BMJ Open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-007433
Article Type:	Research
Date Submitted by the Author:	11-Dec-2014
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Mental health
Keywords:	EPIDEMIOLOGY, Developmental neurology & neurodisability < PAEDIATRICS, Child & adolescent psychiatry < PSYCHIATRY

Digit Ratio and Autism Spectrum Disorders: A Birth Cohort Study.

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Word count: 3806 words (excluding title page, abstract, references, figures and tables)

Keywords: Avon Longitudinal Study of Parents and Children (ALSPAC); autistic disorder; digit ratio; testosterone; prenatal exposure delayed effects.

OBJECTIVES To investigate whether second-to-fourth digit ratio (2D:4D), a proxy measure of foetal testosterone exposure, is associated with autism spectrum disorders (ASDs), as predicted by the extreme male brain theory of autism.

DESIGN Analysis of the association between digit ratio and autism spectrum disorders in a large UK cohort study.

SETTING The Avon Longitudinal Study of Parents and Children (ALSPAC).

PARTICIPANTS 6015 ALSPAC children with data on digit ratio, at least one outcome measure, and information on potential confounding variables (parental occupational class, maternal education, and age at digit ratio measurement). Digit ratio was measured by the photocopy and calliper method.

OUTCOMES ASD diagnosis (cases have been identified previously by record linkage or maternal report) and four measures that combine optimally within ALSPAC to predict ASD: the Children's Communication Checklist (coherence subscale), the Social and Communication Disorders Checklist, a repetitive behaviour measure, and the Emotionality, Activity and Sociability scale (sociability subscale). These four measures were dichotomised, with 10% defined as the 'risk' group.

RESULTS Using logistic regression, we examined for association of 2D:4D with ASDs and four dichotomised ASD traits: Covariates were occupational class, maternal education and age at 2D:4D measurement. 2D:4D was not associated with ASDs in males (adjusted odds ratio (OR) per 1-standard deviation increase in mean 2D:4D, 0.88 [95% confidence interval (CI) 0.65-1.21], p=0.435) or females (adjusted OR=1.36 [95% CI 0.81-2.28], p=0.245). Similar associations were observed after further adjustment for IQ. There was one weak association between reduced coherence and increased left 2D:4D in males (adjusted OR=1.15 [95% CI 1.02-1.29], p=0.023). Given multiple comparisons, this may be consistent with chance.

CONCLUSIONS In this population-based study, there was no association between 2D:4D and ASD diagnosis. There was weak, inconsistent evidence of association between 2D:4D and coherence. These results are not consistent with the extreme male brain theory.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To date, this is one of the largest population based studies examining the relationship between second-to-fourth digit ratio (2D:4D), which is used as a proxy for foetal testosterone exposure, and autism spectrum disorders, separately by sex.
- This paper also examines the relationship between second-to-fourth digit ratio (2D:4D) and component measures of autism, which may give insight into the less-explored relationship between digit ratio and the individual domains (social and communication, repetitive behaviours) represented in autism.
- In comparison with other small studies based in clinical settings, this study may be less prone to selection bias, and the rich phenotype information within ALSPAC allowed us to control for possible confounding variables.
- Despite its relatively large sample size compared with many studies, the number of autism cases in this study is still small.
- Cohort studies are prone to bias from attrition, and whilst the major sociodemographic predictors of attrition were controlled for in this study, it is also possible that children severely affected by ASD may have been less likely to attend clinics where 2D:4D was measured, which could bias our results.

INTRODUCTION

Autism spectrum disorders (ASD) are pervasive developmental disorders characterised by impaired social communication and reciprocal social interaction; and restricted, repetitive patterns of behaviour, interests, or activities.[1] Whilst twin studies suggest that ASDs are highly heritable, their aetiology remains largely unexplained.[2] Elucidating the causes of ASD may facilitate earlier diagnosis and enhance the potential for primary prevention. Earlier diagnosis may lead to an improvement in educational and behavioural outcomes, a reduction in comorbid psychiatric symptoms, and a decrease in the financial and emotional stresses created by ASDs for individuals and their families.[3]

Various population cohorts have estimated the prevalence of ASD at approximately 1%.[4-7] These studies demonstrate a marked gender imbalance, with male to female ratio estimates ranging from 2:1[5] to 9:1,[8] depending upon age of assessment, length of follow-up, and whether studies screen for ASDs or examine data on pre-existing diagnosis. Although diagnostic bias has been proposed as a partial explanation for this disparity between sexes,[9-10] the consistently observed preponderance amongst males has generated interest into possible biological mechanisms predisposing to ASDs.

The extreme male brain (EMB) theory is the most popular hypothesis put forward to explain the sex difference in ASD. This theory postulates that children with ASD exhibit an exaggerated form of the male cognitive profile,[11] and proposes that prenatal androgens are plausible biological candidates. Some evidence from animal studies suggests that testosterone may mediate cognitive differences between the sexes via organizational effects on the brain.[12-13] Recent evidence supporting the EMB theory found sex steroid levels in amniocentesis samples to be correlated with diagnosis of ASDs.[14]

The index to ring finger ratio (2D:4D) has been widely used as a proxy for foetal testosterone exposure.[15-24] An observation supporting a causal association between 2D:4D and foetal testosterone is that 2D:4D is sexually dimorphic,[25] with males generally having lower 2D:4D (that is to say, a relatively shorter index finger (2D) compared to their ring finger (4D)).[26-27] This sexual dimorphism is apparent from the first trimester of pregnancy, and appears to be largely static after birth, being unaffected by pubertal androgen.[28-32] 2D:4D has also been shown to be sexually dimorphic in endocrine models of elevated (congenital adrenal hyperplasia) and reduced foetal testosterone exposure (complete androgen insensitivity syndrome).[27,33-35]

Although recent reviews have concluded that lower 2D:4D is associated with ASD,[36-37] the published literature consists of relatively small observational studies which are susceptible to selection bias and confounding. We designed a study to examine the association between 2D:4D and ASD and various autistic trait measures in a population-based cohort in the UK. The primary hypothesis being tested was that lower 2D:4D would be associated with ASDs and ASD traits.

METHODS

Study design and population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a birth cohort based in Avon, UK. 14,541 pregnant women with expected delivery dates between 1st April 1991 and 31st December 1992 were initially enrolled, and 13,988 children were alive at one year.[38] Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. The study website contains details of all the data that is available through a fully searchable data dictionary: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary.[39] The primary source of data collection was via self-completion questionnaires administered at four points during the prenatal period then at regular intervals following birth to both parents and the "study child". Since the age of 7 years the whole cohort has been invited to a regular "focus" clinic for a variety of hands-on assessments.

Outcome variables

Autism spectrum disorders

Within ALSPAC, the identification of children with ASD has been described elsewhere.40 Briefly, a previous record linkage study identified 86 ASD cases in ALSPAC, of which there was evidence for a strict multidisciplinary clinical assessment in 71 individuals, and in 15 others with a diagnosis of ASD recorded in school records.[40] In a validation study, a consultant paediatrician reviewed the records of the individuals with ASD to confirm concordance with ICD-10 criteria.[40] In addition to cases identified by record linkage, we further identified individuals with ASD based on maternal report. At 9.5 years, mothers were asked, "Have you ever been told that your child has: autism, Asperger's syndrome or autistic spectrum disorder?" Investigators utilizing maternal reports based on similar questions in other cohorts have reported an acceptable validity of identifying ASD using this approach.[41-43] We cross validated ASD cases confirmed only by maternal report by studying their association with various autistic trait measures and found strong associations (see supplementary Table S1, available online). Of the 56 ASD cases included in this study, 24 were formally diagnosed and 32 were from maternal reports of ASD diagnosis.

Dichotomised ASD traits

A previous factor analysis of 93 measures related to autism reported four individual measures administered in ALSPAC via parental questionnaires that 'combined optimally to predict autism'.[44] The coherence subscale of the Children's Communication Checklist, measured at 115 months, assesses pragmatic abnormalities in social communication.[45] The Social and Communication Disorders Checklist, measured at 91 months, measured social skills (described previously).[46] A Repetitive Behaviour score, measured at 69 months asked whether the child "repeatedly rocks the head or body, has tics or twitches or other unusual behaviour", and included a question from the Rutter scale on tics, mannerisms or twitches.[47] The sociability subscale of the Emotionality Activity and Sociability measure at 38 months measures tendency to affiliate and interact with others.[48] As all the ASD scales considered were highly negatively skewed, and not readily correctable by transformation, each was dichotomised, creating a high risk (for ASDs) group of as close to 10% of the population as possible.

Explanatory variable: 2D:4D

Participants were invited to a focus clinic, when 2D:4D was measured. Mean measurement age was 11.75 years. Whilst 2D:4D was measured *after* the outcome measures (between approximately 2 to 8 years later, depending upon the outcome), it is widely used as a measurement of the prenatal environment, and therefore we feel justified in using it as our exposure in our analysis.

2D:4D was measured by the photocopy and calliper method: the ventral surface of both palms was placed as flatly as possible onto a photocopier, ensuring that the fingers were apart and the palms pressed firmly onto the glass. The lengths of the second (index) and fourth (ring) fingers were measured using the "Mahr digital caliper16 EX", accurate to 0.01mm. Each finger measured has a crease at its base: the index finger (2D) has one crease, the ring finger (4D) probably 3 or 4. The most proximal crease was chosen and by eye, the midpoint of this was determined and the distance from this crease to the distal fingertip measured. 2D measurement was divided by 4D measurement thus giving a 2D:4D measurement for each hand.[39] To assess the legitimacy of using photocopies to calculate 2D:4D, 57 right and 48 left hands in ALSPAC have been measured previously in vivo, with high correlation between these measures and photocopies (r=0.97).[49]

Confounding variables

We adjusted for the potential confounding effect of a number of socio-demographic measures. Each parent's self-reported occupation was coded as one of professional, managerial and technical, skilled non-manual, or manual,[50] and the highest reported class from each child's parent(s) was recorded. Highest self-reported maternal education level was recorded as CSE (certificate of secondary education), vocational, O level, 'A' level or degree. Occupational class and maternal education were used as a proxy for socioeconomic status (SES), which is known to predict loss to follow up in ALSPAC.[38] ASDs are socially patterned,[51] although recent research suggests this may be in a direction opposite to that previously reported.[52] We also adjusted for the age at which the participants attended the 11-year clinic (where the 2D:4D exposure was measured) to account for compliance to clinic invite, which may be related to the outcomes considered.

Statistical analysis

For the main analysis, we included children from singleton pregnancies with left and right 2D:4D measured, who had data for at least one of five outcome measures (recorded presence/absence of ASDs, or data on at least one of the four dichotomised ASD trait measures) and additionally, data on potential confounders, including parental occupational class, maternal education level, and age at 2D:4D measurement (n=6015, including 56 ASD cases, and up to 616 in high risk dichotomised group) (see Fig. 1 and Table 1).

TABLE 1: Characteristics of the study population compared to those ALSPAC participants not included in the study*. n=number of participants.

	Study population (n=6015) (Standard deviations given for means)	Other ALSPAC participants (n≤7602) (Standard deviations given for means)	p value**
% Male	49%	53%	p≤0.001
% Autism spectrum disorder (maternal and formal report)	0.93%	0.96%	p=0.861
Mean 2D:4D (those with left and right 2D:4D, excluding two outliers)	0.964 (0.029)	0.963 (0.030)	p=0.421
Age at 2D:4D measurement, months	140.9 (2.8)	141.3 (3.2)	p≤0.001
% Manual occupational background	14.2%	25.3%	p≤0.001
% Mother educated to degree level	16.3%	9.5%	p≤0.001

*(excluding multiple births, those not alive at 1 year, and those not in the core ALSPAC sample). ALSPAC=Avon Longitudinal Study of Parents and Children. 2D:4D=second-to-fourth digit ratio.

**from chi-squared test for categorical variables, and unpaired t-test (two tailed) for continuous variables

***These four measures were dichotomised, creating a high risk (for ASDs) group of as close to 10% of the population as possible.

Since the correlation between right and left hand 2D:4D was high (males 0.64, females 0.69), we used the mean of left and right hand 2D:4D in our main analysis (see Fig 2.). Two outliers were removed due to their extreme 2D:4D values (<0.75 and >1.25). Sex differences in this composite measure were assessed using a two-tailed t-test. Given the male preponderance in ASDs, and since 2D:4D is sexually dimorphic, all analyses which followed were stratified by sex, in order to assess whether the relationship of 2D:4D and ASD varied between sexes. To enable a clearer comparison of the male and female results, the 2D:4D measure was further standardised within each sex, so that a one unit change in 2D:4D represents a change by 1 standard deviation. Raw scores (i.e. non-standardised scores) are presented for descriptive tables.

A univariable logistic regression model was estimated for each ASD related outcome in turn, using the standardised mean 2D:4D variable as a continuous exposure (see Table 3). Effects were subsequently adjusted for the potential confounders listed above.

A supplementary analysis adjusted for IQ (see supplementary Table S3, available online). As sensitivity analyses, models were repeated using left and then right hand in turn (see supplementary Tables S4a and S4b, available online). All analyses were carried out in Stata (StataCorp, TX), version 13.

RESULTS

The sample population and those ALSPAC participants not in the sample were similar in terms of mean 2D:4D, age at 2D:4D measurement and ASD prevalence, but fewer participants amongst our sample were male (see Table 1). Participants in the study sample were more likely to have parents working in non-manual occupations, were more likely to have a marginally higher IQ, and their mothers were more likely to be educated to degree level, which is consistent with the social patterning of attrition previously observed within ALSPAC.[38] Within the full sample (n=6015) there was strong evidence of sexual dimorphism for 2D:4D, with a mean (SE) of 0.959 (0.001) in males and 0.969 (0.001) in females (t=13.4, d.f.=6013, two-tailed t-test p≤0.001). This sexual dimorphism was still present in the subsample of ASD cases (n=56, males 2D:4D=0.955 (0.003); females=0.979 (0.006), t=3.5, d.f.=54, p≤0.001). Mean 2D:4D values stratified by ASD diagnosis and the other outcomes are shown in Table 2 (see supplementary Table S2, available online, for results shown separately for right and left hand).

TABLE 2: Mean (standard error) of left and right second-to-fourth digit ratio, and numbers (n) in each subgroup (ASD/no ASD; high/low ASD risk), by sex.

Outcome	Age	Risk group	Males		Females	
			Mean (SE)	N	Mean (SE)	N
Autism Spectrum Disorder (ASD) diagnosis	Diagnosed by 11 years	No ASD diagnosis	0.959 (0.001)	2920	0.969 (0.001)	3039
		ASD diagnosis	0.955 (0.003)	42	0.979 (0.006)	14
Children's Communication Checklist (Coherence subscale)	115 months	Lower risk	0.959 (0.001)	2285	0.970 (0.001)	2495
		Higher risk	0.962 (0.002)	302	0.969 (0.002)	167
Social and Communication Developmental Checklist	91 months	Lower risk	0.960 (0.001)	2260	0.970 (0.001)	2444
		Higher risk	0.959 (0.002)	299	0.970 (0.002)	172
Repetitive behaviour	69 months	Lower risk	0.960 (0.001)	2378	0.970 (0.001)	2509
		Higher risk	0.958 (0.002)	199	0.969 (0.003)	130
Emotionality Activity, Sociability Temperament Scale	38 months	Lower risk	0.959 (0.001)	2378	0.970 (0.001)	2540
		Higher risk	0.959 (0.002)	352	0.968 (0.002)	264

(Sociability subscale)						

Table 3 shows unadjusted and adjusted estimates for the increase in odds of each outcome per 1 standard deviation increase in mean 2D:4D ratio. There was no evidence of an association between mean 2D:4D and any of the five outcomes in either sex.

TABLE 3: Logistic regression of ASD vs no ASD, or high risk dichotomised trait vs low risk, against mean 2D:4D (left/right hands combined), stratified by sex. Unadjusted and adjusted[a] models are presented. NB Odds ratios are for one unit change in Z score (i.e. 1 standard deviation). N=numbers of participants.

Sex	Males				Females			
Model	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]
Autism Spectrum Disorder (ASD) diagnosis	0.87 [0.64, 1.19] p=0.393	0.88 [0.65, 1.21] p=0.435	2962	42	1.39 [0.83, 2.34] p=0.206	1.36 [0.81, 2.28] p=0.245[c]	3053	14
Children's Communication Checklist (Coherence subscale)	1.10 [0.97, 1.24] p=0.124	1.10 [0.98, 1.24] p=0.118	2587	302	0.98 [0.84, 1.15] p=0.790	0.98 [0.84, 1.15] p=0.809	2662	167
Social and Communication Developmental Checklist	0.96 [0.85, 1.09] p=0.553	0.96 [0.85, 1.09] p=0.550	2559	299	1.03 [0.88, 1.20] p=0.698	1.04 [0.89, 1.22] p=0.601	2616	172
Repetitive behaviour	0.95 [0.82, 1.10] p=0.466	0.95 [0.82, 1.09] p=0.458	2577	199	0.98 [0.82, 1.17] p=0.796	0.98 [0.82, 1.17] p=0.840	2639	130
Emotionality, Activity, Sociability (Sociability subscale)	0.98 [0.87, 1.09] p=0.670	0.97 [0.87, 1.08] p=0.583	2730	352	0.94 [0.83, 1.07] p=0.325	0.94 [0.83, 1.07] p=0.357	2804	264

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D (second-to-fourth digit ratio) measurement.

[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait.

[c]To avoid perfect prediction, the CSE (Certificate of Secondary Education) and vocational classes of maternal education were collapsed for this analysis.

We included Intelligence Quotient (IQ) as measured by a modified version of the Wechsler Intelligence Scale for Children, as a potential confounder in a supplementary analysis. We considered this separately from our main analysis to prevent a substantial loss to our sample size, since the IQ measure was available on only 44/56 of included participants with ASDs. Another supplementary analysis examined the effect of 2D:4D by left and right hand.

