found that scores on a latent factor representing shared variance of five hormones measured in amniotic fluid (cortisol, 17a-hydroxy-progesterone, androstenedione, progesterone and testosterone) were higher in 128 males diagnosed with autism than 217 matched male controls (Baron-Cohen et al., 2015), although calculations based on descriptive statistics provided in the study suggested no statistically significant difference in amniotic testosterone between the two groups. Based on a subsample from the same cohort (98 males with autism and 177 control males), another study reported elevated amniotic oestrogens in the males with autism (Baron-Cohen et al., 2019). The elevated amniotic hormones were suggested to have masculinised the brain (Baron-Cohen et al., 2015, 2019). However, the finding suggesting a link between amniotic oestrogens and autism in males contradicts prior research showing no correlations between amniotic oestradiol and autistic traits in boys or girls (Auyeung et al., 2010, 2012) or between umbilical cord blood oestrogens and autistic traits in men or women (Jamnadass et al., 2015). Furthermore, it is unclear how these varied hormones might act together to influence human neurobehavioural sexual differentiation. Cortisol, 17a-hydroxy-progesterone, androstenedione, progesterone and testosterone are relatively distant from one another along the biosynthetic pathway, and there is no strong evidence supporting the hypothesis that oestrogens masculinise human brain development (McCarthy, 2019). It has been suggested that the reported elevation in these hormones in males with autism might reflect placental dysfunction or maternal disrupted steroid metabolism, rather than masculinisation effects (McCarthy, 2019). Further research is needed to replicate the association between elevated amniotic hormones and autism in both males and females and to investigate the mechanisms via which elevated concentrations of hormones, other than androgens, in amniotic fluid, might contribute to autism.

The current study might be limited by its sample size. However, significant correlations between measures of early androgen exposure and autistic traits have been reported based on much smaller samples (Auyeung et al., 2010, 2012; Saenz & Alexander, 2013; see Tables S2 and S3 for details). Moreover, some of the correlations in the current study were not in the expected direction.

Following most of the prior studies on this topic, the current study focuses on autistic traits scores instead of autism diagnosis. The number of high-scoring individuals is small in the current study, and individuals with a high score on the AQ-10 Child do not necessarily have an autism diagnosis. It therefore remains possible that early measurements of AGD and penile length or other early hormonal measures may predict autism diagnosis. In addition, the structure and validity of versions of the AQ as measures of autistic traits in the general population have been challenged (English, Gignac, Visser, Whitehouse, & Maybery, 2020; Lundin, Kosidou, & Dalman, 2019). Nonetheless, versions of the AQ have been widely employed to assess autistic traits, and the use of the AQ-10 Child and its original scoring method in the current study enables comparisons across studies.

Another methodological concern is that AGD has been associated with exposure to endocrine disrupting chemicals (Thankamony et al., 2016), which may affect the actions and production of androgens and other hormones. Further research may usefully examine the relations among exposure to endocrine disrupting chemicals, AGD and autistic traits. Further research may also assess autistic traits at a younger age, because autistic traits may be influenced by sociocognitive experience and the effects of early androgen exposure on autistic traits may be more salient earlier in life.

A strength of the current study is that it employed measures that reflect the cumulative effects of early androgen exposure during both the presumed prenatal and early postnatal critical windows. Different measures have somewhat different strengths and weaknesses (for details, see Introduction), so it is important to assess early androgen exposure using a range of methods when addressing research questions. Because AGD and penile length can be used to assess both prenatal androgen exposure and early postnatal androgen exposure, these measures enable researchers to evaluate the relative importance of the two presumed critical windows. Also, since the measurements at birth may be influenced by placental or maternal hormones during pregnancy, the inclusion of postnatal measurements at 3 months in the current study enables more specific assessments of early influences of hormones made by the child. Although the current study shows no relationship between AGD or penile length and autistic traits, a prior report from the same cohort found that AGD at birth and penile growth between birth and 3 months of age positively and independently predicted male-typical play behaviour in preschool boys (Pasterski et al., 2015). Further research may usefully evaluate whether early measurements of AGD and penile length can predict gender-related differences in disorder-relevant traits and psychiatric conditions in general population samples. Compared with hormonal assays and procedures such as amniocentesis, measuring AGD and penile length is relatively noninvasive and inexpensive and can be done routinely at hospitals and clinics, and thus, these putative biomarkers of early androgen exposure may be included in future large-scale longitudinal population studies.

