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Associations Between the 2nd to 4th Digit Ratio and Autism Spectrum Disorder in Population-Based Samples of Boys and Girls: Findings from the Study to Explore Early Development

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Abstract

The ratio of the index (2nd) finger to ring (4th) finger lengths (2D:4D) is a proxy for fetal testosterone and estradiol. Studies suggesting 2D:4D is inversely associated with autism spectrum disorder (ASD) in males were limited by lack of confounder and subgroup assessments. Studies of females are sparse. We examined associations between ASD and 2D:4D among children in the Study to Explore Early Development; we considered case subgroups and numerous potential demographic and maternal-perinatal health confounders. We observed a modest inverse association between ASD and right-hand 2D:4D in males; subgroup analyses indicated associations were limited to ASD cases with birth defects/genetic syndromes or dysmorphic features. We observed a positive association between ASD and left-hand 2D:4D in females, overall and within most case subgroups.

Keywords

Autism spectrum disorder; Testosterone; Estradiol; Fetal development

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Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all participants in this study.

Conflict of interest All authors report no conflicts of interest for this study.

Introduction

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental condition estimated to occur in 1–2% of children (Christensen et al. 2016; Blumberg et al. 2013). The defining characteristics of ASD include social and communication deficits and restricted stereotyped patterns of behavior. Population prevalence studies indicate a marked male:female sex ratio with the ASD prevalence 4–5 times higher in males than females (Christensen et al. 2016; Blumberg et al. 2013).

While much remains unknown about the underlying causes of ASD, available evidence strongly indicates it has a complex, multifactorial etiology with involvement of both genetic and environmental factors (Miles 2011; Kim and Leventhal 2015; Ornoy et al. 2016; Arndt et al. 2005). Studies also implicate the prenatal period as the critical window of exposure (Arndt et al. 2005). Given the core neurodevelopmental features and marked male predominance of ASD, one etiologic hypothesis put forth by Baron-Cohen and colleagues (2011) is the “Extreme Male Brain” (EMB) theory. EMB theory asserts that individuals with ASD have a very high systemizing drive and very low empathizing drive, which is caused by exposure to high concentrations of testosterone during fetal development.

Fetal testosterone has been linked to neurodevelopment in both animal and human studies. Animal studies demonstrate that exposure to high concentrations of testosterone can impact brain structure, cognition, and behavior (Taylor et al. 2017; Jacome et al. 2016; Hu et al. 2015; Dela Cruz and Pereira 2012; Thornton et al. 2009). Human studies, which necessarily examined fetal testosterone concentrations within a more limited range (i.e., the typical range of prenatal variability), are also suggestive of impacts on neurodevelopment, including several traits related to ASD. These include inverse associations between amniotic testosterone concentration and eye contact at 12 months (Lutchmaya et al. 2002a) and 18 months (Saenz and Alexander 2013), vocabulary size at 18–24 months (Lutchmaya et al. 2002b), social cognition at 4 years (Knickmeyer et al. 2005) and level of empathy at 4 and 8 years (Chapman et al. 2006; Knickmeyer et al. 2006) and positive associations between amniotic testosterone and non-social domains, such as restricted interests, at 4 years (Knickmeyer et al. 2005). Additionally, a recent study demonstrated that amniotic levels of several steroidal hormones, including testosterone, were elevated in individuals who went on to develop ASD (Baron-Cohen et al. 2015).

Other studies have assessed associations between the ratio of the length of the index finger (2nd digit) to the ring finger (4th digit) (2D:4D) as a proxy for fetal testosterone concentration. In utero development of the metacarpals and phalangeal bones of the hands are regulated by the same *HOX* cluster genes that regulate development of the male reproductive tract, as evidenced by mutations in *HOXA13* and *HOXD13* causing hand-foot-genital syndrome (Quinonez and Innis 2014). These genes respond to circulating androgens (Kondo et al. 1997; Manning and Bundred 2000; Zheng and Cohn 2011). Specifically, fetal testosterone stimulates elongation of the 4th digit relative to the 2nd digit, such that the 2D:4D is inversely associated with fetal testosterone concentration. Additionally there is a suggestion that 2D:4D may be positively associated with fetal estradiol concentration and

inversely associated with the ratio of fetal testosterone to estradiol (Manning et al. 1998; Lutchmaya et al. 2004).

Given the difficulty in obtaining amniotic specimens, 2D:4D is a key proxy for fetal testosterone and possibly fetal estradiol concentrations. The validity of 2D:4D as a proxy is supported by limited direct empiric evidence (Lutchmaya et al. 2004; Ventura et al. 2013) and indirect evidence of observed inverse associations between 2D:4D and both male sex (McIntyre 2006; Manning and Bundred 2000; Zheng and Cohn 2011; Ventura et al. 2013) and congenital adrenal hyperplasia among females (McIntyre 2006). While limited, the available evidence generally supports that 2D:4D is likely a superior proxy to other biologic measurements, such as umbilical cord or gravid maternal serum testosterone concentrations, which have both been found to be poorly correlated with fetal amniotic testosterone concentrations at critical periods of development (McIntyre 2006; van de Beek et al. 2004).

The findings from studies examining associations between 2D:4D and ASD have been inconsistent. While most studies of male samples (Al-Zaid et al. 2015; Masuya et al. 2015; Krajmer et al. 2011; de Bruin et al. 2006) or samples in which the vast majority of participants were male (Noipayak 2009; Sugie et al. 2010; Milne et al. 2006; Manning et al. 2001) have reported inverse associations between 2D:4D and ASD diagnosis, other studies of males reported no association between 2D:4D and ASD diagnosis (Falter et al. 2008) or reported a positive association (Bejerot et al. 2012). A related study reported no association between 2D:4D and scores on standardized assessments of autistic traits in males (Barona et al. 2015); however, in that study, the authors did observe an inverse association in post hoc analyses examining the most extreme digit ratio measure—digit ratio in the lowest 10th percentile (Barona et al. 2015).

Even among studies showing an association, there were inconsistencies and limited data. Among eight studies reporting inverse associations between ASD or autistic traits and 2D:4D in male or nearly-all male samples, two reported associations with right-hand 2D:4D but not left-hand (Barona et al. 2015; Masuya et al. 2015); two reported associations with both hands (Noipayak 2009; de Bruin et al. 2006); two assessed right hand only (Al-Zaid et al. 2015; Sugie et al. 2010); and two assessed the average of right hand 2D:4D and left-hand 2D:4D but did not present separate results for each hand (Milne et al. 2006; Manning et al. 2001). The study reporting a positive association between ASD and 2D:4D reported similar results for both hands (Bejerot et al. 2012). A meta-analysis that included several of the aforementioned studies reported a significant inverse association between ASD and right-hand 2D:4D but not left hand (Teatero and Netley 2013).