Supplementary tables show (i) little impact of further adjustment for IQ (supplementary Table S3, available online), and (ii) a broadly consistent pattern of findings when examining the effect of 2D:4D ratio for each hand in turn (supplementary Tables S4a and S4b, available online). One sub-analysis suggested a weak association between reduced coherence (as measured by the Children's Communication Checklist) with left 2D:4D in males (adjusted OR 1.15 [95% CI 1.02, 1.29], p=0.023, see supplementary Table S4a). There was also a trend towards increased right 2D:4D being associated with ASD diagnosis in females (adjusted OR 1.60 [95% CI 0.97, 2.65] p=0.068, see supplementary Table S4b). However, these results are in the opposite direction to that predicted by the prevailing theory of the extreme male brain. One sub-analysis found a trend towards masculinized 2D:4D being associated with the repetitive behaviours measure OR 0.87 [95% CI 0.75, 1.01] p=0.061. Yet, given the number of comparisons, we consider that these results are likely to be consistent with chance.

DISCUSSION

In this large UK-based birth cohort, we did not find evidence to support the hypothesis that lower second to fourth digit ratio (2D:4D) is associated with an increased risk of autism spectrum disorders (ASDs). In this study, we also examined the relationship of digit ratio with dichotomised component trait measures of ASDs, including social cognition, coherence, repetitive behaviours and sociability. There was one instance of a very weak relationship between increased 2D:4D and reduced coherence. This association is discordant with the extreme male theory of autism, and given the number of comparisons made, and the lack of association between ASD diagnosis and 2D:4D, we consider that it could be consistent with chance.

Two recent reviews concluded that there was support for an association between masculinised 2D:4D and ASD.[36-37] Amongst the studies examining the 2D:4D-ASD diagnosis relationship (i.e. not ASD trait measures), most studies recruited cases from clinical settings,[17,21-23] which may provide results of limited external validity. Others recruited from mixed clinical/non-clinical populations,[16] schools,[18,20] and a charity.[15] ASD case numbers in all of these studies varied from 23[20] to 72.[15] The study with the largest number of cases to date was a population-based study, and these authors found an association between masculinised 2D:4D and ASD.[16] Overall, results of published studies examining the 2D:4D–ASD relationship have been mixed, although the majority of them have reported an association concordant with the extreme male brain theory.[15,17,19-22] Others have found no association,[18,23] or the inverse association to the prevailing hypothesis.[16] It is also notable that many studies have examined case populations that are entirely or almost entirely male.[17-20,23]

Although most case-control studies selected age- and sex-matched controls, the possibility remains that the composition of the ALSPAC population may differ to these reference populations. The strengths of this study are that it is a population-based study, which is less susceptible to selection bias. The population studied had a comparatively high proportion of female ASD cases. We analysed the association between 2D:4D and ASD risk separately by sex, which may be important, since 2D:4D is a sexually dimorphic trait. However, despite this, the number of female ASD cases in our study was also small (n=14). Although 2D:4D is sexually dimorphic, it has been found that this dimorphism may not be apparent in individuals with ASD.[15] In our study, 2D:4D was sexually

dimorphic in individuals with or without ASD. Further strengths of this study are that we were able to study various component measures of ASD in a large population.[36,53] Although one large study has found an association between ASD traits and 2D:4D,[53] reviews of previous literature have asserted that there is little overall evidence for the relationship between 2D:4D and ASD traits in healthy subjects,[36-37] some suggesting that foetal testosterone may exhibit an interaction effect, being a risk factor for ASDs only in brains of those who are in some other way predisposed. However, our findings do not support this theory considering there was no consistent association observed for ASD diagnosis and trait measures.

The ALSPAC cohort provides a rich resource of data, from which we selected variables that may have confounded an association between 2D:4D and ASD. Although our sample was population based, the possibility of selection bias in relation to baseline recruitment or attrition in the sample over time cannot be excluded. We tried to address this possible attrition bias by controlling for the characteristics known to be predictive of attrition in ALSPAC, such as socioeconomic status. However, it is also possible that those children severely affected by ASD may have been less likely to attend clinics where 2D:4D was measured – this could therefore result in our sample being weighted towards Asperger Syndrome and other high-functioning ASDs. This may be relevant given that previous studies have observed a less profound difference in 2D:4D in children with Asperger Syndrome compared to ASD.[15] Finally, although the method used to measure 2D:4D has been shown to be reliable,[49,54] and used by some of the previous studies on this topic,[15,17] the possibility of error in measurement of 2D:4D cannot be excluded. Yet, it is unlikely that this would be differential for people with and without a diagnosis of ASD.

One recent study reported an association between foetal steroidogenic activity (including testosterone) and ASD, providing some direct evidence in support of the biological basis of the EMB theory of autism.[14] Yet, as foetal testosterone cannot be readily measured, papers assessing the relationship between foetal testosterone and ASDs, including our study, require the existence of a proxy, for example, 2D:4D. One study cites direct evidence of 2D:4D being related to foetal testosterone-to-oestradiol ratio in humans.[55] Some of the strongest indirect evidence for the foetal testosterone-2D:4D relationship comes from studies associating 2D:4D with increased (congenital adrenal hyperplasia)[27,33,35] and decreased (complete androgen insensitivity syndrome)[34]

endocrine models of foetal testosterone exposure. Animal studies have provided mixed results, with some in favour of the foetal testosterone-2D:4D relationship,[56-57] and others supporting the inverse association,[58] or no association.[59] Studies of repeats in the androgen receptor (AR) gene (correlated to testosterone) and 2D:4D have similarly provided mixed results.[49,60-62] A genome-wide scan of 2889 children (including ALSPAC participants) found one SNP in LIN28B (linked to age of menarche)[63] to be associated with 2D:4D. This association was in the opposite direction to that predicted by previous work, in which lower 2D:4D was associated with delayed menarche, putatively via higher foetal testosterone exposure.[49] However, regardless as to whether 2D:4D ratio is causally determined by testosterone, 2D:4D is clearly sexually dimorphic, and thus may still act as a marker of sex-related traits.

To conclude, in this large population based study, we found no evidence for an association between lower 2D:4D ratio and increased risk of ASDs, and very little, inconsistent evidence for an association between 2D:4D and ASD component traits. These findings are not consistent with the extreme male brain theory of autism.

ACKNOWLEDGMENTS

We are extremely grateful to the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council and the Wellcome Trust (Grant ref: 092731) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors, who will serve as guarantors for the contents of this paper. JH was the statistical expert for this paper.

FUNDING

The research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. AG is in receipt of a Wellcome Trust PhD studentship (Grant ref: 102433).

REFERENCE LIST

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Arlington, VA: American Psychiatric Publishing 2013.
2. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* 1977;18:297–321.
3. Koegel LK, Koegel RL, Ashbaugh K, et al. The importance of early identification and intervention for children with or at risk for autism spectrum disorders. *Int J Speech Lang Pathol* 2014;16:50-6.
4. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* 2014;63:1-21.
5. Mattila ML, Kielinen M, Linna SL, et al. Autism spectrum disorders according to DSM-IV-TR and comparison with DSM-5 draft criteria: an epidemiological study. *J Am Acad Child Adolesc Psychiatry* 2011;50:583-592.e511.
6. Baird G, Simonoff E, Pickles A, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet* 2006;368:210-5.
7. Idring S, Rai D, Dal H, et al. Autism spectrum disorders in the Stockholm Youth Cohort: design, prevalence and validity. *PLoS One* 2012;7:e41280. doi: 10.1371/journal.pone.0041280. [published Online First: 20 July 2012].
8. The NHS Information Centre for Health and Social Care. Autism spectrum disorders in adults living in households throughout England. *Report from the Adult Psychiatric Morbidity Survey 2007.* http://www.hscic.gov.uk/catalogue/PUB01131/aut-sp-dis-adu-liv-ho-a-p-m-sur-eng-2007-rep.pdf. Published 22 September 2009. Accessed 28 August 2014.
9. Kothari R, Skuse D, Wakefield J, et al. Gender differences in the relationship between social communication and emotion recognition. *J Am Acad Child Adolesc Psychiatry* 2013;52:1148-1157.

10. Dworzynski K, Ronald A, Bolton P, et al. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry* 2012;51:788-797.
11. Baron-Cohen S. The extreme male brain theory of autism. *Trends Cogn Sci* 2002;6:248-254.
12. Geschwind N, Galaburda A. Cerebral Lateralization: biological mechanisms, associations and pathology: I. A hypothesis and a program for research. Arch Neurol 1985;42:428-59.
13. Rhees R, Shryne J, Gorski R. Onset of the hormone-sensitive perinatal period for sexual differentiation of the sexually dimorphic nucleus of the preoptic area. *J Neurobiol* 1990;21:781-6.
14. Baron-Cohen S, Auyeung B, Norgaard-Pedersen B, et al. Elevated fetal steroidogenic activity in autism. *Mol Psychiatry* Published Online First: 3 June 2014. doi: 10.1038/mp.2014.48.
15. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43:160-4.
16. Bejerot S, Eriksson JM, Bonde S, et al. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. *Br J Psychiatry* 2012;201:116-23.
17. De Bruin EI, Verheij F, Wiegman T, et al. Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. *Dev Med Child Neurol* 2006;48:962-5.
18. Falter CM, Plaisted KC, Davis G. Visuo-spatial processing in autism - Testing the predictions of extreme male brain theory. *J Autism Dev Disord* 2008;38:507-515.
19. Krajmer P, Spajdel M, Kubranska A, et al. 2D:4D finger ratio in Slovak autism spectrum disorder population. *Bratisl Lek Listy* 2011;112:377-9.
20. Milne E, White S, Campbell R, et al. Motion and form coherence detection in autistic spectrum disorder: Relationship to motor control and 2:4 digit ratio. *J Autism Dev Disord* 2006;36:225-37.
21. Noipayak P. The ratio of 2nd and 4th digit length in autistic children. *J Med Assoc Thai* 2009;92:1040-5.
22. Sugie Y, Sugie H, Fukuda T, et al. Study of HOXD genes in autism particularly regarding the ratio of second to fourth digit length. *Brain Dev* 2010;32:356-61.

23. Aksu F, Baykara B, Ergin C, et al. [Phenotypic features in autistic individuals: the finger length ratio (2D:4D), hair whorl, and hand dominance]. *Turk Psikiyatri Derg 2013*;24:94-100.
24. Kothari R, Gafton J, Treasure J, et al. 2D:4D ratio in children at familial high-risk for eating disorders: The role of prenatal testosterone exposure. *Am J Hum Biol* 2014;26:176-182.
25. Ecker A. Einige Bemerkungen über einen schwankenden Charakter in der Hand des Menschen. *Archiv für Anthropologie* 1875;8:67-74.
26. George R. Human finger types. *The Anatomical Record* 1930;46:199–204.
27. Honekopp J, Watson S. Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. *Am J Hum Biol* 2010;22:619-30.
28. Knickmeyer RC, Baron-Cohen S. Fetal testosterone and sex differences in typical social development and in autism. *J Child Neurol* 2006;21:825-45.
29. Garn SM, Burdi AR, Babler WJ, et al. Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *Am J Phys Anthropol* 1975;43:327-32.
30. Honekopp J, Bartholdt L, Beier L, et al. Second to fourth digit length ratio (2D:4D) and adult sex hormone levels: New data and a meta-analytic review. *Psychoneuroendocrinology* 2007;32:313-21.
31. McIntyre MH, Ellison PT, Lieberman DE, et al. The development of sex differences in digital formula from infancy in the Fels Longitudinal Study. *Proc Biol Sci* 2005;272:1473-9.
32. Manning JT, Scutt D, Wilson J, et al. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum Reprod.* 1998;13:3000-4.
33. Buck JJ, Williams RM, Hughes IA, et al. In-utero androgen exposure and 2nd to 4th digit length ratio - Comparisons between healthy controls and females with classical congenital adrenal hyperplasia. *Hum Reprod* 2003;18:976-9.
34. Berenbaum SA, Bryk KK, Nowak N, et al. Fingers as a marker of prenatal androgen exposure. *Endocrinology* 2009;150:5119-24.
35. Brown WM, Hines M, Fane BA, et al. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Horm Behav* 2002;42:380-6.
36. Honekopp J. Digit ratio 2D:4D in relation to autism spectrum disorders, empathizing, and systemizing: a quantitative review. *Autism Res* 2012;5:221-30.

37. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43:2664-76.

38. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;42:111-27.

39. Avon Longitudinal Study of Parents and Children. ALSPAC: Data Dictionary. ALSPAC. 2014; http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Updated 21 April, 2014, Accessed 28 August 2014.

40. Williams E, Thomas K, Sidebotham H, et al. Prevalence and characteristics of autistic spectrum disorders in the ALSPAC cohort. *Dev Med Child Neurol* 2008;50: 672-77.

41. Center for Disease Control and Prevention (CDC). Mental health in the United States: parental report of diagnosed autism in children aged 4-17 years--United States, 2003-2004. *MMWR Morb Mortal Wkly Rep* 2006;55:481-486.

42. Lyall K, Pauls DL, Spiegelman D, et al. Fertility therapies, infertility and autism spectrum disorders in the Nurses' Health Study II. *Paediatr Perinat Epidemiol* 2012;26:361-72.

43. Russell G, Rodgers LR, Ukoumunne OC, et al. Prevalence of parent-reported ASD and ADHD in the UK: findings from the Millennium Cohort Study. *J Autism Dev Disord* 2014;44:31-40.

44. Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. *PLoS One* 2010;5:e12633.

45. Bishop DV. Development of the Children's Communication Checklist (CCC): a method for assessing qualitative aspects of communicative impairment in children. *J Child Psychol Psychiatry* 1998;39:879-91.

46. Skuse DH, James RS, Bishop DV, et al. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 1997;387:705-8.

47. Rutter M, Tizard J, Whitmore K. *Education, Health & Behaviour.* London, UK: Longman 1970.

48. Buss A, Plomin R. Temperament: Early developing personality traits. Hillsdale, NJ: Lawrence Erlbaum 1984.

49. Medland SE, Zayats T, Glaser B, et al. A variant in LIN28B is associated with 2D:4D finger-length ratio, a putative retrospective biomarker of prenatal testosterone exposure. *Am J Hum Genet* 2010;86:519-25.

50. Office of Population Censuses and Surveys. Standard Occupational Classification, Vol 3. London, UK: Her Majesty's Stationery Office 1991.

51. Durkin MS, Maenner MJ, Meaney FJ, et al. Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. *PLoS One* 2010;5:e11551.

52. Rai D, Lewis G, Lundberg M, et al. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. *J Am Acad Child Adolesc Psychiatry* 2012;51:467-476.e6.

53. Manning JT, Baron-Cohen S, Wheelwright S, et al. Is digit ratio (2D:4D) related to systemizing and empathizing? Evidence from direct finger measurements reported in the BBC internet survey. *Pers Individ Diff.* 2010;48:767-71.

54. Robinson SJ, Manning JT. The ratio of 2nd to 4th digit length and male homosexuality. *Evol Hum Behav* 2000;21:333-45.

55. Lutchmaya S, Baron-Cohen S, Raggatt P, et al. 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Hum Dev* 2004;77:23-8.

56. Talarovicova A, Krskova L, Blazekova J. Testosterone enhancement during pregnancy influences the 2D:4D ratio and open field motor activity of rat siblings in adulthood. *Horm Behav* 2009;55:235-9.

57. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. *Proc Natl Acad Sci U S A* 2011;108:16289-94.

58. Abbott AD, Colman RJ, Tiefenthaler R, et al. Early-to-mid gestation fetal testosterone increases right hand 2D:4D finger length ratio in polycystic ovary syndrome-like monkeys. *PLoS One* 2012;7:e42372.

59. Lilley T, Laaksonen T, Huitu O, et al. Maternal corticosterone but not testosterone level is associated with the ratio of second-to-fourth digit length (2D:4D) in field vole offspring (Microtus agrestis). *Physiol Behav* 2010;99:433-7.

60. Honekopp J. No Evidence that 2D:4D is Related to the Number of CAG Repeats in the Androgen Receptor Gene. *Front Endocrinol (Lausanne)* 2013;4:185.

61. Manning J, Bundred P, Newton D, et al. The second to fourth digit ratio and variation in the androgen receptor gene. *Evol Hum Behav* 2003;24:399–405.

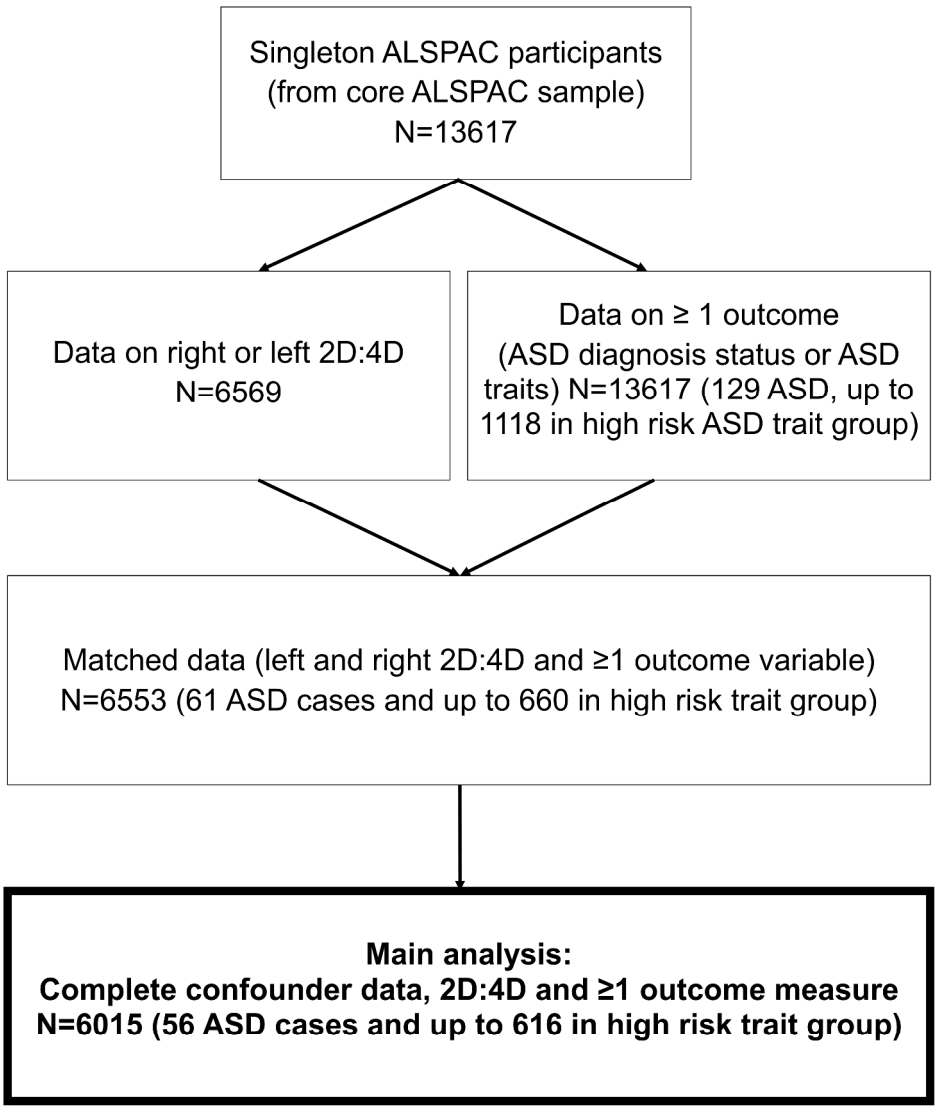
62. Hurd PL, Vaillancourt KL, Dinsdale NL. Aggression, digit ratio and variation in androgen receptor and monoamine oxidase A genes in men. *Behav Genet* 2011;41:543-56.