Conclusion

The current study found no associations between early measurements of AGD or penile length and subsequent autistic traits in boys or girls. Accumulating evidence from research studies using different methods and study populations suggests that there is no clear relationship between early androgen exposure and autistic traits. This body of research suggests that the hypothesised relationship is likely to be weak or nonexistent. Further research may continue to explore the use of AGD and penile length in assessing early androgen exposure and predicting autism diagnosis and other gender-related psychopathology.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1. Details of prior studies on early androgen exposure and autistic traits.
Appendix S2. Comparisons between continuing and attrited participants.
Appendix S3. Supplementary analyses and findings.

Correspondence

Karson Kung, Department of Psychology, University of Hong Kong, Pokfulam, Hong Kong; Email: karson.kung@hku.hk

---

Key points

- **What's known?** Some prior research suggests that early androgen exposure contributes to autistic traits.
- **What's new?** This is the first study relating early measurements of anogenital distance and penile length, which are putative biomarkers of androgen exposure, to autistic traits. Repeated measurements were taken to assess both the prenatal and the early postnatal periods. The current study found no significant relationship between prenatal or early postnatal androgen exposure and autistic traits.
- **What's relevant?** The current study highlights the need to exercise caution when communicating any potential effects of early androgen exposure on risk of autism. Measurements of anogenital distance and penile length, which are relatively noninvasive and inexpensive, may be included in larger future studies.

Note

1. In this report, 'no correlation/association/difference' and other similar expressions indicate statistically nonsignificant effects but not zero effects.

References

Allison, C., Auyeung, B., & Baron-Cohen, S. (2012). Toward brief "red flags" for autism screening: The short autism spectrum quotient and the short quantitative checklist in 1,000 cases and 3,000 controls. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*, 202–212.

Allison, C., Baron-Cohen, S., Wheelwright, S., Charman, T., Richler, J., Pasco, G., & Brayne, C. (2008). The Q-CHAT (Quantitative CHecklist for Autism in Toddlers): A normally distributed quantitative measure of autistic traits at 18–24 months of age: Preliminary report. *Journal of Autism and Developmental Disorders*, *38*, 1414–1425.

Arnold, A.P. (2009). The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Hormones and Behavior*, *55*, 570–578.

Auyeung, B., Ahluwalia, J., Thomson, L., Taylor, K., Hackett, G., O'Donnell, K.J., & Baron-Cohen, S. (2012). Prenatal versus postnatal sex steroid hormone effects on autistic traits in children at 18 to 24 months of age. *Molecular Autism*, *3*, 17.

Auyeung, B., Baron-Cohen, S., Chapman, E., Knickmeyer, R., Taylor, K., & Hackett, G. (2009). Foetal testosterone and autistic traits. *British Journal of Psychology*, *100*, 1–22.

Auyeung, B., Baron-Cohen, S., Wheelwright, S., & Allison, C. (2008). The Autism Spectrum Quotient: Children's version (AQ-Child). *Journal of Autism and Developmental Disorders*, *38*, 1230–1240.

Auyeung, B., Taylor, K., Hackett, G., & Baron-Cohen, S. (2010). Foetal testosterone and autistic traits in 18 to 24-month-old children. *Molecular Autism*, *1*, 11.

Baron-Cohen, S. (2002). The extreme male brain theory of autism. *Trends in Cognitive Sciences*, *6*, 248–254.