There is a paucity of studies examining associations between ASD and 2D:4D in females. Of the limited studies that separately assessed samples of females, results have been inconsistent; one found no association between ASD and 2D:4D of either hand (Bejerot et al. 2012), one showed no association between autistic traits and 2D:4D of either hand (Barona et al. 2015), and one showed a positive association between ASD and right-hand 2D:4D but no association with left-hand 2D:4D (Masuya et al. 2015).

Digit ratio studies among both males and females must be interpreted in context of several important methodologic limitations. The majority of studies included small samples of children or adults (< 100 ASD cases) recruited from clinics, schools, or ASD support organizations with varying methods and rigor in defining ASD. Assessment of potential confounding factors was very limited. Only one study (of autistic traits as measured on the Social Communication Questionnaire) adjusted for sociodemographic factors; and none of the studies to date considered other important biologic pregnancy exposures that might influence fetal testosterone and/or estradiol concentrations. Although data are limited or not available on direct fetal testosterone effects in humans, and as noted above umbilical cord and maternal prenatal testosterone levels are not necessarily reflective of fetal testosterone exposure, numerous maternal and perinatal factors should nonetheless be investigated as potential confounders given there has been some suggestion of an association with either fetal or maternal testosterone or estradiol concentrations. These include maternal smoking in pregnancy (Rizwan et al. 2007; Toriola et al. 2011), maternal infertility conditions, such as polycystic ovarian syndrome (Dumesic et al. 2014; Makieva et al. 2014; Abbott et al. 2012), conception after ovarian stimulation treatments (Järvelä et al. 2014), prenatal progesterone treatments (Pointis et al. 1987), advanced maternal age (Toriola et al. 2011), parity (Toriola et al. 2011), race-ethnicity (Knickmeyer et al. 2011), preterm birth (Makieva et al. 2014) and multiple gestation pregnancy (Kuijper et al. 2015). Finally, ASD represents a spectrum of severity levels and often co-occurs with other conditions, including many genetic syndromes and birth defects (Ornoy et al. 2016); it is important to assess whether associations between ASD and 2D:4D are uniform across various ASD subtypes or are specific to a particular subset of children with ASD. These findings might provide clues as to the underlying explanation for the observed digit ratio associations.

The Study to Explore Early Development (SEED) (Schendel et al. 2012; Wiggins et al. 2015a) provides a unique opportunity to more fully assess associations between ASD and 2D:4D than has been achieved in other studies. SEED has much larger sample sizes of both males and females than nearly all past studies. Additionally, it was designed to enroll individuals from diverse population subgroups and included systematic recruitment of ASD cases and population controls. SEED case classification was based on rigorous research-reliable developmental assessments conducted in person. Moreover, data on various facets of ASD phenotype, including data from an in depth dysmorphology assessment, were collected, which allowed for ASD subgroup analyses. Finally, SEED included detailed data collection of maternal health conditions before and during pregnancy and maternal exposures and behaviors during pregnancy, allowing for assessment of and control for other factors (beyond ASD) that might be associated with fetal testosterone and estradiol concentrations and thus, impact a child’s 2D:4D.

Methods

SEED was implemented in 2007 in six sites located in California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania. Institutional review boards at CDC and each study site approved the SEED protocol.

Details of the SEED methodology were previously published (Schendel et al. 2012). Each site followed a common protocol, including enrollment of three study groups: children with ASD, children with other non-ASD developmental disabilities (DD group), and non-ASD children from the general population (POP group). Children for the ASD and DD groups were identified from multiple special education and clinical sources that provide services to children with disabilities. Children recruited from each source were those with select special education or International Classification of Diseases codes indicative of autism/ASD or other DDs often seen as precursors or co-occurring diagnoses in children eventually diagnosed with ASD. POP children were selected from random samples of the birth certificates within a given site's defined geographic study area.

Recruitment, enrollment, and data collection activities for the first phase of SEED occurred from 2007 to 2012. Eligible children were born between 2003 and 2006, lived in the respective site's study area both at birth and at study enrollment, and lived with a caregiver since 6 months of age who could provide legal consent and was capable of communicating in English (all sites) or Spanish (two sites). For 98% of children, the caregiver was the biological mother. Children were enrolled when they were 2–5 years of age. Across sites, 22% of the potential ASD or DD families who were contacted were found to be ineligible, 34% refused participation before eligibility could be determined, and 43% were eligible and enrolled. Among potential POP families contacted, 34% were ineligible, 40% refused before eligibility could be determined, and 25% were eligible and enrolled (Schendel et al. 2012).

Case identification and classification were key strengths of SEED. As described above, case finding included identification of young children with a range of developmental delays from numerous sources serving diverse population subgroups, thus casting a "wide net" to identify possible ASD cases (including yet undiagnosed cases) and a second non-ASD DD case group. Final case classification was based on in-depth standardized in-person developmental assessments administered by research-reliable clinical study staff, rather than reports of past diagnoses.

Thus, although children were initially identified as potentially being eligible for a given group—ASD, DD, or POP—the final study group classification was determined from standardized research developmental assessments (Schendel et al. 2012; Wiggins et al. 2015a). Upon enrollment, caregivers of all children were administered the Social Communication Questionnaire (SCQ) to screen for possible autism characteristics in their children. Children with SCQ scores above the predetermined threshold of 11 were designated as potential ASD cases regardless of how they were initially identified. This threshold was intentionally set low to maximize case-finding. Additionally, all children who had a previous ASD diagnosis or autism special education classification were designated as potential ASD cases regardless of their SCQ scores. Children in the potential ASD group were additionally administered the Autism Diagnostic Observation Schedule (ADOS) and their caregivers were administered the Autism Diagnostic Interview—Revised (ADI-R). Final ASD case classification was based on the ADOS and ADI-R scores. Children who had been designated as potential ASD cases, who did not meet the criteria for classification as an ASD case after the ADOS and ADI-R, received a final classification of either DD or POP depending on their original identification source (education/clinical source vs. birth

certificate sample, respectively). Among the children classified as ASD cases, 85% were identified from special education or clinical sources and had a previous ASD diagnosis, 14% were identified from special education or clinical sources in which they were being served for another DD, and 1% were initially identified from random samples of birth certificate. Among children with a final classification of POP, 94% screened negative on the SCQ for autism characteristics and the remaining 6% screened positive for autism characteristics but did not meet case classification criteria on the ADOS and ADI-R. (See Wiggins et al. 2015a for further detail.)