63. He C, Kraft P, Chen C, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet* 2009;41:724-8.

FIGURE 1: Description of case selection. ASD: autism spectrum disorders. ALSPAC: Avon Longitudinal Study of Parents and Children. 2D:4D: Second-to-fourth digit ratio.

FIGURE 2: Histograms and scatterplots of left and right 2D:4D (second-to-fourth digit ratio), separately for males and females.

a) Histogram showing distribution of right and left 2D:4D in males. Mean (standard deviation) of right 2D:4D: 0.958 (0.032); left 2D:4D 0.960 (0.032). b) Histogram showing distribution of right and left 2D:4D in females. Mean (standard deviation) of right 2D:4D 0.969 (0.033); left 0.970 (0.032). c) Scatterplot of right and left 2D:4D in males; correlation between right and left hands: 0.64. d) Scatterplot of right and left 2D:4D in females; correlation between right and left hands: 0.69.



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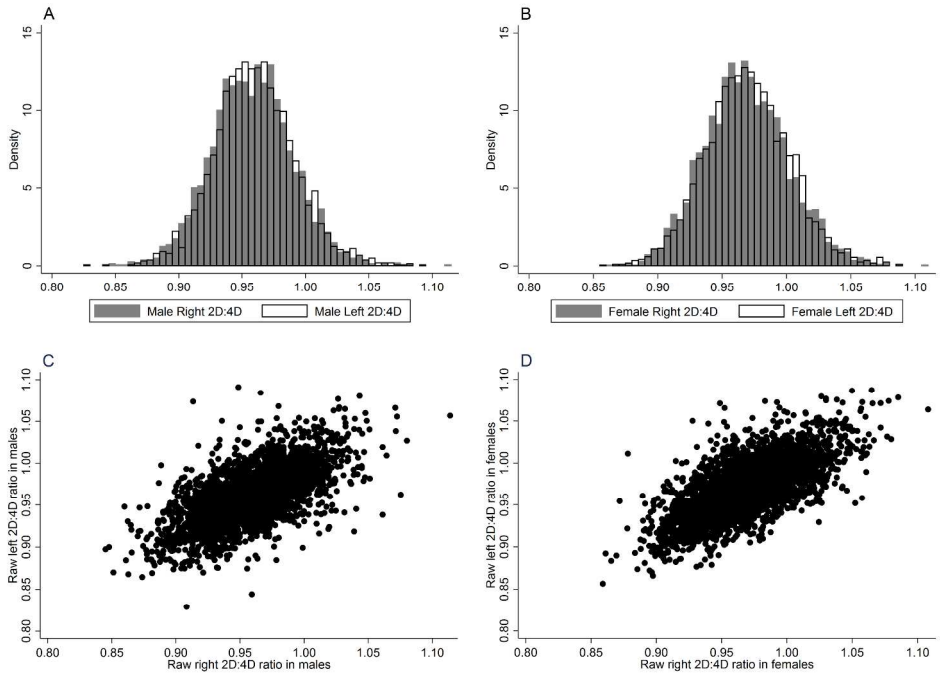

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595x441mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	✓
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	✓
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓
		(*b*) Describe any methods used to examine subgroups and interactions	✓
		(*c*) Explain how missing data were addressed	✓
		(*d*) If applicable, explain how loss to follow-up was addressed	✓
		(*e*) Describe any sensitivity analyses	✓
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	✓
		(c) Consider use of a flow diagram	✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	✓
		(c) Summarise follow-up time (eg, average and total amount)	✓
Outcome data	15*	Report numbers of outcome events or summary measures over time	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓

Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Table S1: Logistic regression of ASD (Autism Spectrum Disorder) cases status diagnosed by two methods (maternal report only or formal diagnosis), against risk group for each dichotomised ASD trait measure.

ASD trait measure	Maternally reported ASD only versus no ASD	Formal ASD diagnosis versus no ASD
	OR [95% CI], p value	OR [95% CI], p value
Children's Communication Checklist (Coherence subscale) (115 months)	9.41 [4.64, 19.07] p≤0.001	†
Social and Communication Developmental Checklist (91 months)	8.98 [4.33, 18.63] p≤0.001	73.13 [21.73, 246.11] p≤0.001
Repetitive behaviour (69 months)	10.23 [4.78, 21.88] p≤0.001	11.75 [5.11, 27.01] p≤0.001
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	2.40 [1.03, 5.60] p=0.042	5.73 [2.56, 13.07] p≤0.001

†All children with formally reported ASD and a valid Children's Communication Checklist score were in the high risk group.

Table S2: Left and right 2D:4D (second-to-fourth digit ratio): mean (standard error), and numbers (n) in each subgroup, separately by sex.[a]

Outcome	Age at measurement	Risk group	Left hand		Right hand	
			Males	Females	Males	Females
ASD diagnosis	Diagnosed by 11 years	No ASD diagnosis	0.960 (0.001) n = 2920	0.970 (0.001) n = 3039	0.958 (0.001) n = 2920	0.968 (0.001) n = 3039
		ASD diagnosis	0.957 (0.003) n = 42	0.974 (0.007) n = 14	0.954 (0.004) n = 42	0.985 (0.007) n = 14
Children's Communication Checklist (Coherence subscale)	115 months	Low risk	0.960 (0.001) n = 2285	0.970 (0.001) n = 2495	0.958 (0.001) n = 2285	0.969 (0.001) n = 2495
		High risk	0.964 (0.002) n = 302	0.970 (0.002) n = 167	0.959 (0.002) n = 302	0.968 (0.002) n = 167
Social and Communication Developmental Checklist	91 months	Low risk	0.961 (0.001) n = 2260	0.970 (0.001) n = 2444	0.958 (0.001) n = 2260	0.969 (0.001) n = 2444
		High risk	0.959 (0.002) n = 299	0.972 (0.03) n = 172	0.958 (0.002) n = 299	0.969 (0.003) n = 172
Repetitive behaviour	69 months	Low risk	0.961 (0.001) n = 2378	0.970 (0.001) n = 2509	0.959 (0.001) n = 2378	0.969 (0.001) n = 2509
		High risk	0.962 (0.002) n = 199	0.971 (0.003) n = 130	0.954 (0.002) n = 199	0.967 (0.002) n = 130
Emotionality Activity, Sociability Temperament Scale (Sociability subscale)	38 months	Low risk	0.960 (0.001) n = 2378	0.970 (0.001) n = 2540	0.958 (0.001) n = 2378	0.969 (0.001) n = 2540
		High risk	0.961 (0.002) n = 352	0.968 (0.002) n = 264	0.957 (0.002) n = 352	0.967 (0.002) n = 264

[a] Subgroups are split by ASD (Autism Spectrum Disorder) diagnosis, or by high and low risk groups for each of the four dichotomised measures predictive of ASD.

Table S3: IQ-adjusted logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) vs no diagnosis, or high risk dichotomised trait measure group vs low risk, against mean 2D:4D (second-to-fourth digit ratio) (left and right hand combined), stratified by sex. Unadjusted and adjusted[a] models are presented. NB Odds ratios are for one unit change in Z score (i.e. 1 standard deviation). N=numbers of participants.

Outcome	Males				Females			
	Unadjusted OR [95% CI] P value	Adjusted[a] OR [95% CI] P value	Total N	Risk N[b]	Unadjusted OR [95% CI] P value	Adjusted[a] OR [95% CI] P value	Total N	Risk N[b]
ASD diagnosis (diagnosed by 11 years)	0.81 [0.57, 1.15] p=0.242	0.81 [0.57, 1.16] p=0.253	2543	33	1.51 [0.84, 2.69] p=0.165	1.50 [0.83, 2.70] p=0.181[c]	2676	11
Children's Communication Checklist (Coherence subscale) (115 months)	1.10 [0.97, 1.26] p=0.144	1.10 [0.96, 1.26] p=0.159	2319	254	0.94 [0.79, 1.11] p=0.452	0.93 [0.78, 1.10] p=0.405	2418	151
Social and Communication Developmental Checklist (91 months)	0.95 [0.84, 1.08] p=0.446	0.95 [0.84, 1.08] p=0.440	2295	269	1.01 [0.85, 1.19] p=0.933	1.01 [0.86, 1.20] p=0.855	2380	150
Repetitive behaviour (69 months)	0.92 [0.79, 1.07] p=0.285	0.92 [0.79, 1.08] p=0.300	2282	175	0.96 [0.80, 1.17] p=0.712	0.98 [0.80, 1.18] p=0.753	2372	112
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	0.96 [0.85, 1.08] p=0.489	0.95 [0.85, 1.08] p=0.452	2391	312	0.94 [0.82, 1.08] p=0.366	0.94 [0.82, 1.08] p=0.402	2495	233

[a]Adjusted for highest parental occupational class, maternal education, age at 2D:4D measurement and intelligence quotient (IQ).
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure.
[c]To avoid perfect prediction, skilled non-manual and manual occupation groups, and CSE (Certificate of Secondary Education) and vocational maternal education groups were collapsed.

Table S4a: Logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) versus no diagnosis, or high risk dichotomised trait measure group versus low risk, against 2D:4D (second-to-fourth digit ratio), separately by left and right hand, in males only. Unadjusted and adjusted[a] models are presented. Odds ratios are for one unit change in Z score (one standard deviation). N=numbers of participants.

Outcome	Males					
	Left		Right		Total N	Risk N[b]
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]		
	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value		
ASD diagnosis (diagnosed by 11 years)	0.89 [0.65, 1.21] p=0.447	0.89 [0.65, 1.22] p=0.469	0.88 [0.65, 1.20] p=0.431	0.90 [0.66, 1.23] p=0.494	2962	42
Children's Communication Checklist (Coherence subscale) (115 months)	1.15 [1.02, 1.29] p=0.024	1.15 [1.02, 1.29] p=0.023	1.03 [0.91, 1.17] p=0.602	1.03 [0.92, 1.17] p=0.584	2587	302
Social and Communication Developmental Checklist (91 months)	0.95 [0.84, 1.07] p=0.392	0.95 [0.84, 1.07] p=0.375	0.99 [0.87, 1.11] p=0.827	0.99 [0.88, 1.12] p=0.847	2559	299
Repetitive behaviour (69 months)	1.04 [0.90, 1.20] p=0.576	1.04 [0.90, 1.20] p=0.592	0.87 [0.75, 1.01] p=0.060	0.87 [0.75, 1.01] p=0.061	2577	199
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	1.01 [0.90, 1.13] p=0.892	1.00 [0.89, 1.12] p=0.997	0.95 [0.85, 1.06] p=0.363	0.94 [0.84, 1.06] p=0.322	2730	352

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D measurement.
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure.

Table S4b: Logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) versus no diagnosis, or high risk dichotomised trait measure group versus low risk, against 2D:4D (second-to-fourth digit ratio), separately by left and right hand, in females only. Unadjusted and adjusted[a] models are presented. Odds ratios are for one unit change in Z score (one standard deviation). N=numbers of participants.

Outcome	Females					
	Left		Right		Total N	Risk N[b]
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]		
	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value		
ASD diagnosis (diagnosed by 11 years)	1.12 [0.66, 1.89] p=0.681	1.08 [0.64, 1.82] p=0.770[c]	1.63 [0.99, 2.69] p=0.057	1.60 [0.97, 2.65] p=0.068[c]	3053	14
Children's Communication Checklist (Coherence subscale) (115 months)	0.99 [0.85, 1.16] p=0.914	0.99 [0.85, 1.16] p=0.916	0.97 [0.83, 1.14] p=0.703	0.97 [0.83, 1.14] p=0.736	2662	167
Social and Communication Developmental Checklist (91 months)	1.06 [0.91, 1.24] p=0.455	1.07 [0.92, 1.26] p=0.373	1.00 [0.86, 1.16] p=0.979	1.01 [0.86, 1.17] p=0.936	2610	172
Repetitive behaviour (69 months)	1.03 [0.86, 1.23] p=0.739	1.04 [0.86, 1.24] p=0.704	0.93 [0.78, 1.11] p=0.426	0.94 [0.78, 1.12] p=0.462	2639	130
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	0.93 [0.82, 1.06] p=0.254	0.93 [0.82, 1.06] p=0.283	0.96 [0.84, 1.09] p=0.505	0.96 [0.85, 1.09] p=0.535	2804	264

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D measurement.
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure group.
[c]To avoid perfect prediction, the CSE (Certificate of Secondary Education) and vocational classes of maternal education were collapsed for this analysis.

BMJ Open

Digit Ratio and Autism Spectrum Disorders in the Avon Longitudinal Study of Parents and Children: A Birth Cohort Study.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-007433.R1
Article Type:	Research
Date Submitted by the Author:	27-Mar-2015
Complete List of Authors:	Guyatt, Anna; University of Bristol, Centre for Academic Mental Health, School of Social & Community Medicine; University of Bristol, Bristol Genetic Epidemiology Laboratories, School of Social and Community Medicine Heron, Jon; University of Bristol, Centre for Academic Mental Health, School of Social & Community Medicine Knight, Bernice; University of Bristol, Centre for Academic Mental Health, School of Social & Community Medicine Golding, Jean; University of Bristol, Centre for Child and Adolescent Health, School of Social & Community Medicine Rai, Dheeraj; University of Bristol, Centre for Academic Mental Health, School of Social & Community Medicine; Avon and Wiltshire Mental Health Partnership National Health Service Trust,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Mental health
Keywords:	EPIDEMIOLOGY, Developmental neurology & neurodisability < PAEDIATRICS, Child & adolescent psychiatry < PSYCHIATRY

Digit Ratio and Autism Spectrum Disorders in the Avon Longitudinal Study of Parents and Children: A Birth Cohort Study.

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Word count: 4049 words (excluding title page, abstract, references, figures and tables)

Keywords: Avon Longitudinal Study of Parents and Children (ALSPAC); autistic disorder; digit ratio; testosterone; prenatal exposure delayed effects.

OBJECTIVES To investigate whether second-to-fourth digit ratio (2D:4D), a measure commonly used as a proxy for fetal testosterone exposure, is associated with autism spectrum disorders (ASDs), as predicted by the extreme male brain theory of autism.

DESIGN A birth cohort study.

SETTING The Avon Longitudinal Study of Parents and Children (ALSPAC).

PARTICIPANTS 6015 ALSPAC children with data on digit ratio, at least one outcome measure, and information on potential confounding variables (parental occupational class, maternal education, and age at digit ratio measurement). Digit ratio was measured by the photocopy and calliper method.

OUTCOMES ASD diagnosis (cases were identified previously by record linkage or maternal report) and four measures that combine optimally within ALSPAC to predict ASD: the Children's Communication Checklist (coherence subscale), the Social and Communication Disorders Checklist, a repetitive behaviour measure, and the Emotionality, Activity and Sociability scale (sociability subscale). These measures were dichotomised, with approximately 10% defined as the 'risk' group.

RESULTS Using logistic regression, we examined the association of 2D:4D with ASDs and four dichotomised ASD traits. Covariates were occupational class, maternal education and age at 2D:4D measurement. 2D:4D was not associated with ASDs in males (adjusted odds ratio (OR) per 1-standard deviation increase in mean 2D:4D, 0.88 [95% confidence interval (95%CI) 0.65-1.21], p=0.435) or females (adjusted OR=1.36 [95%CI 0.81-2.28], p=0.245). Similar results were observed after adjustment for IQ. There was one weak association between reduced coherence and increased left 2D:4D in males, in the opposite direction to that predicted by the extreme male brain theory (adjusted OR=1.15 [95%CI 1.02-1.29], p=0.023). Given multiple comparisons, this is consistent with chance.

CONCLUSIONS In this population-based study, there was no strong evidence of an association between 2D:4D and ASD diagnosis or traits, although the confidence intervals were wide. These results are not consistent with the extreme male brain theory.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To date, this is one of the largest population based studies examining the relationship between second-to-fourth digit ratio (2D:4D), which is used as a proxy for fetal testosterone exposure, and autism spectrum disorders, separately by sex.
- This paper also examines the relationship between second-to-fourth digit ratio (2D:4D) and component measures of autism, which may give insight into the less-explored relationship between digit ratio and the individual domains (social and communication, repetitive behaviours) represented in autism.
- In comparison with other small studies based in clinical settings, this study may be less prone to selection bias; the rich phenotype information within ALSPAC allowed us to control for possible confounding variables.
- Despite its relatively large overall sample size compared with many studies, the number of autism cases in this study is still small.
- Cohort studies are prone to bias from attrition, and whilst the major sociodemographic predictors of attrition were controlled for in this study, it is also possible that children severely affected by ASD may have been less likely to attend clinics where 2D:4D was measured, which could bias our results.