Baron-Cohen, S., Auyeung, B., Ashwin, E., & Knickmeyer, R. (2009). Fetal testosterone and autistic traits: A response to three fascinating commentaries. *British journal of Psychology, 100*, 39–47.

Baron-Cohen, S., Auyeung, B., Nørgaard-Pedersen, B., Hougaard, D.M., Abdallah, M.W., Melgaard, L., ... & Lombardo, M.V. (2015). Elevated fetal steroidogenic activity in autism. *Molecular Psychiatry, 20*, 369–376.

Baron-Cohen, S., Knickmeyer, R., & Belmonte, M.K. (2005). Sex differences in the brain: Implications for explaining autism. *Science, 310*, 819–823.

Baron-Cohen, S., Tsompanidis, A., Auyeung, B., Nørgaard-Pedersen, B., Hougaard, D.M., Abdallah, M., ... & Pohl, A. (2019). Foetal oestrogens and autism. *Molecular Psychiatry.* https://doi.org/10.1038/s41380-019-0454-9

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The Autism Spectrum Quotient (AQ): Evidence from Asperger syndrome/high functioning autism, males and females, scientists and mathematicians. *Journal of Autism and Developmental Disorders, 31*, 5–17.

Boas, M., Boisen, K.A., Virtanen, H.E., Kaleva, M., Suomi, A.-M., Schmidt, I.M., ... & Main, K.M. (2006). Postnatal penile length and growth rate correlate to serum testosterone levels: A longitudinal study of 1962 normal boys. *European Journal of Endocrinology, 154*, 125–129.

English, M.C., Gignac, G.E., Visser, T.A., Whitehouse, A.J., & Maybery, M.T. (2020). A comprehensive psychometric analysis of autism-spectrum quotient factor models using two large samples: Model recommendations and the influence of divergent traits on total-scale scores. *Autism Research, 13*, 45–60.

Forest, M.G., Sizonenko, P.C., Cathiard, A.M., & Bertrand, J. (1974). Hypophyso-gonadal function in humans during the first year of life: I. Evidence for testicular activity in early infancy. *Journal of Clinical Investigation, 53*, 819–828.

Gallo, C.B., Costa, W.S., Furriel, A., Bastos, A.L., & Sampaio, F.J. (2013). Development of the penis during the human fetal period (13 to 36 weeks after conception). *Journal of Urology, 190*, 1876–1883.

Halladay, A.K., Bishop, S., Constantino, J.N., Daniels, A.M., Koenig, K., Palmer, K., ... & Taylor, J.L. (2015). Sex and gender differences in autism spectrum disorder: Summarizing evidence gaps and identifying emerging areas of priority. *Molecular Autism, 6*, 36.

Hines, M. (1982). Prenatal gonadal hormones and sex differences in human behavior. *Psychological Bulletin, 92*, 56–80.

Hines, M. (2020). Neuroscience and sex/gender: Looking back and forward. *Journal of Neuroscience, 40*, 37–43.

Jamnadass, E.S.L., Keelan, J.A., Hollier, L.P., Hickey, M., Maybery, M.T., & Whitehouse, A.J.O. (2015). The perinatal androgen:estrogen ratio and autistic-like traits in the general population: A longitudinal pregnancy cohort study. *Journal of Neurodevelopmental Disorders, 7*, 17.

Johnson, P., & Maxwell, D. (2000). Fetal penile length. *Ultrasound in Obstetrics and Gynecology, 15*, 308–310.

Knickmeyer, R.C., Baron-Cohen, S., Fane, B.A., Wheelwright, S., Mathews, G.A., Conway, G.S., & Hines, M. (2006). Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia. *Hormones and Behavior, 50*, 148–153.

Kung, K.T.F., Constantinescu, M., Browne, W.V., Noorderhaven, R.M., & Hines, M. (2016). No relationship between early postnatal testosterone and autistic Traits in 18 to 30-month-old Children. *Molecular Autism, 7*, 15.