A wealth of data was collected from all study groups over the course of several months. Data collection included an extensive telephone interview with the caregiver about family socio-demographics and, if the caregiver was the biological mother, her reproductive history and information about her pregnancy with the index child. Caregivers were also asked to complete self-administered forms on maternal, paternal, and child health history and child development. All children were also seen in person for a developmental assessment and collection of biosamples. Additionally, at this visit or a separate second in person visit, a detailed dysmorphology examination was conducted on all children; this included scanned images of both hands (“hand scans”) obtained using research reliable methods (see below). Study personnel (dysmorphology aides) at each site were trained by a clinical geneticist to perform a standardized dysmorphology protocol and dysmorphology aides were supervised by an on-site clinician. The dysmorphology protocol consisted of six parts: (1) in-person height, weight, and head circumference measurements of the child; (2) in-person standardized examination of the child by visual inspection and under Woods lamp; (3) obtaining a standard series of photographs of the child, along with additional photographs of unusual findings; (4) obtaining hand scans of both hands; (5) filling out a standardized Dysmorphology Examination Form with the collected data observations from the examination; and (6) following the in-person evaluation, performing a set of measurements from photographs and hand scans, recording those measurements on the Dysmorphology Exam Form, and determining and recording percentiles for all measurements obtained. Each site also compiled and submitted analytic variables from children’s birth certificates.

Sample Selection

This analysis is limited to children who completed the study and received a final case classification of ASD or POP. We did not include children with a final classification of DD because due to resource constraints, we did not complete assessments important to this study for this group of children. Additionally, we excluded children who did not have hand scans taken (~ 12% of the sample of ASD and POP children); most of these children did not have hand scans because image scanning equipment was not available at the time of the examination. One additional child’s right-hand data were excluded because the calculated digit ratio (0.67) was considered outside the valid range.

2D:4D Digit Ratio

Study staff at each SEED site were trained in performing hand scans of children using a common protocol and a color scanner with a minimum resolution of 3200 dpi. The protocol described how a child’s hands were to be placed on the scanner next to a sticker or a flat

plastic ruler of a standard length (internal measurement). If a child was not able to place his or her hands completely flat on the scanner, study staff, often with the assistance of a family member, held the child's palms and digits flat on the scanner bed, typically by placing their adult hand over the child's and pressing evenly over the course of scanning the hand. Most children, regardless of the study group, needed at least light reassuring pressure of an adult's hands resting on and positioning the child's hands so that they lay completely flat on the scanner bed. Digital images from the hand scans were analyzed by study staff at each SEED site, trained in using FAS Facial Photographic Analysis Software (http://depts.washington.edu/fasdpn/htmls/order-forms.htm) to measure the following hand structures on both hands and compare those measurements to the internal standard to obtain and record actual measurements: second digit length, third digit length, fourth digit length, palm length, and total hand length.

In order to maintain quality control for hand scan measurements across the six SEED sites, every month a common set of hand scan images was distributed to study staff at each site responsible for measuring digit length from hand scans. Two study staff at the Georgia SEED site, both skilled in obtaining digit measurements, would calculate the digit lengths and agree on a reliable measurement for each digit. Study staff at the other five SEED sites would independently perform the same digit measurements and submit the values for comparison to the Georgia SEED standard. Measurements could not differ from the standard by more than 5%. Study staff who did not achieve the concordance threshold were retrained in obtaining measurements and retested for reliability with Georgia SEED standard measurements.

Previous studies that have assessed the validity of using 2D:4D measures based on hand scans vs direct measurement reported good agreement between the two (Barona et al. 2015; Manning et al. 2001).

Confounders

We considered the following variables as potential confounders or effect modifiers: child sex, child race-ethnicity, child age at scan, birth order, maternal age at birth, maternal education at birth, maternal pre-pregnancy body mass index (BMI), maternal diagnosis of an infertility disorder prior to the index pregnancy, use of ovulation stimulation medications to achieve the index pregnancy, use of progesterone just before or during pregnancy, maternal smoking during pregnancy, preterm delivery, and multiple-gestation pregnancy.

Statistical Analyses

Given the established differences in testosterone and estradiol concentrations in pregnancies with male and female fetuses, we conducted all analyses separately for males and females. We calculated mean 2D:4D for both left and right hands and the mean difference between ASD and POP children for left- and right-hand digit ratios separately. We calculated adjusted ASD–POP differences in mean left- and right-hand 2D:4D from ANOVA models that included child race-ethnicity, child age at hand scan, birth order, maternal age and education at birth, and maternal pre-pregnancy BMI. We did not include all potential confounding factors in ANOVA models because of the instability of some models when all

factors were included, particularly among females, due to small sample sizes for certain subgroups. Rather, we assessed the impact of certain maternal and perinatal factors that pertained to a relatively small percentage of children in our sample through a series of analyses whereby we restricted our sample to exclude children with a given factor: preterm delivery, multiple-gestation pregnancy, prepregnancy maternal infertility disorder, use of ovulation stimulation medications for pregnancy conception, use of progesterone before or during pregnancy, and smoking during pregnancy. We conducted analyses in which we excluded children with each factor separately and in a sample in which we excluded children with any of the above factors (referred heretofore as the “complete restricted sample”). We assessed both unadjusted and adjusted mean digit ratio differences for the complete restricted sample.