INTRODUCTION

Autism spectrum disorders (ASD) are pervasive developmental disorders characterised by impaired social communication and reciprocal social interaction; and restricted, repetitive patterns of behaviour, interests, or activities.[1] Whilst twin studies suggest that ASDs are highly heritable, their aetiology remains largely unexplained.[2] Elucidating the causes of ASD may facilitate earlier diagnosis and enhance the potential for primary prevention. Earlier diagnosis may lead to an improvement in educational and behavioural outcomes, a reduction in comorbid psychiatric symptoms, and a decrease in the emotional and financial stresses created by ASDs for individuals and their families.[3]

Various population cohorts have estimated the prevalence of ASD at approximately 1%.[4-7] These studies demonstrate a marked gender imbalance, with male to female ratio estimates ranging from 2:1[5] to 9:1,[8] depending upon age of assessment, length of follow-up, and whether studies screen for ASDs or examine data on pre-existing diagnosis. Although diagnostic bias has been proposed as a partial explanation for this disparity between sexes,[9-10] the consistently observed preponderance amongst males has generated interest into possible biological mechanisms predisposing to ASDs.

The extreme male brain (EMB) theory is the most popular hypothesis put forward to explain the sex difference in ASD. This theory postulates that children with ASD exhibit an exaggerated form of the male cognitive profile,[11] and proposes that prenatal androgens are plausible biological candidates. Some evidence from animal studies suggests that testosterone may mediate cognitive differences between the sexes via organisational effects on the brain.[12-13] Recent evidence supporting the EMB theory found sex steroid levels in amniocentesis samples to be correlated with the diagnosis of ASDs.[14]

The index to ring finger ratio (2D:4D) has been widely used as a proxy for fetal testosterone exposure in autism research.[15-25] An observation supporting a causal association between 2D:4D and fetal testosterone is that 2D:4D has been shown to be sexually dimorphic,[26] with males generally having lower 2D:4D (that is to say, a relatively shorter index finger (2D) compared to their ring finger (4D)),[27-28] although this is not a unanimously reported finding [25]. This sexual dimorphism is apparent from the first trimester of pregnancy, and appears to be largely static after birth, with most,[29-33] but not all[25] studies finding that it is unaffected by pubertal androgen. 2D:4D has also been shown to be sexually dimorphic in endocrine models of elevated (congenital adrenal

hyperplasia) and reduced fetal testosterone exposure (complete androgen insensitivity syndrome).[28,34-36]

Although recent reviews have concluded that lower 2D:4D is associated with ASD,[37-38] many published studies are within relatively small, clinical populations which may be susceptible to selection bias and confounding. We designed a study to examine the association between 2D:4D and ASD and various autistic trait measures in a population-based cohort in the UK. The primary hypothesis being tested was that lower 2D:4D would be associated with ASDs and ASD traits.

METHODS

Study design and population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a birth cohort based in Avon, UK. 14,541 pregnant women with expected delivery dates between 1st April 1991 and 31st December 1992 were initially enrolled, and 13,988 children were alive at one year.[39] Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. The study website contains details of all the data that are available through a fully searchable data dictionary: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary.[40] The primary source of data collection was via self-completion questionnaires administered at four points during the prenatal period, then at regular intervals following birth, to both parents and the "study child". Since the age of 7 years the whole cohort has been invited to a regular "focus" clinic for a variety of hands-on assessments.

Outcome variables

Autism spectrum disorders

Within ALSPAC, the identification of children with ASD has been described elsewhere.[41] Briefly, a previous record linkage study identified 86 ASD cases in ALSPAC, of which there was evidence for a strict multidisciplinary clinical assessment in 71 individuals, and in 15 others with a diagnosis of ASD recorded in school records.[41] In a validation study, a consultant paediatrician reviewed the records of the individuals with ASD to confirm concordance with ICD-10 criteria.[41] In addition to cases identified by record linkage, we further identified individuals with ASD based on maternal report. At 9.5 years, mothers were asked, "Have you ever been told that your child has: autism, Asperger's syndrome or autistic spectrum disorder?" Investigators utilising maternal reports based on similar questions in other cohorts have reported an acceptable validity of identifying ASD using this approach.[42-44] We cross validated ASD cases confirmed only by maternal report by studying their association with various autistic trait measures and found strong associations (see supplementary Table S1, available online). Of the 56 ASD cases included in this study, 24 were identified by record linkage, and 32 were identified from maternal reports of ASD diagnosis.

Dichotomised ASD traits

A previous factor analysis of 93 measures related to autism also reported four individual measures administered in ALSPAC via parental questionnaires that 'combined optimally to predict autism'.[45] The coherence subscale of the Children's Communication Checklist, measured at 115 months, assesses pragmatic abnormalities in social communication.[46] The Social and Communication Disorders Checklist, measured at 91 months, measured social skills (described previously).[47] A Repetitive Behaviour score, measured at 69 months asked whether the child "repeatedly rocks the head or body, has tics or twitches or other unusual behaviour", and included a question from the Rutter scale on tics, mannerisms or twitches.[48] The sociability subscale of the Emotionality Activity and Sociability measure at 38 months measures tendency to affiliate and interact with others.[49] As all the ASD scales considered were highly negatively skewed, and not readily correctable by transformation, each was dichotomised, creating a high risk (for ASDs) group of as close to 10% of the population as possible.

Explanatory variable: 2D:4D

Participants were invited to a focus clinic, when 2D:4D was measured. Mean measurement age was 11.75 years. Whilst 2D:4D was measured *after* the outcome measures (between approximately 2 to 8 years later, depending upon the outcome), it is widely used as a measurement of the prenatal environment, and therefore we feel justified in using it as our exposure in our analysis.

2D:4D was measured by the photocopy and calliper method: the ventral surface of each palm was placed as flatly as possible onto a photocopier, ensuring that the fingers were apart and the palms pressed firmly onto the glass. The lengths of the second (index) and fourth (ring) fingers were measured using the "Mahr digital caliper16 EX", accurate to 0.01mm. Each finger measured has a crease at its base: the index finger (2D) has one crease, the ring finger (4D) probably 3 or 4. The most proximal crease was chosen and by eye, the midpoint of this was determined and the distance from this crease to the distal fingertip measured. 2D measurement was divided by 4D measurement thus giving a 2D:4D measurement for each hand.[40] To assess the legitimacy of using photocopies to calculate 2D:4D, 57 right and 48 left hands in ALSPAC have been measured previously in vivo, with high correlation between these measures and photocopies (r=0.97).[50]

Confounding variables

We adjusted for the potential confounding effect of a number of socio-demographic measures. Each parent's self-reported occupation was coded as one of professional, managerial and technical, skilled non-manual, or manual,[51] and the highest reported class from each child's parent(s) was recorded. Highest self-reported maternal education level was recorded as CSE (certificate of secondary education), vocational, 'O' level, 'A' level or degree. Occupational class and maternal education were used as a proxy for socioeconomic status (SES), which is known to predict loss to follow up in ALSPAC.[38] ASDs are socially patterned,[52] although recent research suggests this may be in a direction opposite to that previously reported.[53] We also adjusted for the age at which the participants attended the 11-year clinic (where the 2D:4D exposure was measured) to account for compliance to clinic invite, which may be related to the outcomes considered.

Statistical analysis

For the main analysis, we included children from singleton pregnancies with left and right 2D:4D measured, who had data for at least one of five outcome measures (recorded presence/absence of ASDs, or data on at least one of the four dichotomised ASD trait measures) and additionally, data on potential confounders, including parental occupational class, maternal education level, and age at 2D:4D measurement (n=6015, including 56 ASD cases, and up to 616 in a high risk dichotomised group) (see Fig. 1 and Table 1).

TABLE 1: Characteristics of the study sample compared to those ALSPAC participants not included in the study*. n=number of participants.

	Study sample (n=6015) (Standard deviations given for means)	Other ALSPAC participants (n≤7602) (Standard deviations given for means)	p value**
% Male	49%	53%	p≤0.001
% Autism spectrum disorder (maternal and formal report)	0.93%	0.96%	p=0.861
Mean 2D:4D (those with left and right 2D:4D, excluding two outliers)	0.964 (0.029)	0.963 (0.030)	p=0.421
Age at 2D:4D measurement, months	140.9 (2.8)	141.3 (3.2)	p≤0.001
% Manual occupational background	14.2%	25.3%	p≤0.001
% Mother educated to degree level	16.3%	9.5%	p≤0.001

*(excluding multiple births, those not alive at 1 year, and those not in the core ALSPAC sample). ALSPAC=Avon Longitudinal Study of Parents and Children.

2D:4D=second-to-fourth digit ratio.

**from chi-squared test for categorical variables, and unpaired t-test (two tailed) for continuous variables

Since the correlation between right and left hand 2D:4D was high (males 0.64, females 0.69), we used the mean of left and right hand 2D:4D in our main analysis (see Fig 2.). Two outliers were removed due to their extreme 2D:4D values (<0.75 and >1.25). Sex differences in this composite measure were assessed using a two-tailed t-test. Given the male preponderance in ASDs, and since 2D:4D is sexually dimorphic, all analyses which followed were stratified by sex, in order to assess whether the relationship of 2D:4D and ASD varied between sexes. To enable a clearer comparison of the male and female results, the 2D:4D measure was further standardised within each sex, so that a one unit change in 2D:4D represents a change by 1 standard deviation. Raw scores (i.e. non-standardised scores) are presented for descriptive tables.

A univariable logistic regression model was estimated for each ASD related outcome in turn, using the standardised mean 2D:4D variable as a continuous exposure (see Table 3). Effects were subsequently adjusted for the potential confounders listed above.

A supplementary analysis adjusted for IQ (see supplementary Table S3, available online). As sensitivity analyses, models were repeated using left and then right hand in turn (see supplementary Tables S4a and S4b, available online). All analyses were carried out in Stata (StataCorp, TX), version 13.

RESULTS

The study sample and those ALSPAC participants not in this particular study were similar in terms of mean 2D:4D, age at 2D:4D measurement and ASD prevalence, but fewer participants in our study were male (see Table 1). Amongst our study sample, there was an excess of ASD cases identified from maternal report of diagnosis only, in comparison to cases identified by record linkage, despite the equivalent prevalence of ASDs between our study sample and the remaining ALSPAC participants (see Table 1). Participants in the study sample were more likely to have parents working in non-manual occupations, were more likely to have a marginally higher IQ, and their mothers were more likely to be educated to degree level, which is consistent with the social patterning of attrition previously observed within ALSPAC (see Table 1).[39] Within the full sample (n=6015) there was strong evidence of sexual dimorphism for 2D:4D, with a mean (SE) of 0.959 (0.001) in males and 0.969 (0.001) in females (t=13.4, d.f.=6013, two-tailed t-test p≤0.001). This sexual dimorphism was still present in the subsample of ASD cases (n=56, males 2D:4D=0.955 (0.003); females=0.979 (0.006), t=3.5, d.f.=54, p≤0.001). Mean 2D:4D values stratified by ASD diagnosis and the other outcomes are shown in Table 2 (see also supplementary Table S2, available online, for results shown separately for right and left hand, and for results stratified by record linkage versus maternal report of ASD diagnosis).

TABLE 2: Mean (standard error) of left and right second-to-fourth digit ratio, and numbers (n) in each subgroup (ASD/no ASD; high/low ASD risk), by sex.

Outcome	Age	Risk group	Males		Females	
			Mean (SE)	N	Mean (SE)	N
Autism Spectrum Disorder (ASD) diagnosis	Diagnosed by 11 years	No ASD diagnosis	0.959 (0.001)	2920	0.969 (0.001)	3039
		ASD diagnosis	0.955 (0.003)	42	0.979 (0.006)	14
Children's Communication Checklist (Coherence subscale)	115 months	Lower risk	0.959 (0.001)	2285	0.970 (0.001)	2495
		Higher risk	0.962 (0.002)	302	0.969 (0.002)	167
Social and Communication Developmental Checklist	91 months	Lower risk	0.960 (0.001)	2260	0.970 (0.001)	2444
		Higher risk	0.959 (0.002)	299	0.970 (0.002)	172
Repetitive behaviour	69 months	Lower risk	0.960 (0.001)	2378	0.970 (0.001)	2509
		Higher risk	0.958 (0.002)	199	0.969 (0.003)	130
Emotionality Activity, Sociability Temperament Scale	38 months	Lower risk	0.959 (0.001)	2378	0.970 (0.001)	2540
		Higher risk	0.959 (0.002)	352	0.968 (0.002)	264

(Sociability subscale)						

Table 3 shows unadjusted and adjusted estimates for the increase in odds of each outcome per 1 standard deviation increase in mean 2D:4D ratio. There was no evidence of an association between mean 2D:4D and any of the five outcomes in either sex.

TABLE 3: Logistic regression of ASD vs no ASD, or high risk dichotomised trait vs low risk, against mean 2D:4D (left/right hands combined), stratified by sex. Unadjusted and adjusted[a] models are presented. NB Odds ratios are for one unit change in Z score (i.e. 1 standard deviation). N=numbers of participants.

Sex	Males				Females			
Model	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]
Autism Spectrum Disorder (ASD) diagnosis	0.87 [0.64, 1.19] p=0.393	0.88 [0.65, 1.21] p=0.435	2962	42	1.39 [0.83, 2.34] p=0.206	1.36 [0.81, 2.28] p=0.245[c]	3053	14
Children's Communication Checklist (Coherence subscale)	1.10 [0.97, 1.24] p=0.124	1.10 [0.98, 1.24] p=0.118	2587	302	0.98 [0.84, 1.15] p=0.790	0.98 [0.84, 1.15] p=0.809	2662	167
Social and Communication Developmental Checklist	0.96 [0.85, 1.09] p=0.553	0.96 [0.85, 1.09] p=0.550	2559	299	1.03 [0.88, 1.20] p=0.698	1.04 [0.89, 1.22] p=0.601	2616	172
Repetitive behaviour	0.95 [0.82, 1.10] p=0.466	0.95 [0.82, 1.09] p=0.458	2577	199	0.98 [0.82, 1.17] p=0.796	0.98 [0.82, 1.17] p=0.840	2639	130
Emotionality, Activity, Sociability (Sociability subscale)	0.98 [0.87, 1.09] p=0.670	0.97 [0.87, 1.08] p=0.583	2730	352	0.94 [0.83, 1.07] p=0.325	0.94 [0.83, 1.07] p=0.357	2804	264

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D (second-to-fourth digit ratio) measurement.

[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait.

[c]To avoid perfect prediction, the CSE (Certificate of Secondary Education) and vocational classes of maternal education were collapsed for this analysis.

We included Intelligence Quotient (IQ) as measured by a modified version of the Wechsler Intelligence Scale for Children, as an additional covariate in a supplementary analysis. We considered this separately from our main analysis to prevent a substantial loss to our sample size, since the IQ measure was available on only 44/56 of included participants with ASDs. Another supplementary analysis examined the effect of 2D:4D by left and right hand.

Supplementary tables show (i) little impact of further adjustment for IQ (supplementary Table S3, available online), and (ii) a broadly consistent pattern of findings when examining the effect of 2D:4D ratio for each hand in turn (supplementary Tables S4a and S4b, available online). One sub-analysis suggested a weak association between reduced coherence (as measured by the Children's Communication Checklist) with left 2D:4D in males (adjusted OR 1.15 [95% CI 1.02, 1.29], p=0.023, see supplementary Table S4a). There was also a trend towards increased right 2D:4D being associated with ASD diagnosis in females (adjusted OR 1.60 [95% CI 0.97, 2.65] p=0.068, see supplementary Table S4b). However, these results are in the opposite direction to that predicted by the prevailing theory of the extreme male brain. One sub-analysis found a trend towards masculinized 2D:4D being associated with the repetitive behaviours measure OR 0.87 [95% CI 0.75, 1.01] p=0.061. Yet, given the number of comparisons and differing directions of association, we consider that these results are consistent with chance.

DISCUSSION

In this large UK-based birth cohort, we did not find evidence to support the hypothesis that lower second to fourth digit ratio (2D:4D) is associated with an increased risk of autism spectrum disorders (ASDs). We also examined the relationship of digit ratio with dichotomised component trait measures of ASDs, including social cognition, coherence, repetitive behaviours and sociability. There were several suggestions of very weak relationships between 2D:4D and ASD traits, when the hands were studied separately. These associations were mostly directionally discordant with the extreme male theory of autism, and given the number of comparisons made, and the lack of consistent association between ASD diagnosis and 2D:4D in this study, we consider that they were due to chance.

Recent reviews have concluded that there is support for an association between masculinised 2D:4D and ASD.[37-38] The central estimate from the most recent quantitative review was a Cohen's d of -0.43, representing a moderately lower digit ratio amongst individuals (mostly males were studied) with autism.[38] Using a t-test (comparing 2D:4D between males with and without ASD - the most common comparison in the literature), we calculated that we would have had 79% power to detect this central estimate. However, we chose logistic regression methods for our primary analysis rather than a t-test, since we were interested in adjusting for the effect of potential confounders. For consistency with previous literature, we repeated our analyses (mean 2D:4D in males with and without ASD) using a t-test, and found a Cohen's d of -0.13 [95% CI -0.44, 0.17]. It should be noted that the d of -0.43, reported in the literature[38] is within the confidence interval of this estimate, albeit at the extreme lower bound.