Kung, K.T.F., Spencer, D., Pasterski, V., Neufeld, S., Glover, V., O'Connor, T.G., ... & Hines, M. (2016). 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. *Journal of Child Psychology and Psychiatry, 57*, 1455–1462.

Lundin, A., Kosidou, K., & Dalman, C. (2019). Measuring autism traits in the adult general population with the brief autism-spectrum quotient, AQ-10: Findings from the Stockholm public health cohort. *Journal of Autism and Developmental Disorders, 49*, 773–780.

McCarthy, M. (2019). Extreme male brain theory of autism rests on shaky ground. Available from: https://www.spectrumnews.org/opinion/viewpoint/extreme-male-brain-theory-of-autism-rests-on-shaky-ground/.

Mottron, L., Duret, P., Mueller, S., Moore, R.D., Forgeot, B., Jacquemont, S., ... & Xiong, L. (2015). Sex differences in brain plasticity: A new hypothesis for sex ratio bias in autism. *Molecular Autism, 6*, 33.

Pasterski, V., Acerini, C.L., Dunger, D.B., Ong, K.K., Hughes, I.A., Thankamony, A., & Hines, M. (2015). Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: Evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. *Hormones and Behavior, 69*, 98–105.

Prentice, P., Acerini, C.L., Eleftheriou, A., Hughes, I.A., Ong, K.K., & Dunger, D.B. (2016). Cohort profile: The Cambridge baby growth study (CBGS). *International Journal of Epidemiology, 45*, 35.

Reyes, F.I., Boroditsky, R.S., Winter, J.S.D., & Faiman, C. (1974). Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. *Journal of Clinical Endocrinology & Metabolism, 38*, 612–617.

Rodeck, C.H., Gill, D., Rosenberg, D.A., & Collins, W.P. (1985). Testosterone levels in mid-trimester maternal and foetal plasma and amniotic fluid. *Prenatal Diagnosis, 5*, 175–181.

Saenz, J., & Alexander, G.M. (2013). Postnatal testosterone levels and disorder relevant behavior in the second year of life. *Biological Psychology, 94*, 152–159.

Thankamony, A., Ong, K.K., Dunger, D.B., Acerini, C.L., & Hughes, I.A. (2009). Anogenital distance from birth to 2 years: A population study. *Environmental Health Perspectives, 117*, 1786–1790.

Thankamony, A., Pasterski, V., Ong, K.K., Acerini, C.L., & Hughes, I.A. (2016). Anogenital distance as a marker of androgen exposure in humans. *Andrology, 4*, 616–625.

Ulijaszek, S.J., & Kerr, D.A. (1999). Anthropometric measurement error and the assessment of nutritional status. *British Journal of Nutrition, 82*, 165–177.

Whitehouse, A.J. (2016). Commentary: Are we expecting too much from the extreme male brain theory of autism? A reflection on Kung et al (2016). *Journal of Child Psychology and Psychiatry, 57*, 1463–1464.

Whitehouse, A.J.O., Mattes, E., Maybery, M.T., Dissanayake, C., Sawyer, M., Jones, R.M., ... & Hickey, M. (2012). Perinatal testosterone exposure and autistic-like traits in the general population: A longitudinal pregnancy-cohort study. *Journal of Neurodevelopmental Disorders, 4*, 25.

Winter, J.S., Hughes, I.A., Reyes, F.I., & Faiman, C. (1976). Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *Journal of Clinical Endocrinology and Metabolism, 42*, 679–686.

Wudy, S.A., Dörr, H.G., Solleder, C., Djalali, M., & Homoki, J. (1999). Profiling steroid hormones in amniotic fluid of midpregnancy by routine stable isotope dilution/gas chromatography-mass spectrometry: Reference values and concentrations in fetuses at risk for 21-hydroxylase deficiency 1. *Journal of Clinical Endocrinology and Metabolism, 84*, 2724–2728.