In addition to our overall case-control comparisons, we conducted the same set of analyses for ASD subgroups compared to the full POP control group. We divided our male and female case samples into three sub-groupings: whether or not the child had intellectual disability (ID) in addition to ASD; whether or not the child had a birth defect, Down syndrome, fragile X syndrome, Rett syndrome, or tuberous sclerosis diagnosis in addition to ASD; and whether or not the child met SEED study criteria for classification as dysmorphic. Intellectual disability was defined as a score < 70 on the Mullen Scales of Early Learning (MSEL) cognitive scales. Birth defects and genetic conditions were ascertained via maternal report of diagnoses from a healthcare provider. Children were classified as dysmorphic or non-dysmorphic based on comprehensive standard assessment of 397 individual physical features by expert clinical geneticists (Shapira et al. 2015). Sample sizes for these analyses were reduced to varying extents relative to the sample sizes for the total ASD–POP comparisons because of missing values for data needed to determine case subgroup: 1, 3, and 23% missing data for ID, birth defects/genetic diagnoses, and dysmorphology classifications, respectively. Due to resource constraints, dysmorphology classifications were not obtained on all children who participated in the study. Dysmorphology classifications were restricted to three racial/ethnic groups—non-Hispanic White, non-Hispanic Black, and Hispanic—and to participants who had completed nearly all other data collection components of the study. Among males in the ASD case group with non-missing data, 59% had ID, 6% had a birth defect or genetic disorder, and 14% were classified as dysmorphic. Among females in the ASD case group with non-missing data, 69% had ID, 15% had a birth defect or genetic disorder, and 22% were classified as dysmorphic.

In all analyses, our comparison group was the full POP sample. We designed this analysis to understand how 2D:4D among children in the total ASD case group and each case subgroup compared to expectations based on a general population referent. We did not exclude from the POP group children with low MSEL scores, maternal report of birth defects or genetic conditions, or dysmorphic features. A previous analysis confirmed that the final POP sample for SEED was developmentally representative of children in the general population. Mean scores on the MSEL indicated average cognitive ability, internalizing and externalizing behavioral problems were infrequent, and mothers reported very few specific developmental diagnoses (see Wiggins et al. 2015b). Additionally, in our study sample for this analysis only 2% of POP group children met the criteria for ID, 3% had a birth defect or genetic condition,

and 5% met our criteria for dysmorphic. Each of these proportions is in line with expectations for U.S. children generally.

For all analyses of means, statistical significance of differences in mean 2D:4D were assessed with t-tests for unadjusted analyses, and f-tests for adjusted analyses.

For males, we also assessed a binary variable for digit ratio difference in the most extreme end of the distribution, i.e., the lowest 10th percentile. We defined lowest 10th percentile based on distribution in the composite ASD + POP sample. We calculated adjusted odds ratios (aOR) for associations between ASD and 2D:4D < 10th percentile from logistic regression models. Confounding factors included in the model were the same as those included in ANOVA models. Small sample size precluded these analyses for females.

For readability, we present all measures of 2D:4D and 2D:4D difference as measure × 100.

Results

Sample Description

Our analytic sample included 599 children with ASD (592 had data on left-hand 2D:4D and 595 had data on right-hand 2D:4D) and 811 POP controls (807 had valid data on left-hand 2D:4D and 808 had valid data on right-hand 2D:4D). While about half (53%) of the POP control group were males, 81% of children in the ASD case group were males (Table 1). Thus, case sample size for analyses of females was much smaller (n = 112) than that for analyses of males (n = 487). Compared to controls, cases were more likely to have been born preterm or in a multiple-gestation pregnancy. They were also more likely to be non-Hispanic black, Hispanic or Asian. Case mothers were less likely than control mothers to have been 35 years of age or older or to have a college education or higher at the time of their child's birth; they were more likely to have had an infertility disorder, prepregnancy obesity, and to have smoked during pregnancy.

Assessment of Males: ASD Group Versus POP Group

Among males, the ASD–POP difference in mean left-hand 2D:4D (×100) was − 0.36 (p = 0.162), indicating a lower 2D:4D in males with ASD compared to controls. This difference was attenuated after adjustment for child race-ethnicity, age at scan, maternal age at birth, education at birth, and pre-pregnancy BMI (− 0.21) (Table 2). In most analyses in which children with maternal and perinatal health factors that might have impacted fetal testosterone exposure were excluded, ASD–POP differences in mean left-hand 2D:4D ranged from − 0.25 to − 0.39 (all non-significant). However, the difference after exclusion of children whose mothers smoked during pregnancy (− 0.63) was statistically significant. Nonetheless, in the complete restricted sample (singleton term births with no maternal infertility disorders, no use of maternal ovulation medications or progesterone, and no maternal smoking during pregnancy), the ASD–POP difference in left-hand 2D:4D (− 0.50) was not statistically significant and as with the total sample, the difference was attenuated after adjustment (− 0.28).

The ASD–POP difference in mean right-hand 2D:4D was − 0.51 (p = 0.033). This difference was also attenuated after adjustment (− 0.34, p = 0.172). ASD–POP differences in 2D:4D in restricted sample analyses ranged from − 0.43 to − 0.73 and several were statistically significant. Nonetheless, the difference in right-hand 2D:4D in the complete restricted sample (− 0.64) was attenuated after adjustment (− 0.48) and no longer reached statistical significance (p = 0.145).

The odds of 2D:4D < 10th percentile among male cases was similar to that of controls. The aORs for the association between ASD and left-hand 2D:4D < 10th percentile were 1.29 (95% confidence interval (CI) 0.82–2.01) for the full sample and 1.28 (95% CI 0.69–2.40) for the complete restricted sample. The aORs for the association between ASD and right-hand 2D:4D < 10th percentile were 1.36 (95% CI 0.85–2.19) for the full sample and 0.93 (95% CI 0.50–1.73) for the complete restricted sample.

Assessment of Males: ASD Subgroups versus POP Group

We observed marked heterogeneity in the findings when we divided our ASD case group into more homogenous subgroups (Table 3). The findings for several subgroups were similar to those from the ASD versus POP assessments presented in Table 2. For both left- and right-hand analyses, we observed only small, non-significant differences in mean 2D:4D between POP controls and (1) children with ASD but not ID, (2) children with ASD without a birth defect/genetic disorder, and (3) children with non-dysmorphic ASD. The findings were similar in adjusted, and complete restricted sample adjusted analyses with one exception; the adjusted mean difference in 2D:4D for non-dysmorphic ASD versus POP groups (− 0.77) was statistically significant; however, this finding was greatly attenuated and no longer significant in the complete restricted sample.