Amongst the studies examining the 2D:4D-ASD diagnosis relationship (i.e. not ASD trait measures), most studies recruited cases from clinical settings,[17,21-23] which may potentially limit the external validity of results, if cases recruited in a clinical setting are qualitatively different to the larger population from which they are sampled. Others recruited from mixed clinical/non-clinical populations,[16][25] schools,[18,20] and a charity.[15] ASD case numbers in these studies varied from 23[20] to 216.[25] Of two studies with the largest number of cases to date, one was a population-based study, and these authors found an association between masculinised 2D:4D and ASD.[15] Another was a clinically recruited sample, which found no relationship between 2D:4D and ASD.[25] Overall, results of published studies examining the 2D:4D–ASD relationship have therefore been

mixed, although the majority of them have reported an association concordant with the extreme male brain theory.[15,17,19-22] Others have found no association,[18,23,25] or the inverse association to the prevailing hypothesis.[16] It is also notable that many studies have examined case populations that are entirely or almost entirely male.[17-20,23]

Although most case-control studies selected age- and sex-matched controls, the possibility remains that the composition of the ALSPAC population may differ from these reference populations. The strengths of this study are that it is population-based, which is a design that is generally less susceptible to selection bias. We analysed the association between 2D:4D and ASD risk separately by sex, which may be important, since 2D:4D is a sexually dimorphic trait. The population studied had a comparatively high proportion of female ASD cases, however, despite this, the absolute number of female ASD cases in our study was also small (n=14). Although 2D:4D is sexually dimorphic, it has been found that this dimorphism may not be apparent in individuals with ASD.[15] In our study, 2D:4D was sexually dimorphic in individuals with or without ASD. Further strengths of this study are that we were able to study various component measures of ASD in a large population.[37,54] Although one large study has found an association between 2D:4D and ASD traits,[54] reviews of previous literature have asserted that there is little overall evidence for the relationship between 2D:4D and ASD traits in healthy subjects.[37, 38] Our study also found no consistent association between 2D:4D and ASD trait measures.

The ALSPAC cohort provides a rich resource of data, from which we selected variables that may have confounded an association between 2D:4D and ASD. Although our sample was population based, the possibility of selection bias in relation to baseline recruitment or attrition in the sample over time cannot be entirely excluded. We tried to address this possible attrition bias by controlling for the characteristics known to be predictive of attrition in ALSPAC, such as socioeconomic status. However, it is also possible that those children severely affected by ASD may have been less likely to attend clinics where 2D:4D was measured – this could therefore result in our sample being weighted towards Asperger Syndrome and other high-functioning ASD cases. This may be relevant given that previous studies have observed a less profound difference in 2D:4D in children with Asperger Syndrome compared to ASD.[15] Finally, although the method used to measure 2D:4D has been shown to be reliable,[50,55] and used by some of the previous studies on this topic,[15,17] the

possibility of error in measurement of 2D:4D cannot be excluded. Yet, it is unlikely that this would be differential for people with and without a diagnosis of ASD.

One recent study reported an association between fetal steroidogenic activity (including testosterone) and ASD, providing some direct evidence in support of the biological basis of the EMB theory of autism.[14] Yet, as fetal testosterone cannot be readily measured, papers assessing the relationship between fetal testosterone and ASDs, including our study, require the existence of a proxy, for example, 2D:4D. One study cites direct evidence of 2D:4D being related to fetal testosterone-to-oestradiol ratio in humans.[56] Some of the strongest indirect evidence for the fetal testosterone-2D:4D relationship comes from studies associating 2D:4D with increased (congenital adrenal hyperplasia)[28,34,36] and decreased (complete androgen insensitivity syndrome)[35] endocrine models of fetal testosterone exposure. Animal studies have provided mixed results, with some in favour of the fetal testosterone-2D:4D relationship,[57-58] and others supporting the inverse association,[59] or no association.[60] Studies of repeats in the androgen receptor (AR) gene (correlated to testosterone) and 2D:4D have similarly provided mixed results.[50,61-63] A genome-wide scan (including ALSPAC participants) found the minor allele of one SNP in LIN28B (linked to delayed age at menarche)[64] to be associated with increased 2D:4D.[50] This association was in the opposite direction to that predicted from previous work, in which lower 2D:4D was associated with delayed age at menarche, putatively via higher fetal testosterone exposure.[65] The authors therefore suggested that the relationship between fetal testosterone and 2D:4D may be more complex than first proposed, which is in agreement with previous work.[56] However, regardless as to whether 2D:4D ratio is mainly a marker of fetal testosterone, 2D:4D is clearly sexually dimorphic and related to hormonal traits within sexes[65], and thus may still be useful in the study of other sex-related traits.

To conclude, in this large population-based study, we found no consistent evidence supporting an association between lower 2D:4D ratio and increased risk of ASDs, or ASD component traits. These findings are not consistent with the extreme male brain theory of autism.

ACKNOWLEDGEMENTS

We are extremely grateful to the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We thank Dr Kate Northstone for her help with the data preparation. This publication is the work of the authors, who will serve as guarantors for the contents of this paper. JH was the statistical expert for this paper.

CONTRIBUTORSHIP STATEMENT

AG undertook the analysis, drafted the manuscript and wrote the final version of the manuscript submitted. DR designed the study, and both JH and DR supervised AG in the statistical analysis and interpretation of the results, assisted with the drafting of the manuscript, and approved the final version of the manuscript submitted. BK provided helpful clinical insight, assisted with the drafting of the manuscript, and approved the final version of the manuscript submitted. JG supervised AG in interpreting the results, assisted with the redrafting of the manuscript, and approved the final version of the manuscript submitted.

COMPETING INTERESTS

None.

FUNDING

The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. The research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. AG is in receipt of a Wellcome Trust PhD studentship (Grant ref: 102433/Z/13/Z).

DATA SHARING STATEMENT

ALSPAC data is accessible to bona fide researchers, and the study website details conditions of use and access procedures: http://www.bristol.ac.uk/alspac/researchers/data-access/policy/ .

REFERENCE LIST

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Arlington, VA: American Psychiatric Publishing 2013.
2. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* 1977;18:297–321.
3. Koegel LK, Koegel RL, Ashbaugh K, et al. The importance of early identification and intervention for children with or at risk for autism spectrum disorders. *Int J Speech Lang Pathol* 2014;16:50-6.
4. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* 2014;63:1-21.
5. Mattila ML, Kielinen M, Linna SL, et al. Autism spectrum disorders according to DSM-IV-TR and comparison with DSM-5 draft criteria: an epidemiological study. *J Am Acad Child Adolesc Psychiatry* 2011;50:583-592.e511.
6. Baird G, Simonoff E, Pickles A, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet* 2006;368:210-5.
7. Idring S, Rai D, Dal H, et al. Autism spectrum disorders in the Stockholm Youth Cohort: design, prevalence and validity. *PLoS One* 2012;7:e41280. doi: 10.1371/journal.pone.0041280. [published Online First: 20 July 2012].
8. The NHS Information Centre for Health and Social Care. Autism spectrum disorders in adults living in households throughout England. *Report from the Adult Psychiatric Morbidity Survey 2007.* http://www.hscic.gov.uk/catalogue/PUB01131/aut-sp-dis-adu-liv-ho-a-p-m-sur-eng-2007-rep.pdf. Published 22 September 2009. Accessed 28 August 2014.
9. Kothari R, Skuse D, Wakefield J, et al. Gender differences in the relationship between social communication and emotion recognition. *J Am Acad Child Adolesc Psychiatry* 2013;52:1148-1157.

10. Dworzynski K, Ronald A, Bolton P, et al. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry* 2012;51:788-797.
11. Baron-Cohen S. The extreme male brain theory of autism. *Trends Cogn Sci* 2002;6:248-254.
12. Geschwind N, Galaburda A. Cerebral Lateralization: biological mechanisms, associations and pathology: I. A hypothesis and a program for research. Arch Neurol 1985;42:428-59.
13. Rhees R, Shryne J, Gorski R. Onset of the hormone-sensitive perinatal period for sexual differentiation of the sexually dimorphic nucleus of the preoptic area. *J Neurobiol* 1990;21:781-6.
14. Baron-Cohen S, Auyeung B, Norgaard-Pedersen B, et al. Elevated fetal steroidogenic activity in autism. *Mol Psychiatry* Published Online First: 3 June 2014. doi: 10.1038/mp.2014.48.
15. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43:160-4.
16. Bejerot S, Eriksson JM, Bonde S, et al. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. *Br J Psychiatry* 2012;201:116-23.
17. De Bruin EI, Verheij F, Wiegman T, et al. Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. *Dev Med Child Neurol* 2006;48:962-5.
18. Falter CM, Plaisted KC, Davis G. Visuo-spatial processing in autism - Testing the predictions of extreme male brain theory. *J Autism Dev Disord* 2008;38:507-515.
19. Krajmer P, Spajdel M, Kubranska A, et al. 2D:4D finger ratio in Slovak autism spectrum disorder population. *Bratisl Lek Listy* 2011;112:377-9.
20. Milne E, White S, Campbell R, et al. Motion and form coherence detection in autistic spectrum disorder: Relationship to motor control and 2:4 digit ratio. *J Autism Dev Disord* 2006;36:225-37.
21. Noipayak P. The ratio of 2nd and 4th digit length in autistic children. *J Med Assoc Thai* 2009;92:1040-5.
22. Sugie Y, Sugie H, Fukuda T, et al. Study of HOXD genes in autism particularly regarding the ratio of second to fourth digit length. *Brain Dev* 2010;32:356-61.

23. Aksu F, Baykara B, Ergin C, et al. [Phenotypic features in autistic individuals: the finger length ratio (2D:4D), hair whorl, and hand dominance]. *Turk Psikiyatri Derg 2013*;24:94-100.
24. Kothari R, Gafton J, Treasure J, et al. 2D:4D ratio in children at familial high-risk for eating disorders: The role of prenatal testosterone exposure. *Am J Hum Biol* 2014;26:176-182.
25. Hauth I, de Bruijn YGE, Staal W et al. Testing the extreme male brain theory of autism spectrum disorder in a familial design. *Autism Res* 2014;7:491-500.
26. Ecker A. Einige Bemerkungen über einen schwankenden Charakter in der Hand des Menschen. *Archiv für Anthropologie* 1875;8:67-74.
27. George R. Human finger types. *The Anatomical Record* 1930;46:199–204.
28. Honekopp J, Watson S. Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. *Am J Hum Biol* 2010;22:619-30.
29. Knickmeyer RC, Baron-Cohen S. Fetal testosterone and sex differences in typical social development and in autism. *J Child Neurol* 2006;21:825-45.
30. Garn SM, Burdi AR, Babler WJ, et al. Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *Am J Phys Anthropol* 1975;43:327-32.
31. Honekopp J, Bartholdt L, Beier L, et al. Second to fourth digit length ratio (2D:4D) and adult sex hormone levels: New data and a meta-analytic review. *Psychoneuroendocrinology* 2007;32:313-21.
32. McIntyre MH, Ellison PT, Lieberman DE, et al. The development of sex differences in digital formula from infancy in the Fels Longitudinal Study. *Proc Biol Sci* 2005;272:1473-9.
33. Manning JT, Scutt D, Wilson J, et al. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum Reprod.* 1998;13:3000-4.
34. Buck JJ, Williams RM, Hughes IA, et al. In-utero androgen exposure and 2nd to 4th digit length ratio - Comparisons between healthy controls and females with classical congenital adrenal hyperplasia. *Hum Reprod* 2003;18:976-9.
35. Berenbaum SA, Bryk KK, Nowak N, et al. Fingers as a marker of prenatal androgen exposure. *Endocrinology* 2009;150:5119-24.
36. Brown WM, Hines M, Fane BA, et al. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Horm Behav* 2002;42:380-6.

37. Honekopp J. Digit ratio 2D:4D in relation to autism spectrum disorders, empathizing, and systemizing: a quantitative review. *Autism Res* 2012;5:221-30.

38. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43:2664-76.

39. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;42:111-27.

40. Avon Longitudinal Study of Parents and Children. ALSPAC: Data Dictionary. ALSPAC. 2014; http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Updated 21 April, 2014, Accessed 28 August 2014.

41. Williams E, Thomas K, Sidebotham H, et al. Prevalence and characteristics of autistic spectrum disorders in the ALSPAC cohort. *Dev Med Child Neurol* 2008;50: 672-77.

42. Center for Disease Control and Prevention (CDC). Mental health in the United States: parental report of diagnosed autism in children aged 4-17 years--United States, 2003-2004. *MMWR Morb Mortal Wkly Rep* 2006;55:481-486.

43. Lyall K, Pauls DL, Spiegelman D, et al. Fertility therapies, infertility and autism spectrum disorders in the Nurses' Health Study II. *Paediatr Perinat Epidemiol* 2012;26:361-72.

44. Russell G, Rodgers LR, Ukoumunne OC, et al. Prevalence of parent-reported ASD and ADHD in the UK: findings from the Millennium Cohort Study. *J Autism Dev Disord* 2014;44:31-40.

45. Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. *PLoS One* 2010;5:e12633.

46. Bishop DV. Development of the Children's Communication Checklist (CCC): a method for assessing qualitative aspects of communicative impairment in children. *J Child Psychol Psychiatry* 1998;39:879-91.

47. Skuse DH, James RS, Bishop DV, et al. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 1997;387:705-8.

48. Rutter M, Tizard J, Whitmore K. *Education, Health & Behaviour.* London, UK: Longman 1970.

49. Buss A, Plomin R. Temperament: Early developing personality traits. Hillsdale, NJ: Lawrence Erlbaum 1984.

50. Medland SE, Zayats T, Glaser B, et al. A variant in LIN28B is associated with 2D:4D finger-length ratio, a putative retrospective biomarker of prenatal testosterone exposure. *Am J Hum Genet* 2010;86:519-25.

51. Office of Population Censuses and Surveys. Standard Occupational Classification, Vol 3. London, UK: Her Majesty's Stationery Office 1991.

52. Durkin MS, Maenner MJ, Meaney FJ, et al. Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. *PLoS One* 2010;5:e11551.

53. Rai D, Lewis G, Lundberg M, et al. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. *J Am Acad Child Adolesc Psychiatry* 2012;51:467-476.e6.

54. Manning JT, Baron-Cohen S, Wheelwright S, et al. Is digit ratio (2D:4D) related to systemizing and empathizing? Evidence from direct finger measurements reported in the BBC internet survey. *Pers Individ Diff.* 2010;48:767-71.

55. Robinson SJ, Manning JT. The ratio of 2nd to 4th digit length and male homosexuality. *Evol Hum Behav* 2000;21:333-45.

56. Lutchmaya S, Baron-Cohen S, Raggatt P, et al. 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Hum Dev* 2004;77:23-8.

57. Talarovicova A, Krskova L, Blazekova J. Testosterone enhancement during pregnancy influences the 2D:4D ratio and open field motor activity of rat siblings in adulthood. *Horm Behav* 2009;55:235-9.

58. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. *Proc Natl Acad Sci U S A* 2011;108:16289-94.

59. Abbott AD, Colman RJ, Tiefenthaler R, et al. Early-to-mid gestation fetal testosterone increases right hand 2D:4D finger length ratio in polycystic ovary syndrome-like monkeys. *PLoS One* 2012;7:e42372.

60. Lilley T, Laaksonen T, Huitu O, et al. Maternal corticosterone but not testosterone level is associated with the ratio of second-to-fourth digit length (2D:4D) in field vole offspring (Microtus agrestis). *Physiol Behav* 2010;99:433-7.

61. Honekopp J. No Evidence that 2D:4D is Related to the Number of CAG Repeats in the Androgen Receptor Gene. *Front Endocrinol (Lausanne)* 2013;4:185.

62. Manning J, Bundred P, Newton D, et al. The second to fourth digit ratio and variation in the androgen receptor gene. *Evol Hum Behav* 2003;24:399–405.

63. Hurd PL, Vaillancourt KL, Dinsdale NL. Aggression, digit ratio and variation in androgen receptor and monoamine oxidase A genes in men. *Behav Genet* 2011;41:543-56.

64. He C, Kraft P, Chen C, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet* 2009;41:724-8.

65. Matchock RL. Low digit ratio (2D:4D) is associated with delayed menarche. *Am J Hum Biol* 2008;20:487-9.

FIGURE 1: Description of case selection. ASD: autism spectrum disorders. ALSPAC: Avon Longitudinal Study of Parents and Children. 2D:4D: Second-to-fourth digit ratio.

FIGURE 2: Histograms and scatterplots of left and right 2D:4D (second-to-fourth digit ratio), separately for males and females.

a) Histogram showing distribution of right and left 2D:4D in males. Mean (standard deviation) of right 2D:4D: 0.958 (0.032); left 2D:4D 0.960 (0.032). b) Histogram showing distribution of right and left 2D:4D in females. Mean (standard deviation) of right 2D:4D 0.969 (0.033); left 0.970 (0.032). c) Scatterplot of right and left 2D:4D in males; correlation between right and left hands: 0.64. d) Scatterplot of right and left 2D:4D in females; correlation between right and left hands: 0.69.

Digit Ratio and Autism Spectrum Disorders in the Avon Longitudinal Study of Parents and Children: A Birth Cohort Study.

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Word count: 40493806 words (excluding title page, abstract, references, figures and tables)

Keywords: Avon Longitudinal Study of Parents and Children (ALSPAC); autistic disorder; digit ratio; testosterone; prenatal exposure delayed effects.

OBJECTIVES To investigate whether second-to-fourth digit ratio (2D:4D), a ~~proxy~~ measure commonly used as a proxy~~of~~ for ~~foetal~~fetal testosterone exposure, is associated with autism spectrum disorders (ASDs), as predicted by the extreme male brain theory of autism.

DESIGN ~~Analysis of the association between digit ratio and autism spectrum disorders in a~~A ~~large UK~~ birth cohort study.

SETTING The Avon Longitudinal Study of Parents and Children (ALSPAC).

PARTICIPANTS 6015 ALSPAC children with data on digit ratio, at least one outcome measure, and information on potential confounding variables (parental occupational class, maternal education, and age at digit ratio measurement). Digit ratio was measured by the photocopy and calliper method.

OUTCOMES ASD diagnosis (cases were~~have been~~ identified previously by record linkage or maternal report) and four measures that combine optimally within ALSPAC to predict ASD: the Children's Communication Checklist (coherence subscale), the Social and Communication Disorders Checklist, a repetitive behaviour measure, and the Emotionality, Activity and Sociability scale (sociability subscale). These ~~four~~ measures were dichotomised, with approximately 10% defined as the 'risk' group.

RESULTS Using logistic regression, we examined ~~for~~ the association of 2D:4D with ASDs and four dichotomised ASD traits.~~:~~ Covariates were occupational class, maternal education and age at 2D:4D measurement. 2D:4D was not associated with ASDs in males (adjusted odds ratio (OR) per 1-standard deviation increase in mean 2D:4D, 0.88 [95% confidence interval (95%CI) 0.65-1.21], p=0.435) or females (adjusted OR=1.36 [95%~~-~~CI 0.81-2.28], p=0.245). Similar results~~associations~~ were observed after ~~further~~ adjustment for IQ.~~:~~ There was one weak association between reduced coherence and increased left 2D:4D in males, in the opposite direction to that predicted by the extreme male brain theory (adjusted OR=1.15 [95%~~-~~CI 1.02-1.29], p=0.023). Given multiple comparisons, this ~~may be~~is consistent with chance.

CONCLUSIONS In this population-based study, there was no strong evidence of an association between 2D:4D and ASD diagnosis or traits, although the confidence intervals were wide.~~ There was weak, inconsistent evidence of association between 2D:4D and coherence.~~ These results are not consistent with the extreme male brain theory.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To date, this is one of the largest population based studies examining the relationship between second-to-fourth digit ratio (2D:4D), which is used as a proxy for ~~foetal~~fetal testosterone exposure, and autism spectrum disorders, separately by sex.
- This paper also examines the relationship between second-to-fourth digit ratio (2D:4D) and component measures of autism, which may give insight into the less-explored relationship between digit ratio and the individual domains (social and communication, repetitive behaviours) represented in autism.
- In comparison with other small studies based in clinical settings, this study may be less prone to selection bias; the rich phenotype information within ALSPAC allowed us to control for possible confounding variables.
- Despite its relatively large overall sample size compared with many studies, the number of autism cases in this study is still small.
- Cohort studies are prone to bias from attrition, and whilst the major sociodemographic predictors of attrition were controlled for in this study, it is also possible that children severely affected by ASD may have been less likely to attend clinics where 2D:4D was measured, which could bias our results.

INTRODUCTION

Autism spectrum disorders (ASD) are pervasive developmental disorders characterised by impaired social communication and reciprocal social interaction; and restricted, repetitive patterns of behaviour, interests, or activities.[1] Whilst twin studies suggest that ASDs are highly heritable, their aetiology remains largely unexplained.[2] Elucidating the causes of ASD may facilitate earlier diagnosis and enhance the potential for primary prevention. Earlier diagnosis may lead to an improvement in educational and behavioural outcomes, a reduction in comorbid psychiatric symptoms, and a decrease in the ~~financial~~ emotional and ~~emotional~~ financial stresses created by ASDs for individuals and their families.[3]

Various population cohorts have estimated the prevalence of ASD at approximately 1%.[4-7] These studies demonstrate a marked gender imbalance, with male to female ratio estimates ranging from 2:1[5] to 9:1,[8] depending upon age of assessment, length of follow-up, and whether studies screen for ASDs or examine data on pre-existing diagnosis. Although diagnostic bias has been proposed as a partial explanation for this disparity between sexes,[9-10] the consistently observed preponderance amongst males has generated interest into possible biological mechanisms predisposing to ASDs.

The extreme male brain (EMB) theory is the most popular hypothesis put forward to explain the sex difference in ASD. This theory postulates that children with ASD exhibit an exaggerated form of the male cognitive profile,[11] and proposes that prenatal androgens are plausible biological candidates. Some evidence from animal studies suggests that testosterone may mediate cognitive differences between the sexes via organi~~s~~zational effects on the brain.[12-13] Recent evidence supporting the EMB theory found sex steroid levels in amniocentesis samples to be correlated with the diagnosis of ASDs.[14]

The index to ring finger ratio (2D:4D) has been widely used as a proxy for ~~foetal~~fetal testosterone exposure in autism research.[15-25] An observation supporting a causal association between 2D:4D and ~~foetal~~fetal testosterone is that 2D:4D has been shown to be ~~is~~ sexually dimorphic,[26] with males generally having lower 2D:4D (that is to say, a relatively shorter index finger- (2D) compared to their ring finger (4D)).~~.~~[27-28] although this is not a unanimously reported finding [25]. This sexual dimorphism is apparent from the first trimester of pregnancy, and appears to be largely static after birth, with most,[29-33] but not all[25] studies finding that it is~~being~~ unaffected by pubertal

androgen.~~[28-32]~~ 2D:4D has also been shown to be sexually dimorphic in endocrine models of elevated (congenital adrenal hyperplasia) and reduced ~~foetal~~fetal testosterone exposure (complete androgen insensitivity syndrome).[28,34-36]

Although recent reviews have concluded that lower 2D:4D is associated with ASD,[37-38] many~~the~~ published studies~~literature consists~~ are within~~of~~ relatively small, clinical populations ~~observational studies~~ which may be~~are~~ susceptible to selection bias and confounding. We designed a study to examine the association between 2D:4D and ASD and various autistic trait measures in a population-based cohort in the UK. The primary hypothesis being tested was that lower 2D:4D would be associated with ASDs and ASD traits.

METHODS

Study design and population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a birth cohort based in Avon, UK. 14,541 pregnant women with expected delivery dates between 1st April 1991 and 31st December 1992 were initially enrolled, and 13,988 children were alive at one year.[39] Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. The study website contains details of all the data that are~~is~~ available through a fully searchable data dictionary: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary.[40] The primary source of data collection was via self-completion questionnaires administered at four points during the prenatal period, then at regular intervals following birth, to both parents and the "study child". Since the age of 7 years the whole cohort has been invited to a regular "focus" clinic for a variety of hands-on assessments.

Outcome variables

Autism spectrum disorders

Within ALSPAC, the identification of children with ASD has been described elsewhere.[41] Briefly, a previous record linkage study identified 86 ASD cases in ALSPAC, of which there was evidence for a strict multidisciplinary clinical assessment in 71 individuals, and in 15 others with a diagnosis of ASD recorded in school records.[41] In a validation study, a consultant paediatrician reviewed the records of the individuals with ASD to confirm concordance with ICD-10 criteria.[41] In addition to cases identified by record linkage, we further identified individuals with ASD based on maternal report. At 9.5 years, mothers were asked, "Have you ever been told that your child has: autism, Asperger's syndrome or autistic spectrum disorder?" Investigators utilis~~z~~ing maternal reports based on similar questions in other cohorts have reported an acceptable validity of identifying ASD using this approach.[42-44] We cross validated ASD cases confirmed only by maternal report by studying their association with various autistic trait measures and found strong associations (see supplementary Table S1, available online). Of the 56 ASD cases included in this study, 24 were ~~formally diagnosed~~ identified by record linkage, and 32 were identified from maternal reports of ASD diagnosis.

Dichotomised ASD traits

A previous factor analysis of 93 measures related to autism also reported four individual measures administered in ALSPAC via parental questionnaires that 'combined optimally to predict autism'.[45] The coherence subscale of the Children's Communication Checklist, measured at 115 months, assesses pragmatic abnormalities in social communication.[46] The Social and Communication Disorders Checklist, measured at 91 months, measured social skills (described previously).[47] A Repetitive Behaviour score, measured at 69 months asked whether the child "repeatedly rocks the head or body, has tics or twitches or other unusual behaviour", and included a question from the Rutter scale on tics, mannerisms or twitches.[48] The sociability subscale of the Emotionality Activity and Sociability measure at 38 months measures tendency to affiliate and interact with others.[49] As all the ASD scales considered were highly negatively skewed, and not readily correctable by transformation, each was dichotomised, creating a high risk (for ASDs) group of as close to 10% of the population as possible.

Explanatory variable: 2D:4D

Participants were invited to a focus clinic, when 2D:4D was measured. Mean measurement age was 11.75 years. Whilst 2D:4D was measured *after* the outcome measures (between approximately 2 to 8 years later, depending upon the outcome), it is widely used as a measurement of the prenatal environment, and therefore we feel justified in using it as our exposure in our analysis.

2D:4D was measured by the photocopy and calliper method: the ventral surface of ~~both~~each palm~~s~~ was placed as flatly as possible onto a photocopier, ensuring that the fingers were apart and the palms pressed firmly onto the glass. The lengths of the second (index) and fourth (ring) fingers were measured using the "Mahr digital caliper16 EX", accurate to 0.01mm. Each finger measured has a crease at its base: the index finger (2D) has one crease, the ring finger (4D) probably 3 or 4. The most proximal crease was chosen and by eye, the midpoint of this was determined and the distance from this crease to the distal fingertip measured. 2D measurement was divided by 4D measurement thus giving a 2D:4D measurement for each hand.[40] To assess the legitimacy of using photocopies to calculate 2D:4D, 57 right and 48 left hands in ALSPAC have been measured previously in vivo, with high correlation between these measures and photocopies (r=0.97).[50]

Confounding variables

We adjusted for the potential confounding effect of a number of socio-demographic measures. Each parent's self-reported occupation was coded as one of professional, managerial and technical, skilled non-manual, or manual,[51] and the highest reported class from each child's parent(s) was recorded. Highest self-reported maternal education level was recorded as CSE (certificate of secondary education), vocational, 'O' level, 'A' level or degree. Occupational class and maternal education were used as a proxy for socioeconomic status (SES), which is known to predict loss to follow up in ALSPAC.[38] ASDs are socially patterned,[52] although recent research suggests this may be in a direction opposite to that previously reported.[53] We also adjusted for the age at which the participants attended the 11-year clinic (where the 2D:4D exposure was measured) to account for compliance to clinic invite, which may be related to the outcomes considered.

Statistical analysis

For the main analysis, we included children from singleton pregnancies with left and right 2D:4D measured, who had data for at least one of five outcome measures (recorded presence/absence of ASDs, or data on at least one of the four dichotomised ASD trait measures) and additionally, data on potential confounders, including parental occupational class, maternal education level, and age at 2D:4D measurement (n=6015, including 56 ASD cases, and up to 616 in a high risk dichotomised group) (see Fig. 1 and Table 1).

TABLE 1: Characteristics of the study samplepopulation compared to those ALSPAC participants not included in the study*. n=number of participants.

	Study samplepopulation (n=6015) (Standard deviations given for means)	Other ALSPAC participants (n≤7602) (Standard deviations given for means)	p value**
% Male	49%	53%	p≤0.001
% Autism spectrum disorder (maternal and formal report)	0.93%	0.96%	p=0.861
Mean 2D:4D (those with left and right 2D:4D, excluding two outliers)	0.964 (0.029)	0.963 (0.030)	p=0.421
Age at 2D:4D measurement, months	140.9 (2.8)	141.3 (3.2)	p≤0.001
% Manual occupational background	14.2%	25.3%	p≤0.001
% Mother educated to degree level	16.3%	9.5%	p≤0.001

*(excluding multiple births, those not alive at 1 year, and those not in the core ALSPAC sample). ALSPAC=Avon Longitudinal Study of Parents and Children.

2D:4D=second-to-fourth digit ratio.

**from chi-squared test for categorical variables, and unpaired t-test (two tailed) for continuous variables

~~***These four measures were dichotomised, creating a high risk (for ASDs) group of as close to 10% of the population as possible.~~

Since the correlation between right and left hand 2D:4D was high (males 0.64, females 0.69), we used the mean of left and right hand 2D:4D in our main analysis (see Fig 2.). Two outliers were removed due to their extreme 2D:4D values (<0.75 and >1.25). Sex differences in this composite measure were assessed using a two-tailed t-test. Given the male preponderance in ASDs, and since 2D:4D is sexually dimorphic, all analyses which followed were stratified by sex, in order to assess whether the relationship of 2D:4D and ASD varied between sexes. To enable a clearer comparison of the male and female results, the 2D:4D measure was further standardised within each sex, so that a one unit change in 2D:4D represents a change by 1 standard deviation. Raw scores (i.e. non-standardised scores) are presented for descriptive tables.

A univariable logistic regression model was estimated for each ASD related outcome in turn, using the standardised mean 2D:4D variable as a continuous exposure (see Table 3). Effects were subsequently adjusted for the potential confounders listed above.

A supplementary analysis adjusted for IQ (see supplementary Table S3, available online). As sensitivity analyses, models were repeated using left and then right hand in turn (see supplementary Tables S4a and S4b, available online). All analyses were carried out in Stata (StataCorp, TX), version 13.

RESULTS

The ~~sample population~~study sample and those ALSPAC participants not in this particular~~e~~ ~~sample~~ study were similar in terms of mean 2D:4D, age at 2D:4D measurement and ASD prevalence, but fewer participants in~~amongst~~ our study ~~sample~~ were male (see Table 1). Amongst our study sample, there was an excess of ASD cases identified from maternal report of diagnosis only, in comparison to cases identified by record linkage, despite the equivalent prevalence of ASDs between our study sample and the remaining ALSPAC participants (see Table 1). Participants in the study sample were more likely to have parents working in non-manual occupations, were more likely to have a marginally higher IQ, and their mothers were more likely to be educated to degree level, which is consistent with the social patterning of attrition previously observed within ALSPAC (see Table 1).[39] Within the full sample (n=6015) there was strong evidence of sexual dimorphism for 2D:4D, with a mean (SE) of 0.959 (0.001) in males and 0.969 (0.001) in females (t=13.4, d.f.=6013, two-tailed t-test p≤0.001). This sexual dimorphism was still present in the subsample of ASD cases (n=56, males 2D:4D=0.955 (0.003); females=0.979 (0.006), t=3.5, d.f.=54, p≤0.001). Mean 2D:4D values stratified by ASD diagnosis and the other outcomes are shown in Table 2 (see also supplementary Table S2, available online, for results shown separately for right and left hand, and for results stratified by record linkage versus maternal report of ASD diagnosis).

TABLE 2: Mean (standard error) of left and right second-to-fourth digit ratio, and numbers (n) in each subgroup (ASD/no ASD; high/low ASD risk), by sex.

Outcome	Age	Risk group	Males		Females	
			Mean (SE)	N	Mean (SE)	N
Autism Spectrum Disorder (ASD) diagnosis	Diagnosed by 11 years	No ASD diagnosis	0.959 (0.001)	2920	0.969 (0.001)	3039
		ASD diagnosis	0.955 (0.003)	42	0.979 (0.006)	14
Children's Communication Checklist (Coherence subscale)	115 months	Lower risk	0.959 (0.001)	2285	0.970 (0.001)	2495
		Higher risk	0.962 (0.002)	302	0.969 (0.002)	167
Social and Communication Developmental Checklist	91 months	Lower risk	0.960 (0.001)	2260	0.970 (0.001)	2444
		Higher risk	0.959 (0.002)	299	0.970 (0.002)	172
Repetitive behaviour	69 months	Lower risk	0.960 (0.001)	2378	0.970 (0.001)	2509
		Higher risk	0.958 (0.002)	199	0.969 (0.003)	130
Emotionality Activity, Sociability Temperament Scale	38 months	Lower risk	0.959 (0.001)	2378	0.970 (0.001)	2540
		Higher risk	0.959 (0.002)	352	0.968 (0.002)	264

(Sociability subscale)						

Table 3 shows unadjusted and adjusted estimates for the increase in odds of each outcome per 1 standard deviation increase in mean 2D:4D ratio. There was no evidence of an association between mean 2D:4D and any of the five outcomes in either sex.

TABLE 3: Logistic regression of ASD vs no ASD, or high risk dichotomised trait vs low risk, against mean 2D:4D (left/right hands combined), stratified by sex. Unadjusted and adjusted[a] models are presented. NB Odds ratios are for one unit change in Z score (i.e. 1 standard deviation). N=numbers of participants.

Sex	Males				Females			
Model	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]	Unadjusted OR [95% CI]	Adjusted[a] OR [95% CI]	Total N	Risk N[b]
Autism Spectrum Disorder (ASD) diagnosis	0.87 [0.64, 1.19] p=0.393	0.88 [0.65, 1.21] p=0.435	2962	42	1.39 [0.83, 2.34] p=0.206	1.36 [0.81, 2.28] p=0.245[c]	3053	14
Children's Communication Checklist (Coherence subscale)	1.10 [0.97, 1.24] p=0.124	1.10 [0.98, 1.24] p=0.118	2587	302	0.98 [0.84, 1.15] p=0.790	0.98 [0.84, 1.15] p=0.809	2662	167
Social and Communication Developmental Checklist	0.96 [0.85, 1.09] p=0.553	0.96 [0.85, 1.09] p=0.550	2559	299	1.03 [0.88, 1.20] p=0.698	1.04 [0.89, 1.22] p=0.601	2616	172
Repetitive behaviour	0.95 [0.82, 1.10] p=0.466	0.95 [0.82, 1.09] p=0.458	2577	199	0.98 [0.82, 1.17] p=0.796	0.98 [0.82, 1.17] p=0.840	2639	130
Emotionality, Activity, Sociability (Sociability subscale)	0.98 [0.87, 1.09] p=0.670	0.97 [0.87, 1.08] p=0.583	2730	352	0.94 [0.83, 1.07] p=0.325	0.94 [0.83, 1.07] p=0.357	2804	264

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D (second-to-fourth digit ratio) measurement.

[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait.

[c]To avoid perfect prediction, the CSE (Certificate of Secondary Education) and vocational classes of maternal education were collapsed for this analysis.

We included Intelligence Quotient (IQ) as measured by a modified version of the Wechsler Intelligence Scale for Children, as an additional covariate~~potential confounder~~ in a supplementary analysis. We considered this separately from our main analysis to prevent a substantial loss to our sample size, since the IQ measure was available on only 44/56 of included participants with ASDs. Another supplementary analysis examined the effect of 2D:4D by left and right hand.