In contrast, in both left- and right-hand analyses, we observed large and statistically significant differences in mean 2D:4D between children with ASD + a birth defect/genetic disorder and POP controls. Adjusted mean differences in 2D:4D were − 1.82 and − 1.83 for left- and right-hand analyses, respectively. We could not further analyze this ASD subgroup in the complete restricted sample. In left-hand analyses, we also observed notable mean 2D:4D differences between the dysmorphic ASD and POP groups; however, although the mean difference remained elevated in the complete restricted sample adjusted analysis (− 1.53), it was no longer statistically significant (p = 0.065). The findings for dysmorphic ASD versus POP group comparisons were less clear-cut in analyses of right-hand 2D:4D. Additionally, while we initially observed significant differences in left- and right-hand 2D:4D between children with ASD + ID and POP controls, the findings were attenuated and no longer significant after adjustment.

Assessment of Females: ASD Group Versus POP Group

In contrast to males, females with ASD had slightly higher mean left-hand 2D:4D than female POP controls (Table 4). In the full sample, the unadjusted and adjusted differences were 0.31 and 0.76, with the adjusted difference close to being statistically significant. The differences were much greater in the complete restricted sample (1.16 unadjusted and 1.75 adjusted) and were statistically significant. Conversely, case-control differences in right-

hand 2D:4D were small and non-significant, both in the full and complete restricted samples of females.

Assessment of Females: ASD Subgroups Versus POP Group

Most of the findings from ASD subgroup assessments in females (Table 5) were comparable to those from the ASD versus POP assessments presented in Table 4. In the complete restricted sample, we observed statistically significant differences in mean left-hand 2D:4D between POP controls and (1) children with ASD + ID, (2) children with ASD but not ID, (3) children with ASD without a birth defect/ genetic disorder, and (4) children with non-dysmorphic ASD. Right-hand mean 2D:4D was not significantly different between POP controls and any of the aforementioned groups.

Small sample sizes ($n \leq 6$) precluded us from assessing differences between POP controls and either children with ASD + a birth defect/genetic disorder or children with dysmorphic ASD in the complete restricted sample. However, we observed very large differences (> 2.0) in both left- and right-hand 2D:4D between children with ASD + a birth defect/genetic disorder and POP controls in the full (not restricted) sample both before and after adjustment.

Discussion

Our findings of decreased right-hand 2D:4D in male children with ASD in comparison to male children from the general population are generally consistent with several previous investigations (Al-Zaid et al. 2015; Masuya et al. 2015; Krajmer et al. 2011; Noipayak 2009; Sugie et al. 2010; Milne et al. 2006; de Bruin et al. 2006; Manning et al. 2001). However, the magnitude of the difference we observed between cases and controls was smaller than past studies showing an inverse association. In the full sample of > 400 male cases and > 400 male controls we detected a case-control difference of − 0.51. Other studies documented differences ranging from − 1.3 to − 8.0 (Al-Zaid et al. 2015; Masuya et al. 2015; Krajmer et al. 2011; Noipayak 2009; Sugie et al. 2010; Milne et al. 2006; de Bruin et al. 2006; Manning et al. 2001). One possible reason for the differing magnitudes of effect between our study and others is age at assessment. SEED includes very young children (ages 3–5 years), while the average ages of children in other studies of ASD and 2D:4D were older (see Teatero and Netley 2013 for a summary). Given that some studies indicate 2D:4D changes slightly as children age (Trivers et al. 2006), it is possible that 2D:4D differences between children with and without ASD become more evident at older ages. The SEED in-depth data collection of maternal and perinatal factors allowed us to examine the impact of many important potential confounders not previously studied. We found that our results for left-hand 2D:4D were stronger after sample restriction to children whose mothers did not smoke during pregnancy, which suggests that the effect of maternal smoking on fetal testosterone might have masked an association between ASD and 2D:4D. Nonetheless, the studies examining the association between maternal smoking and maternal testosterone levels or child 2D:4D report mixed results (Rizwan et al. 2007; Toriola et al. 2011; Velez et al. 2017) and in the current study, we also found that controlling for other confounders slightly attenuated associations initially observed.

SEED also allowed us to assess case subgroups and these analyses were more instructive. The largest left- and right-hand 2D:4D differences were observed for cases with a clear or potential genetic link—those with a co-occurring diagnosis of a birth defect or genetic disorder and those who were classified as dysmorphic. Further, we observed only modest (non-significant) case-control difference in either left- or right-hand 2D:4D for male ASD cases without ID, without a birth defect or genetic disorder, or for males with non-dysmorphic ASD. These findings were generally similar before or after adjustment and sample restriction.

Altogether these findings suggest that in males, while ASD is associated with lower 2D:4D, the association may be primarily explained by genetic and associated dysmorphology differences between cases and controls rather than indicating a causal association between ASD and fetal exposure to high concentrations of testosterone. Thus, the lower right-hand 2D:4D observed in our male case sample does not necessarily support the EMB theory.

Even though previous studies document high levels of amniotic fluid testosterone levels in males (Baron-Cohen et al. 2015) who have gone on to develop ASD, the causal pathway has not been clearly established. For instance, the Baron-Cohen et al. 2015 study indicates ASD is associated with a range of steroidal hormones in addition to testosterone—including progesterone and cortisol. Thus, other potential mechanisms related to these steroid hormones should also be considered. Likewise, it has been postulated that rather than a direct testosterone effect, a common gene or set of genes might be involved in the control both brain development and organ-specific responses to androgen and estrogenic activity (Zheng and Cohn 2011). Finally, it is important to note that while previous studies of males have primarily indicated an inverse association between 2D:4D and ASD diagnosis, studies that specifically investigated empathizing and systemizing measures in neuro-typical populations have shown much weaker effects or no effects with 2D:4D (Teatero and Netley 2013).

Although our finding of increased left-hand 2D:4D in females with ASD appears inconsistent with one previous study showing no association (Maysuya et al. 2015) and one previous study showing an association between ASD and right- but not left-hand 2D:4D (Bejerot et al. 2012), the direction and magnitude of the differences we observed in this study were nonetheless similar to non-statistically significant differences reported in the two other studies of females. As with our analyses of males, we observed the largest case-control differences after restricting the sample to children not exposed to other factors potentially linked to in utero hormonal concentrations. However, unlike the findings for males, the case-control differences for females were positive and were strengthened by further confounder adjustment rather than attenuated. Additionally, unlike our findings in males, in females we observed significant case-control differences whether or not cases had co-occurring ID, a birth defect or genetic disorder, or were dysmorphic. Thus, the higher 2D:4D in female cases might represent an association between ASD and either decreased in utero testosterone concentrations, increased in utero estradiol concentrations, or both. We did not have data to further explore the underlying reasons for this finding. One possible area of interest is the role of endocrine disrupting chemicals, which are found in a wide array of food and

consumer products and pesticides, and have been linked to various neurodevelopmental outcomes and symptoms (Schug et al. 2015).

Our findings must be interpreted in the context of study limitations. SEED includes a sample of young children (3–5 years of age) and thus, if bone growth occurs in an asynchronous manner, 2D:4D might not be reflective of in utero hormonal exposures. However, associations between 2D:4D and amniotic testosterone and estradiol concentrations have been established in children as young as 2 years of age (Lutchmaya et al. 2004). Moreover, several previous studies showing an association between ASD and 2D:4D were based on samples of pre-pubescent children (Al-Zaid et al. 2015; Milne et al. 2006; Manning et al. 2001) with a much wider age range than our sample. Our digit ratio measurements were not taken directly but rather were taken from hand scans. Previous studies that included validation components reported high concordance between 2D:4D measured directly and from hand scans (Barona et al. 2015; Manning et al. 2001). However, scans have been found to yield lower (more masculinized) ratios (Ribeiro et al. 2016). Presumably, this would impact cases and controls consistently, but we could not assess this. Due to resource limitations we were not able to collect and code dysmorphology data for the full POP sample or for most of the other (non-ASD) DD group. Additionally, because the DD group is a heterogeneous mix of multiple disabilities we did not assess this group in any of our analyses, which somewhat limits interpretation of the findings from ASD case subgroup analyses.

In contrast to many other ASD studies, SEED sought to enroll a diverse population-based sample by identifying potentially eligible case children from multiple clinical and education sources serving children throughout each site’s catchment area and identifying potential control children from birth certificate samples. One drawback to this approach is that numerous families targeted for potential recruitment could not be located or contacted. It is likely that many of these families were actually ineligible for inclusion since our a priori eligibility criteria required children to have current as well as birth residence in the study catchment area; four sites also required parents to be able to communicate well in English. Given the low response rate, selection bias should be considered. However, in a separate study (currently unpublished) we have conducted a detailed analysis to assess whether non-response was likely to have resulted in selection bias by assessing various risk estimates using data from one site that had access to some data on all families initially invited. We found that while certain demographic factors were associated with non-response—younger maternal age, lower maternal education, Hispanic ethnicity—other perinatal factors such as preterm birth, birth order, and infertility disorders and treatments were not (unpublished data). We adjusted all analyses in the current study for maternal age and education and child race-ethnicity and we initially assessed all associations within demographic strata to ensure there were no effect modifications by these factors. We also conducted adjusted analyses in which we included numerous biological risk factors. We have no reason to believe that a child’s digit lengths or digit ratios in and of themselves would have affected a parent’s decision to participate in SEED.

Despite these limitations, SEED offers one of the largest and most diverse samples available to assess the association between ASD and 2D:4D. Moreover, it allowed for a much more

detailed analysis of this research question than any previous study. Our findings suggest that among males, there is a small inverse association between ASD and 2D:4D, but it may be explained by genetic factors, presumed related to the presence of known syndromes and birth defects or the increased prevalence of dysmorphic features generally in children with ASD. In females there appears to be a positive association between ASD and left-hand 2D:4D, which is not explained by confounding by demographic factors or maternal and perinatal health-related exposures and is not limited to select subgroups of children with genetic syndromes or increased prevalence of dysmorphic features. Thus, the association in females may be indicative of an association between ASD and in utero hormonal exposures. However, the finding of high 2D:4D in females with ASD is in the opposite direction of what would be predicted based on EMB theory and findings from the literature on fetal testosterone. Further study to understand fetal hormonal effects on neurodevelopment is particularly needed among females.

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Table 1

Characteristics of cases and controls, study to explore early development

	ASD (N = 599) %	POP (N = 811) %
Child sex		
Male	81.30	53.14
Female	18.70	46.86
Child race/ethnicity		
Non-Hispanic White	54.75	68.38
Non-Hispanic Black	17.80	10.92
Hispanic	13.39	11.17
Asian	5.93	3.26
Mixed	8.14	6.27
Age at hand scan (months)		
< 48	5.56	7.60
48–59	37.54	32.88
60+	56.90	59.53
Maternal age at birth (years)		
≤ 34	71.81	68.60
35+	28.19	31.40
Maternal education at birth (years)		
≤ 15	48.41	33.83
16+	51.59	66.17
Birth order		
First birth	48.71	45.56
Second birth or later	51.29	54.44
Gestational age		
Term	83.22	90.84
Preterm (< 37 weeks)	16.78	9.16
Birth plurality		
Singleton	91.08	96.24
Multiple birth	8.92	3.76
Any maternal infertility disorder diagnosed prior to index pregnancy		
Yes	20.45	14.95
No	79.55	85.05
Any maternal use ovulation medications to help get pregnant		
Yes	9.94	9.28
No	90.06	90.72
Any maternal use progesterone medications to help get pregnant or maintain pregnancy		
Yes	10.69	9.54
No	89.31	90.46
Maternal pre-pregnancy BMI (kg/m^2)		
< 25	56.33	64.40

	ASD (N = 599) %	POP (N = 811) %
25.0–29.9	25.67	21.85
≥ 30	18.00	13.75
Maternal prenatal smoking		
No	90.97	95.97
Yes	9.03	4.03

ASD Autism spectrum disorder case group, *POP* population control group, *BMI* body mass index

Data excluded due to missing values for individual factors. Missing values were 0% for child sex, 1–5% for maternal age, maternal education, birth order, gestational age and birth plurality, and maternal pre-pregnancy BMI, and 6–9% for maternal infertility conditions, ovulation induction medications, progesterone medications, and smoking