Supplementary tables show (i) little impact of further adjustment for IQ (supplementary Table S3, available online), and (ii) a broadly consistent pattern of findings when examining the effect of 2D:4D ratio for each hand in turn (supplementary Tables S4a and S4b, available online). One sub-analysis suggested a weak association between reduced coherence (as measured by the Children's Communication Checklist) with left 2D:4D in males (adjusted OR 1.15 [95% CI 1.02, 1.29], p=0.023, see supplementary Table S4a). There was also a trend towards increased right 2D:4D being associated with ASD diagnosis in females (adjusted OR 1.60 [95% CI 0.97, 2.65] p=0.068, see supplementary Table S4b). However, these results are in the opposite direction to that predicted by the prevailing theory of the extreme male brain. One sub-analysis found a trend towards masculinized 2D:4D being associated with the repetitive behaviours measure OR 0.87 [95% CI 0.75, 1.01] p=0.061. Yet, given the number of comparisons and differing directions of association, we consider that these results are ~~likely to be~~ consistent with chance.

DISCUSSION

In this large UK-based birth cohort, we did not find evidence to support the hypothesis that lower second to fourth digit ratio (2D:4D) is associated with an increased risk of autism spectrum disorders (ASDs). ~~In this study, w~~We also examined the relationship of digit ratio with dichotomised component trait measures of ASDs, including social cognition, coherence, repetitive behaviours and sociability. There ~~was one instance of a~~were several suggestions of very weak relationships between ~~increased~~ 2D:4D and ~~reduced coherence~~ASD traits, when the hands were studied separately. These~~is~~ associations were mostly directionally~~is~~ discordant with the extreme male theory of autism, and given the number of comparisons made, and the lack of consistent association between ASD diagnosis and 2D:4D in this study, we consider that they ~~it could be~~were ~~consistent with~~ due to chance.

R~~Two r~~ecent reviews have concluded that there ~~i~~was support for an association between masculinised 2D:4D and ASD.[37-38] The central estimate from the most recent quantitative review was a Cohen's d of -0.43, representing a moderately lower digit ratio amongst individuals (mostly males were studied) with autism.[38] Using a t-test (comparing 2D:4D between males with and without ASD - the most common comparison in the literature), we calculated that we would have had 79% power to detect this central estimate. However, we chose logistic regression methods for our primary analysis rather than a t-test, since we were interested in adjusting for the effect of potential confounders. For consistency with previous literature, we repeated our analyses (mean 2D:4D in males with and without ASD) using a t-test, and found a Cohen's d of -0.13 [95% CI -0.44, 0.17]. It should be noted that the d of -0.43, reported in the literature[38] is within the confidence interval~~the bounds~~ of this estimate, albeit at the extreme lower bound.

Amongst the studies examining the 2D:4D-ASD diagnosis relationship (i.e. not ASD trait measures), most studies recruited cases from clinical settings,[17,21-23] which may ~~provide results of~~ potentially limit~~ed~~ the external validity of results, if cases recruited in a clinical setting are qualitatively different to the larger population from which they are sampled. Others recruited from mixed clinical/non-clinical populations,[16][25] schools,[18,20] and a charity.[15] ASD case numbers in ~~all of~~ these studies varied from 23[20] to 216.[25] Of t~~T~~wo ~~he~~ studies~~y~~ with the largest number of cases to date, one was a population-based study, and these authors found an association between masculinised 2D:4D and ASD.[15] Another was a clinically recruited sample, which found no relationship between 2D:4D and

ASD.[25] Overall, results of published studies examining the 2D:4D–ASD relationship have therefore been mixed, although the majority of them have reported an association concordant with the extreme male brain theory.[15,17,19-22] Others have found no association,[18,23,25] or the inverse association to the prevailing hypothesis.[16] It is also notable that many studies have examined case populations that are entirely or almost entirely male.[17-20,23]

Although most case-control studies selected age- and sex-matched controls, the possibility remains that the composition of the ALSPAC population may differ fromto these reference populations. The strengths of this study are that it is a population-based study, which is a design that is generally less susceptible to selection bias. The population studied had a comparatively high proportion of female ASD cases. We analysed the association between 2D:4D and ASD risk separately by sex, which may be important, since 2D:4D is a sexually dimorphic trait. The population studied had a comparatively high proportion of female ASD cases, however, despite this, the absolute number of female ASD cases in our study was also small (n=14). Although 2D:4D is sexually dimorphic, it has been found that this dimorphism may not be apparent in individuals with ASD.[15] In our study, 2D:4D was sexually dimorphic in individuals with or without ASD. Further strengths of this study are that we were able to study various component measures of ASD in a large population.[37,54] Although one large study has found an association between ASD traits2D:4D and ASD traits2D:4D,[54] reviews of previous literature have asserted that there is little overall evidence for the relationship between 2D:4D and ASD traits in healthy subjects.[37, 38] Our study also found some suggesting that foetal testosterone may exhibit an interaction effect, being a risk factor for ASDs only in brains of those who are in some other way predisposed. However, our findings do not support this theory considering there was no consistent association observed for ASD diagnosisbetween 2D:4D and ASD trait measures.

The ALSPAC cohort provides a rich resource of data, from which we selected variables that may have confounded an association between 2D:4D and ASD. Although our sample was population based, the possibility of selection bias in relation to baseline recruitment or attrition in the sample over time cannot be entirely excluded. We tried to address this possible attrition bias by controlling for the characteristics known to be predictive of attrition in ALSPAC, such as socioeconomic status. However, it is also possible that those children severely affected by ASD may have been less likely to

attend clinics where 2D:4D was measured – this could therefore result in our sample being weighted towards Asperger Syndrome and other high-functioning ASD cases. This may be relevant given that previous studies have observed a less profound difference in 2D:4D in children with Asperger Syndrome compared to ASD.[15] Finally, although the method used to measure 2D:4D has been shown to be reliable,[50,55] and used by some of the previous studies on this topic,[15,17] the possibility of error in measurement of 2D:4D cannot be excluded. Yet, it is unlikely that this would be differential for people with and without a diagnosis of ASD.

One recent study reported an association between foetalfetal steroidogenic activity (including testosterone) and ASD, providing some direct evidence in support of the biological basis of the EMB theory of autism.[14] Yet, as foetalfetal testosterone cannot be readily measured, papers assessing the relationship between foetalfetal testosterone and ASDs, including our study, require the existence of a proxy, for example, 2D:4D. One study cites direct evidence of 2D:4D being related to foetalfetal testosterone-to-oestradiol ratio in humans.[56] Some of the strongest indirect evidence for the foetalfetal testosterone-2D:4D relationship comes from studies associating 2D:4D with increased (congenital adrenal hyperplasia)[28,34,36] and decreased (complete androgen insensitivity syndrome)[35] endocrine models of foetalfetal testosterone exposure. Animal studies have provided mixed results, with some in favour of the foetalfetal testosterone-2D:4D relationship,[57-58] and others supporting the inverse association,[59] or no association.[60] Studies of repeats in the androgen receptor (AR) gene (correlated to testosterone) and 2D:4D have similarly provided mixed results.[50,61-63] A genome-wide scan of 2889 children (including ALSPAC participants) found the minor allele of one SNP in LIN28B (linked to delayed age atof menarche)[64] to be associated with increased 2D:4D.[50] This association was in the opposite direction to that predicted fromby previous work, in which lower 2D:4D was associated with delayed age at menarche, putatively via higher foetalfetal testosterone exposure.[65] The authors therefore suggested that the relationship between fetal testosterone and 2D:4D may be more complex than first proposed, which is in agreement with previous work.[56] However, regardless as to whether 2D:4D ratio is mainly a marker ofcausally determined by fetal testosterone, 2D:4D is clearly sexually dimorphic and related to hormonal traits within sexes[65], and thus may still be useful in the study of act as a marker of other sex-related traits.

To conclude, in this large population- -based study, we found no consistent evidence supportingfor an association between lower 2D:4D ratio and increased risk of ASDs, and very little, inconsistent evidence for an association between 2D:4D andor ASD component traits. These findings are not consistent with the extreme male brain theory of autism.

ACKNOWLEDGEMENTS

We are extremely grateful to the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We thank Dr Kate Northstone for her help with the data preparation. ~~The UK Medical Research Council and the Wellcome Trust (Grant ref: 092731) and the University of Bristol provide core support for ALSPAC.~~ This publication is the work of the authors, who will serve as guarantors for the contents of this paper. JH was the statistical expert for this paper.

CONTRIBUTORSHIP STATEMENT

AG undertook the analysis, drafted the manuscript and wrote the final version of the manuscript submitted. DR designed the study, and both JH and DR supervised AG in the statistical analysis and interpretation of the results, assisted with the drafting of the manuscript, and approved the final version of the manuscript submitted. BK provided helpful clinical insight, assisted with the drafting of the manuscript, and approved the final version of the manuscript submitted. JG supervised AG in interpreting the results, assisted with the redrafting of the manuscript, and approved the final version of the manuscript submitted.

COMPETING INTERESTS

None.

FUNDING

The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. The research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. AG is in receipt of a Wellcome Trust PhD studentship (Grant ref: 102433/Z/13/Z~~102433~~).

DATA SHARING STATEMENT

ALSPAC data is accessible to bona fide researchers, and the study website details conditions of use and access procedures: http://www.bristol.ac.uk/alspac/researchers/data-access/policy/ .

REFERENCE LIST

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Arlington, VA: American Psychiatric Publishing 2013.
2. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* 1977;18:297–321.
3. Koegel LK, Koegel RL, Ashbaugh K, et al. The importance of early identification and intervention for children with or at risk for autism spectrum disorders. *Int J Speech Lang Pathol* 2014;16:50-6.
4. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* 2014;63:1-21.
5. Mattila ML, Kielinen M, Linna SL, et al. Autism spectrum disorders according to DSM-IV-TR and comparison with DSM-5 draft criteria: an epidemiological study. *J Am Acad Child Adolesc Psychiatry* 2011;50:583-592.e511.
6. Baird G, Simonoff E, Pickles A, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet* 2006;368:210-5.
7. Idring S, Rai D, Dal H, et al. Autism spectrum disorders in the Stockholm Youth Cohort: design, prevalence and validity. *PLoS One* 2012;7:e41280. doi: 10.1371/journal.pone.0041280. [published Online First: 20 July 2012].
8. The NHS Information Centre for Health and Social Care. Autism spectrum disorders in adults living in households throughout England. *Report from the Adult Psychiatric Morbidity Survey 2007.* http://www.hscic.gov.uk/catalogue/PUB01131/aut-sp-dis-adu-liv-ho-a-p-m-sur-eng-2007-rep.pdf. Published 22 September 2009. Accessed 28 August 2014.
9. Kothari R, Skuse D, Wakefield J, et al. Gender differences in the relationship between social communication and emotion recognition. *J Am Acad Child Adolesc Psychiatry* 2013;52:1148-1157.

10. Dworzynski K, Ronald A, Bolton P, et al. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry* 2012;51:788-797.
11. Baron-Cohen S. The extreme male brain theory of autism. *Trends Cogn Sci* 2002;6:248-254.
12. Geschwind N, Galaburda A. Cerebral Lateralization: biological mechanisms, associations and pathology: I. A hypothesis and a program for research. Arch Neurol 1985;42:428-59.
13. Rhees R, Shryne J, Gorski R. Onset of the hormone-sensitive perinatal period for sexual differentiation of the sexually dimorphic nucleus of the preoptic area. *J Neurobiol* 1990;21:781-6.
14. Baron-Cohen S, Auyeung B, Norgaard-Pedersen B, et al. Elevated fetal steroidogenic activity in autism. *Mol Psychiatry* Published Online First: 3 June 2014. doi: 10.1038/mp.2014.48.
15. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43:160-4.
16. Bejerot S, Eriksson JM, Bonde S, et al. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. *Br J Psychiatry* 2012;201:116-23.
17. De Bruin EI, Verheij F, Wiegman T, et al. Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. *Dev Med Child Neurol* 2006;48:962-5.
18. Falter CM, Plaisted KC, Davis G. Visuo-spatial processing in autism - Testing the predictions of extreme male brain theory. *J Autism Dev Disord* 2008;38:507-515.
19. Krajmer P, Spajdel M, Kubranska A, et al. 2D:4D finger ratio in Slovak autism spectrum disorder population. *Bratisl Lek Listy* 2011;112:377-9.
20. Milne E, White S, Campbell R, et al. Motion and form coherence detection in autistic spectrum disorder: Relationship to motor control and 2:4 digit ratio. *J Autism Dev Disord* 2006;36:225-37.
21. Noipayak P. The ratio of 2nd and 4th digit length in autistic children. *J Med Assoc Thai* 2009;92:1040-5.
22. Sugie Y, Sugie H, Fukuda T, et al. Study of HOXD genes in autism particularly regarding the ratio of second to fourth digit length. *Brain Dev* 2010;32:356-61.

23. Aksu F, Baykara B, Ergin C, et al. [Phenotypic features in autistic individuals: the finger length ratio (2D:4D), hair whorl, and hand dominance]. *Turk Psikiyatri Derg 2013*;24:94-100.

24. Kothari R, Gafton J, Treasure J, et al. 2D:4D ratio in children at familial high-risk for eating disorders: The role of prenatal testosterone exposure. *Am J Hum Biol* 2014;26:176-182.

25. Hauth I, de Bruijn YGE, Staal W et al. Testing the extreme male brain theory of autism spectrum disorder in a familial design. *Autism Res* 2014;7:491-500.

26. Ecker A. Einige Bemerkungen über einen schwankenden Charakter in der Hand des Menschen. *Archiv für Anthropologie* 1875;8:67-74.

27. George R. Human finger types. *The Anatomical Record* 1930;46:199–204.

28. Honekopp J, Watson S. Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. *Am J Hum Biol* 2010;22:619-30.

29. Knickmeyer RC, Baron-Cohen S. Fetal testosterone and sex differences in typical social development and in autism. *J Child Neurol* 2006;21:825-45.

30. Garn SM, Burdi AR, Babler WJ, et al. Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *Am J Phys Anthropol* 1975;43:327-32.

31. Honekopp J, Bartholdt L, Beier L, et al. Second to fourth digit length ratio (2D:4D) and adult sex hormone levels: New data and a meta-analytic review. *Psychoneuroendocrinology* 2007;32:313-21.

32. McIntyre MH, Ellison PT, Lieberman DE, et al. The development of sex differences in digital formula from infancy in the Fels Longitudinal Study. *Proc Biol Sci* 2005;272:1473-9.

33. Manning JT, Scutt D, Wilson J, et al. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum Reprod.* 1998;13:3000-4.

34. Buck JJ, Williams RM, Hughes IA, et al. In-utero androgen exposure and 2nd to 4th digit length ratio - Comparisons between healthy controls and females with classical congenital adrenal hyperplasia. *Hum Reprod* 2003;18:976-9.

35. Berenbaum SA, Bryk KK, Nowak N, et al. Fingers as a marker of prenatal androgen exposure. *Endocrinology* 2009;150:5119-24.

36. Brown WM, Hines M, Fane BA, et al. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Horm Behav* 2002;42:380-6.

37. Honekopp J. Digit ratio 2D:4D in relation to autism spectrum disorders, empathizing, and systemizing: a quantitative review. *Autism Res* 2012;5:221-30.

38. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43:2664-76.

39. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;42:111-27.

40. Avon Longitudinal Study of Parents and Children. ALSPAC: Data Dictionary. ALSPAC. 2014; http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Updated 21 April, 2014, Accessed 28 August 2014.

41. Williams E, Thomas K, Sidebotham H, et al. Prevalence and characteristics of autistic spectrum disorders in the ALSPAC cohort. *Dev Med Child Neurol* 2008;50: 672-77.

42. Center for Disease Control and Prevention (CDC). Mental health in the United States: parental report of diagnosed autism in children aged 4-17 years--United States, 2003-2004. *MMWR Morb Mortal Wkly Rep* 2006;55:481-486.

43. Lyall K, Pauls DL, Spiegelman D, et al. Fertility therapies, infertility and autism spectrum disorders in the Nurses' Health Study II. *Paediatr Perinat Epidemiol* 2012;26:361-72.

44. Russell G, Rodgers LR, Ukoumunne OC, et al. Prevalence of parent-reported ASD and ADHD in the UK: findings from the Millennium Cohort Study. *J Autism Dev Disord* 2014;44:31-40.

45. Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. *PLoS One* 2010;5:e12633.

46. Bishop DV. Development of the Children's Communication Checklist (CCC): a method for assessing qualitative aspects of communicative impairment in children. *J Child Psychol Psychiatry* 1998;39:879-91.

47. Skuse DH, James RS, Bishop DV, et al. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 1997;387:705-8.

48. Rutter M, Tizard J, Whitmore K. *Education, Health & Behaviour.* London, UK: Longman 1970.

49. Buss A, Plomin R. Temperament: Early developing personality traits. Hillsdale, NJ: Lawrence Erlbaum 1984.

50. Medland SE, Zayats T, Glaser B, et al. A variant in LIN28B is associated with 2D:4D finger-length ratio, a putative retrospective biomarker of prenatal testosterone exposure. *Am J Hum Genet* 2010;86:519-25.

51. Office of Population Censuses and Surveys. Standard Occupational Classification, Vol 3. London, UK: Her Majesty's Stationery Office 1991.

52. Durkin MS, Maenner MJ, Meaney FJ, et al. Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. *PLoS One* 2010;5:e11551.

53. Rai D, Lewis G, Lundberg M, et al. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. *J Am Acad Child Adolesc Psychiatry* 2012;51:467-476.e6.

54. Manning JT, Baron-Cohen S, Wheelwright S, et al. Is digit ratio (2D:4D) related to systemizing and empathizing? Evidence from direct finger measurements reported in the BBC internet survey. *Pers Individ Diff.* 2010;48:767-71.

55. Robinson SJ, Manning JT. The ratio of 2nd to 4th digit length and male homosexuality. *Evol Hum Behav* 2000;21:333-45.

56. Lutchmaya S, Baron-Cohen S, Raggatt P, et al. 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Hum Dev* 2004;77:23-8.

57. Talarovicova A, Krskova L, Blazekova J. Testosterone enhancement during pregnancy influences the 2D:4D ratio and open field motor activity of rat siblings in adulthood. *Horm Behav* 2009;55:235-9.

58. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. *Proc Natl Acad Sci U S A* 2011;108:16289-94.