Table 2

Mean digit ratio males, study to explore early development

	ASD Digit ratio × 100	POP Digit ratio × 100	Digit ratio difference ASD–POP	p Value ASD versus POP
Left hand, n = 910				
Total sample				
Unadjusted, n = 910	94.20	94.56	− 0.36	0.162
Adjusted[a], n = 842	93.47	93.68	− 0.21	0.422
Restriction to subgroups (unadjusted)				
Term birth, n = 784	94.15	94.48	− 0.33	0.226
Singleton birth, n = 831	94.08	94.47	− 0.39	0.144
No pre-pregnancy maternal infertility disorders reported, n = 691	94.34	94.59	− 0.25	0.405
No ovulation medications used by mother to help get pregnant, n = 749	94.27	94.52	− 0.25	0.363
No maternal progesterone medications used by mother to help get or maintain pregnancy, n = 744	94.25	94.51	− 0.26	0.366
No maternal smoking during pregnancy, n = 801	93.99	94.62	− 0.63	**0.020**
Complete restricted sample[b]				
Unadjusted, n = 503	94.01	94.51	− 0.50	0.148
Adjusted[a], n = 477	93.16	93.43	− 0.28	0.423
Right hand, n = 914				
Total sample				
Unadjusted, n = 914	94.08	94.58	− 0.51	**0.033**
Adjusted[a], n = 846	93.63	93.97	− 0.34	0.172
Restriction to subgroups (unadjusted)				
Term birth, n = 786	94.04	94.59	− 0.55	**0.033**
Singleton birth, n = 834	94.02	94.54	− 0.52	**0.039**
No pre-pregnancy maternal infertility disorders reported, n = 696	94.12	94.57	− 0.45	0.098
No ovulation medications used by mother to help get pregnant, n = 754	94.12	94.59	− 0.46	0.077
No maternal progesterone medications used by mother to help get or maintain pregnancy, n = 749	94.11	94.54	− 0.43	0.100
No maternal smoking during pregnancy, n = 806	93.90	94.64	− 0.73	**0.004**
Complete restricted sample[b]				
Unadjusted, n = 507	93.96	94.60	− 0.64	**0.044**
Adjusted[a], n = 481	93.49	93.97	− 0.48	0.145

Statistically significant findings are indicated in bold

ASD autism spectrum disorder case group, *POP* population control group

[a]Adjusted models included child race-ethnicity, child age at scan, birth order, maternal age at birth, maternal education at birth and maternal prepregnancy body mass index

[b]Complete restricted sample was restricted to children born in singleton term births with no pre-pregnancy maternal infertility disorders, no use of maternal ovulation medications or progesterone, and no maternal smoking during pregnancy

Table 3

Mean digit ratio males, examination of ASD subtypes, study to explore early development

	Digit ratio × 100 ASD with given condition or characteristic	Digit ratio × 100 ASD without given condition or characteristic	Digit ratio × 100 POP	Digit ratio difference ASD with condition minus POP	p Value ASD with condition versus POP	Digit ratio difference ASD without condition minus POP	p Value ASD without condition versus POP
Left hand							
Intellectual disability							
Total, unadjusted, n = 900	93.75	94.86	94.57	− 0.82	**0.006**	0.28	0.387
Total, adjusted[a], n = 833	93.18	93.87	93.71	− 0.52	0.097	0.16	0.629
Complete restricted, adjusted[a,b], n = 474	93.03	93.30	93.38	− 0.35	0.394	− 0.08	0.856
Diagnosis of birth defect, Down syndrome, fragile X syndrome, Rett syndrome, or tuberous sclerosis							
Total, unadjusted, n = 888	92.67	94.27	94.61	− 1.93	**0.010**	− 0.34	0.195
Total, adjusted[a], n = 841	91.86	93.57	93.68	− 1.82	**0.019**	− 0.11	0.678
Complete restricted, adjusted[a,b], n = 477	_[c]	93.16	93.43	–	–	− 0.28	0.422
Dysmorphic							
Total, unadjusted, n = 556	93.41	94.18	94.63	− 1.22	**0.045**	− 0.46	0.194
Total, adjusted[a], n = 527	92.74	93.57	94.35	− 1.60	**0.007**	− 0.77	**0.032**
Complete restricted, adjusted[a,b], n = 288	92.47	93.63	94.00	− 1.53	0.065	− 0.37	0.434
Right hand							
Intellectual disability							
Total, unadjusted, n = 904	93.79	94.43	94.60	− 0.80	**0.004**	− 0.17	0.584
Total, adjusted[a], n = 837	93.48	93.77	93.99	− 0.51	0.082	− 0.22	0.486
Complete restricted, adjusted[a,b], n = 478	93.48	93.44	93.98	− 0.50	0.204	− 0.53	0.209
Diagnosis of birth defect, Down syndrome, fragile X syndrome, Rett syndrome, or tuberous sclerosis							
Total, unadjusted, n = 892	92.69	94.13	94.60	− 1.91	**0.006**	− 0.47	0.058
Total, adjusted[a], n = 845	92.13	93.72	93.96	− 1.83	**0.011**	− 0.24	0.343
Complete restricted, adjusted[a,b], n = 481	_[c]	93.51	93.96	–	–	− 0.45	0.177
Dysmorphic							
Total, unadjusted, n = 560	93.25	94.11	94.34	− 1.09	**0.047**	− 0.23	0.481

	Digit ratio × 100 ASD with given condition or characteristic	Digit ratio × 100 ASD without given condition or characteristic	Digit ratio × 100 POP	Digit ratio difference ASD with condition minus POP	p Value ASD with condition versus POP	Digit ratio difference ASD without condition minus POP	p Value ASD without condition versus POP
Total, adjusted[a], n = 531	93.08	93.99	94.19	− 1.11	**0.049**	− 0.20	0.562
Complete restricted, adjusted[a,b], n = 291	93.46	93.43	93.66	− 0.20	0.788	− 0.23	0.600

Statistically significant findings are indicated in bold

Total sample sizes for each analysis type indicated in table. Samples sizes for individual study groups varied as follows:
N = 122–282 for ASD with intellectual disability
N = 95–196 for ASD without intellectual disability
N = 7–28 for ASD with a birth defect/genetic disorder
N = 211–440 for ASD without a birth defect/genetic disorder
N = 25–54 for dysmorphic ASD
N = 149–317 for non-dysmorphic ASD
N = 114–426 for the POP group

ASD autism spectrum disorder case group, *POP* population control group

[a]Adjusted models included child race-ethnicity, child age at scan, birth order, maternal age at birth, maternal education at birth and maternal prepregnancy body mass index

[b]Complete restricted sample was restricted to children born in singleton term births with no pre-pregnancy maternal infertility disorders, no use of maternal ovulation medications or progesterone, and no maternal smoking during pregnancy

[c]Data not shown if estimates were of questionable reliability due to sparse data

Table 4

Mean digit ratio females, study to explore early development

	ASD Digit ratio × 100	POP Digit ratio × 100	Digit ratio Difference ASD–POP	p Value ASD versus POP
Left hand, n = 489				
Total sample				
Unadjusted, n = 489	94.90	94.59	0.31	0.422
Adjusted[a], n = 460	94.41	93.65	0.76	0.056
Restriction to subgroups (unadjusted)				
Term birth, n = 437	95.00	94.54	0.46	0.266
Singleton birth, n = 458	94.81	94.59	0.22	0.587
No pre-pregnancy maternal infertility disorders reported, n = 366	95.52	94.70	0.82	0.074
No ovulation medications used by mother to help get pregnant, n = 404	95.26	94.76	0.50	0.242
No maternal progesterone medications used by mother to help get or maintain pregnancy, n = 404	95.25	94.70	0.55	0.206
No maternal smoking during pregnancy, n = 430	94.88	94.53	0.35	0.410
Complete restricted sample[b]				
Unadjusted, n = 271	95.61	94.45	1.16	**0.049**
Adjusted[a], n = 259	95.28	93.53	1.75	**0.006**
Right hand, n = 489				
Total sample				
Unadjusted, n = 489	95.01	94.87	0.14	0.720
Adjusted[a], n = 461	94.51	93.92	0.59	0.153
Restriction to subgroups (unadjusted)				
Term birth, n = 438	95.10	94.79	0.31	0.468
Singleton birth, n = 460	94.70	94.85	− 0.15	0.710
No maternal infertility disorders reported, n = 367	95.16	94.99	0.17	0.720
No ovulation medications used by mother to help get pregnant, n = 406	95.18	95.00	0.18	0.685
No maternal progesterone medications used by mother to help get or maintain pregnancy, n = 406	94.98	94.95	0.03	0.941
No maternal smoking during pregnancy, n = 431	94.73	94.88	− 0.15	0.728
Complete restricted sample[b]				
Unadjusted, n = 272	94.54	94.76	− 0.22	0.709
Adjusted[a], n = 260	94.08	93.91	0.17	0.791

Statistically significant findings are indicated in bold

ASD autism spectrum disorder case group, *POP* population control group

[a]Adjusted models included child race-ethnicity, child age at scan, birth order, maternal age at birth, maternal education at birth and maternal prepregnancy body mass index

[b]Complete restricted sample was restricted to children born in singleton term births with no pre-pregnancy maternal infertility disorders, no use of maternal ovulation medications or progesterone, and no maternal smoking during pregnancy

Table 5

Mean digit ratio females, examination of ASD subtypes, study to explore early development

	Digit ratio × 100 ASD with given condition or characteristic	Digit ratio × 100 ASD without given condition or characteristic	Digit ratio × 100 POP	Digit ratio difference ASD with condition minus POP	p Value ASD with condition vs POP	Digit ratio difference ASD without condition minus POP	p Value ASD without condition versus POP
Left hand							
Intellectual disability							
Total, unadjusted, n = 487	94.68	95.42	94.61	0.07	0.874	0.81	0.193
Total, adjusted[a], n = 458	94.27	94.79	93.70	0.57	0.224	1.09	0.085
Complete restricted, adjusted[a,b], n = 258	95.16	95.49	93.54	1.62	**0.036**	1.95	**0.035**
Diagnosis of birth defect, Down syndrome, fragile X syndrome, Rett syndrome, or tuberous sclerosis							
Total, unadjusted[a], n = 481	96.73	94.52	94.62	2.10	**0.020**	− 0.10	0.809
Total, adjusted, n = 460	96.00	94.16	93.69	2.31	**0.009**	0.47	0.265
Complete restricted, adjusted[a,b], n = 259	–[c]	94.99	93.46	–	–	1.53	**0.018**
Dysmorphic							
Total, unadjusted, n = 244	94.92	95.14	94.36	0.56	0.497	0.77	0.131
Total, adjusted[a], n = 232	95.02	95.05	94.43	0.59	0.488	0.63	0.213
Complete restricted, adjusted[a,b], n = 114	–[c]	95.82	94.21	–	–	1.61	**0.044**
Right hand							
Intellectual disability							
Total, unadjusted, n = 487	94.79	95.44	94.87	− 0.08	0.859	0.57	0.369
Total, adjusted[a], n = 459	94.40	94.65	93.90	0.50	0.304	0.75	0.258
Complete restricted, adjusted[a,b], n = 259	93.63	94.51	93.88	− 0.25	0.747	0.63	0.492
Diagnosis of birth defect, Down syndrome, fragile X syndrome, Rett syndrome, or tuberous sclerosis							
Total, unadjusted, n = 482	96.91	94.64	94.89	2.02	**0.020**	− 0.25	0.552
Total, adjusted[a], n = 461	96.23	94.23	93.97	2.26	**0.013**	0.26	0.555
Complete restricted, adjusted[a,b], n = 260	–[c]	93.72	93.83	–	–	− 0.10	0.872
Dysmorphic							
Total, unadjusted, n = 246	94.39	95.38	94.62	− 0.23	0.796	0.76	0.173
Total, adjusted[a], n = 234	93.98	94.53	94.02	− 0.04	0.968	0.51	0.365

	Digit ratio × 100 ASD with given condition or characteristic	Digit ratio × 100 ASD without given condition or characteristic	Digit ratio × 100 POP	Digit ratio difference ASD with condition minus POP	p Value ASD with condition vs POP	Digit ratio difference ASD without condition minus POP	p Value ASD without condition versus POP
Complete restricted, adjusted[a,b], n = 115	–[c]	94.40	93.84	–	–	0.56	0.516

Statistically significant findings are indicated in bold

Total sample sizes for each analysis type indicated in table. Samples sizes for individual study groups varied as follows:
N = 26–77 for ASD with intellectual disability
N = 17–34 for ASD without intellectual disability
N = 4–17 for ASD with a birth defect/genetic disorder
N = 40–93 for ASD without a birth defect/genetic disorder
N = 5–19 for dysmorphic ASD
N = 30–67 for non-dysmorphic ASD
N = 79–377 for the POP group

ASD autism spectrum disorder case group, *POP* population control group

[a]Adjusted models included child race-ethnicity, child age at scan, birth order, maternal age at birth, maternal education at birth and maternal prepregnancy body mass index

[b]Complete restricted sample was restricted to children born in singleton term births with no pre-pregnancy maternal infertility disorders, no use of maternal ovulation medications or progesterone, and no maternal smoking during pregnancy

[c]Data not shown if estimates were of questionable reliability due to sparse data