59. Abbott AD, Colman RJ, Tiefenthaler R, et al. Early-to-mid gestation fetal testosterone increases right hand 2D:4D finger length ratio in polycystic ovary syndrome-like monkeys. *PLoS One* 2012;7:e42372.

60. Lilley T, Laaksonen T, Huitu O, et al. Maternal corticosterone but not testosterone level is associated with the ratio of second-to-fourth digit length (2D:4D) in field vole offspring (Microtus agrestis). *Physiol Behav* 2010;99:433-7.

61. Honekopp J. No Evidence that 2D:4D is Related to the Number of CAG Repeats in the Androgen Receptor Gene. *Front Endocrinol (Lausanne)* 2013;4:185.

62. Manning J, Bundred P, Newton D, et al. The second to fourth digit ratio and variation in the androgen receptor gene. *Evol Hum Behav* 2003;24:399–405.

63. Hurd PL, Vaillancourt KL, Dinsdale NL. Aggression, digit ratio and variation in androgen receptor and monoamine oxidase A genes in men. *Behav Genet* 2011;41:543-56.

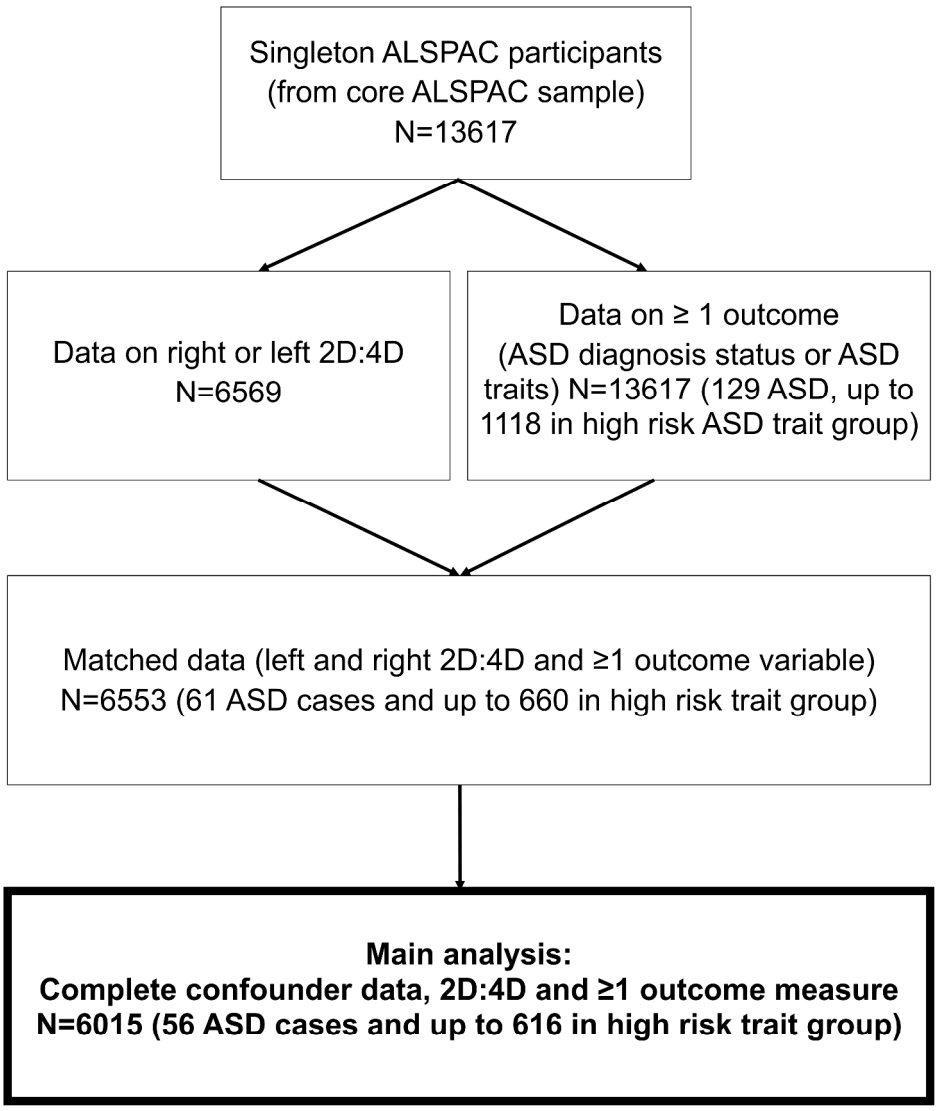
64. He C, Kraft P, Chen C, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet* 2009;41:724-8.

65. Matchock RL. Low digit ratio (2D:4D) is associated with delayed menarche. *Am J Hum Biol* 2008;20:487-9.

FIGURE 1: Description of case selection. ASD: autism spectrum disorders. ALSPAC: Avon Longitudinal Study of Parents and Children. 2D:4D: Second-to-fourth digit ratio.

FIGURE 2: Histograms and scatterplots of left and right 2D:4D (second-to-fourth digit ratio), separately for males and females.

a) Histogram showing distribution of right and left 2D:4D in males. Mean (standard deviation) of right 2D:4D: 0.958 (0.032); left 2D:4D 0.960 (0.032). b) Histogram showing distribution of right and left 2D:4D in females. Mean (standard deviation) of right 2D:4D 0.969 (0.033); left 0.970 (0.032). c) Scatterplot of right and left 2D:4D in males; correlation between right and left hands: 0.64. d) Scatterplot of right and left 2D:4D in females; correlation between right and left hands: 0.69.



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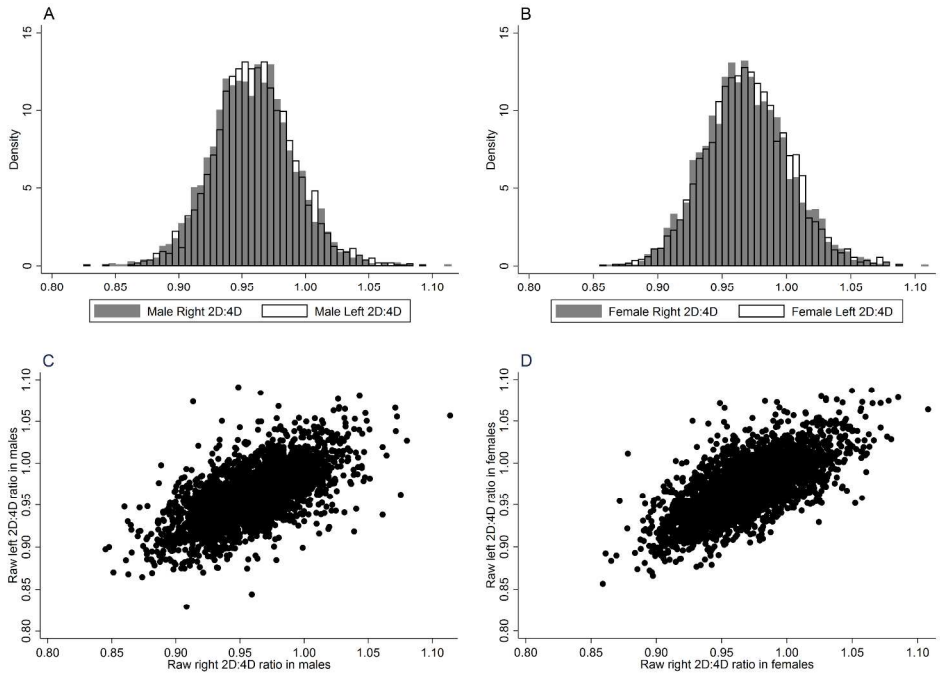
420x489mm (300 x 300 DPI)



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595x441mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓
Objectives	3	State specific objectives, including any prespecified hypotheses	✓
Methods			
Study design	4	Present key elements of study design early in the paper	✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	✓
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓
Bias	9	Describe any efforts to address potential sources of bias	✓
Study size	10	Explain how the study size was arrived at	✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓
		(*b*) Describe any methods used to examine subgroups and interactions	✓
		(*c*) Explain how missing data were addressed	✓
		(*d*) If applicable, explain how loss to follow-up was addressed	✓
		(*e*) Describe any sensitivity analyses	✓
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	✓
		(c) Consider use of a flow diagram	✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	✓
		(c) Summarise follow-up time (eg, average and total amount)	✓
Outcome data	15*	Report numbers of outcome events or summary measures over time	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓

Discussion			
Key results	18	Summarise key results with reference to study objectives	✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Table S1: Logistic regression of ASD (Autism Spectrum Disorder) cases status diagnosed by two methods (maternal report only or formal diagnosis), against risk group for each dichotomised ASD trait measure.

ASD trait measure	Maternally reported ASD only versus no ASD	Formal ASD diagnosis versus no ASD
	OR [95% CI], p value	OR [95% CI], p value
Children's Communication Checklist (Coherence subscale) (115 months)	9.41 [4.64, 19.07] p≤0.001	†
Social and Communication Developmental Checklist (91 months)	8.98 [4.33, 18.63] p≤0.001	73.13 [21.73, 246.11] p≤0.001
Repetitive behaviour (69 months)	10.23 [4.78, 21.88] p≤0.001	11.75 [5.11, 27.01] p≤0.001
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	2.40 [1.03, 5.60] p=0.042	5.73 [2.56, 13.07] p≤0.001

†All children with formally reported ASD and a valid Children's Communication Checklist score were in the high risk group.

Table S2: Left and right 2D:4D (second-to-fourth digit ratio): mean (standard error), and numbers (n) in each subgroup, separately by sex.[a]

Outcome	Age at measurement	Risk group		Left hand		Right hand	
				Males	Females	Males	Females
ASD diagnosis	Diagnosed by 11 years	No ASD diagnosis		0.960 (0.001) n = 2920	0.970 (0.001) n = 3039	0.958 (0.001) n = 2920	0.968 (0.001) n = 3039
		ASD diagnosis	All ASD diagnoses	0.957 (0.003) n = 42	0.974 (0.007) n = 14	0.954 (0.004) n = 42	0.985 (0.007) n = 14
			Linkage ASD diagnosis only	0.958 (0.006) n=19	0.985 (0.014) n=5	0.956 (0.006) n=19	0.981 (0.010) n=5
			Maternal report of ASD diagnosis only	0.955 (0.004) n=23	0.967 (0.006) n=9	0.953 (0.005) n=23	0.987 (0.009) n=9
Children's Communication Checklist (Coherence subscale)	115 months	Low risk		0.960 (0.001) n = 2285	0.970 (0.001) n = 2495	0.958 (0.001) n = 2285	0.969 (0.001) n = 2495
		High risk		0.964 (0.002) n = 302	0.970 (0.002) n = 167	0.959 (0.002) n = 302	0.968 (0.002) n = 167
Social and Communication Developmental Checklist	91 months	Low risk		0.961 (0.001) n = 2260	0.970 (0.001) n = 2444	0.958 (0.001) n = 2260	0.969 (0.001) n = 2444
		High risk		0.959 (0.002) n = 299	0.972 (0.003) n = 172	0.958 (0.002) n = 299	0.969 (0.003) n = 172
Repetitive behaviour	69 months	Low risk		0.961 (0.001) n = 2378	0.970 (0.001) n = 2509	0.959 (0.001) n = 2378	0.969 (0.001) n = 2509
		High risk		0.962 (0.002) n = 199	0.971 (0.003) n = 130	0.954 (0.002) n = 199	0.967 (0.002) n = 130
Emotionality Activity, Sociability Temperament Scale (Sociability subscale)	38 months	Low risk		0.960 (0.001) n = 2378	0.970 (0.001) n = 2540	0.958 (0.001) n = 2378	0.969 (0.001) n = 2540
		High risk		0.961 (0.002) n = 352	0.968 (0.002) n = 264	0.957 (0.002) n = 352	0.967 (0.002) n = 264

[a] Subgroups are split by ASD (Autism Spectrum Disorder) diagnosis (including subgroups – case identification from record linkage or maternal report of diagnosis), or by high/low risk groups for each of the four dichotomised measures predictive of ASD.

Table S3: IQ-adjusted logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) vs no diagnosis, or high risk dichotomised trait measure group vs low risk, against mean 2D:4D (second-to-fourth digit ratio) (left and right hand combined), stratified by sex. Unadjusted and adjusted[a] models are presented. NB Odds ratios are for one unit change in Z score (i.e. 1 standard deviation). N=numbers of participants.

Outcome	Males				Females			
	Unadjusted	Adjusted[a]	Total N	Risk N[b]	Unadjusted	Adjusted[a]	Total N	Risk N[b]
	OR [95% CI] P value	OR [95% CI] P value			OR [95% CI] P value	OR [95% CI] P value		
ASD diagnosis (diagnosed by 11 years)	0.81 [0.57, 1.15] p=0.242	0.81 [0.57, 1.16] p=0.253	2543	33	1.51 [0.84, 2.69] p=0.165	1.50 [0.83, 2.70] p=0.181[c]	2676	11
Children's Communication Checklist (Coherence subscale) (115 months)	1.10 [0.97, 1.26] p=0.144	1.10 [0.96, 1.26] p=0.159	2319	254	0.94 [0.79, 1.11] p=0.452	0.93 [0.78, 1.10] p=0.405	2418	151
Social and Communication Developmental Checklist (91 months)	0.95 [0.84, 1.08] p=0.446	0.95 [0.84, 1.08] p=0.440	2295	269	1.01 [0.85, 1.19] p=0.933	1.01 [0.86, 1.20] p=0.855	2380	150
Repetitive behaviour (69 months)	0.92 [0.79, 1.07] p=0.285	0.92 [0.79, 1.08] p=0.300	2282	175	0.96 [0.80, 1.17] p=0.712	0.98 [0.80, 1.18] p=0.753	2372	112
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	0.96 [0.85, 1.08] p=0.489	0.95 [0.85, 1.08] p=0.452	2391	312	0.94 [0.82, 1.08] p=0.366	0.94 [0.82, 1.08] p=0.402	2495	233

[a]Adjusted for highest parental occupational class, maternal education, age at 2D:4D measurement and intelligence quotient (IQ).
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure.
[c]To avoid perfect prediction, skilled non-manual and manual occupation groups, and CSE (Certificate of Secondary Education) and vocational maternal education groups were collapsed.

Table S4a: Logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) versus no diagnosis, or high risk dichotomised trait measure group versus low risk, against 2D:4D (second-to-fourth digit ratio), separately by left and right hand, in males only. Unadjusted and adjusted[a] models are presented. Odds ratios are for one unit change in Z score (one standard deviation). N=numbers of participants.

Outcome	Males					
	Left		Right		Total N	Risk N[b]
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]		
	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value		
ASD diagnosis (diagnosed by 11 years)	0.89 [0.65, 1.21] p=0.447	0.89 [0.65, 1.22] p=0.469	0.88 [0.65, 1.20] p=0.431	0.90 [0.66, 1.23] p=0.494	2962	42
Children's Communication Checklist (Coherence subscale) (115 months)	1.15 [1.02, 1.29] p=0.024	1.15 [1.02, 1.29] p=0.023	1.03 [0.91, 1.17] p=0.602	1.03 [0.92, 1.17] p=0.584	2587	302
Social and Communication Developmental Checklist (91 months)	0.95 [0.84, 1.07] p=0.392	0.95 [0.84, 1.07] p=0.375	0.99 [0.87, 1.11] p=0.827	0.99 [0.88, 1.12] p=0.847	2559	299
Repetitive behaviour (69 months)	1.04 [0.90, 1.20] p=0.576	1.04 [0.90, 1.20] p=0.592	0.87 [0.75, 1.01] p=0.060	0.87 [0.75, 1.01] p=0.061	2571	199
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	1.01 [0.90, 1.13] p=0.892	1.00 [0.89, 1.12] p=0.997	0.95 [0.85, 1.06] p=0.363	0.94 [0.84, 1.06] p=0.322	2730	352

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D measurement.
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure.

Table S4b: Logistic regression analysis of diagnosis of ASD (Autism Spectrum Disorder) versus no diagnosis, or high risk dichotomised trait measure group versus low risk, against 2D:4D (second-to-fourth digit ratio), separately by left and right hand, in females only. Unadjusted and adjusted[a] models are presented. Odds ratios are for one unit change in Z score (one standard deviation). N=numbers of participants.

Outcome	Females					
	Left		Right		Total N	Risk N[b]
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]		
	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value	OR [95% CI] P value		
ASD diagnosis (diagnosed by 11 years)	1.12 [0.66, 1.89] p=0.681	1.08 [0.64, 1.82] p=0.770[c]	1.63 [0.99, 2.69] p=0.057	1.60 [0.97, 2.65] p=0.068[c]	3053	14
Children's Communication Checklist (Coherence subscale) (115 months)	0.99 [0.85, 1.16] p=0.914	0.99 [0.85, 1.16] p=0.916	0.97 [0.83, 1.14] p=0.703	0.97 [0.83, 1.14] p=0.736	2662	167
Social and Communication Developmental Checklist (91 months)	1.06 [0.91, 1.24] p=0.455	1.07 [0.92, 1.26] p=0.373	1.00 [0.86, 1.16] p=0.979	1.01 [0.86, 1.17] p=0.936	2610	172
Repetitive behaviour (69 months)	1.03 [0.86, 1.23] p=0.739	1.04 [0.86, 1.24] p=0.704	0.93 [0.78, 1.11] p=0.426	0.94 [0.78, 1.12] p=0.462	2639	130
Emotionality Activity, Sociability Temperament Scale (Sociability subscale) (38 months)	0.93 [0.82, 1.06] p=0.254	0.93 [0.82, 1.06] p=0.283	0.96 [0.84, 1.09] p=0.505	0.96 [0.85, 1.09] p=0.535	2804	264

[a]Adjusted for highest parental occupational class, maternal education and age at 2D:4D measurement.
[b]Risk N includes those with ASD diagnosis or in the high risk group for the ASD trait measure group.
[c]To avoid perfect prediction, the CSE (Certificate of Secondary Education) and vocational classes of maternal education were collapsed for this analysis.