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Manuscript Title: Maternal pre-pregnancy weight and autistic-like traits among offspring in the general population

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Scientific Abstract

There is an emerging body of evidence demonstrating that maternal obesity at the time of conception increases the risk for Autism Spectrum Disorders (ASD) among offspring. We explored whether pre-pregnancy weight was related to autistic-like traits among offspring not diagnosed with ASD. A large sample of women, recruited during the second trimester of pregnancy, had their height measured and reported their pre-pregnancy weight. These measurements were then converted to a Body Mass Index (BMI) using the formula: (weight in kilograms)/(height in metres²). At 19-20 years of age, 1238 offspring of these women completed a measure of autistic-like traits, the Autism-Spectrum Quotient (AQ). Regression analyses identified a positive association between increasing maternal pre-pregnancy BMI and increasing AQ Total Score amongst offspring; this association was maintained even after controlling for a range of variables including maternal/obstetric factors (age at conception, education, smoking, alcohol consumption, hypertensive diseases, diabetes, threatened abortion), paternal BMI at pregnancy, and child factors (parity, sex) ($p < .01$, $R^2 = .03$). Chi-square analyses found that women with pre-pregnancy obesity (BMI ≥ 30) were more likely to have offspring with high scores (≥ 26) on the AQ ($p = .01$). Follow-up binary logistic regression analyses also accounting for the same obstetric and sociodemographic variables found that the offspring of women with pre-pregnancy obesity were at a statistically significantly increased risk of having high scores (≥ 26) on the AQ (OR: 2.80; 95% CI: 1.06, 7.43). This study provides further evidence that maternal pre-pregnancy obesity is associated with autism-like behaviors in offspring.

Lay Summary

The current study explored whether pre-pregnancy weight was related to autistic-like traits among offspring not diagnosed with ASD. We found that pre-pregnancy body mass index (BMI) in women is associated with the amount of autistic-like traits in their children in early adulthood. Specifically, women who were obese at the time of conception, were more likely to have a child who had high levels of autistic-like traits in early adulthood.

Keywords: autism spectrum disorder; body mass index; obesity; prenatal; maternal

A substantial body of research has investigated the association between obstetric complications and a range of neurodevelopmental disorders, including autism spectrum disorder (ASD). Specifically, in the context of ASD, several risk factors have been identified, including advanced parental age, caesarean delivery, and birth complications associated with trauma or hypoxia (see Modabbernia et al., 2017 for a review). Maternal weight at the time of conception is another factor that has come under recent scrutiny for its effect on fetal neurodevelopment, with several studies reporting associations between maternal pre-pregnancy weight and developmental outcomes for offspring, such as internalizing (Robinson et al., 2013) and externalizing (Rodriguez, 2008) problems and cognitive impairments (Van Lieshout et al., 2011).

Maternal pre-pregnancy obesity, especially, has demonstrated links to risk for ASD among offspring. A recent meta-analysis found that maternal pre-pregnancy obesity was associated with an increased risk of ASD amongst offspring, with an odds ratio of 1.47 (95% confidence interval: 1.24 – 1.74; Li et al., 2016a). A separate meta-analysis found similar effects when examining studies based on both pre-pregnancy, and pregnancy, body mass index (BMI) in both overweight and obese mothers (Wang et al., 2016). Moreover, several recent large cohort studies have also reported effects that align with these meta-analyses, demonstrating links between maternal pre-pregnancy obesity and an increased risk of ASD amongst offspring (Getz et al., 2016; Li et al., 2016b).

While there is mounting, convergent evidence for the link between maternal pre-pregnancy obesity and ASD risk, studies to date have predominately focused on dichotomous clinical outcomes (ASD vs no-ASD) to examine this link. One methodological approach with increasing traction in ASD research is to examine autistic-like traits in the broader community. There is accumulating evidence that autistic-like traits are on a continuum in the general population, with twin and population studies suggesting there is etiological similarity between autistic-like traits within normal variation, and traits at the extreme end of the distribution (clinical ASD; Robinson et al., 2011; Lundström et al., 2012; Robinson et al., 2016). Understanding developmental factors that are associated with variation in autistic-like traits in the general population may provide important insights into the biological mechanisms underpinning the clinical disorder. Specifically, understanding how maternal pre-pregnancy weight, particularly obesity, influences ASD-traits in the general population may further contribute to our understanding of the ways in which maternal pre- and perinatal factors can shape offspring developmental outcomes. This is a particularly pertinent area of research considering the world-wide increase in the prevalence of obesity (Kelly et al., 2008) and the increasing recognition of environmental risk factors in ASD.

In the current study, we hypothesized that increased maternal pre-pregnancy weight, and particularly pre-pregnancy obesity, would be related to increased levels of autistic-like traits among offspring in a large, non-clinical, general population sample.

Methods

Participants

The Western Australian Pregnancy Cohort (Raine) Study is a longitudinal investigation of pregnant women and their offspring consecutively recruited from King Edward Memorial Hospital (KEMH) in Perth (Australia) or nearby private practices between 1989 and 1991. The criteria for enrollment were gestational age between 16 and 20 weeks, English language skills sufficient to understand the implications of participation, an expectation to deliver at KEMH, and an intention to remain in Western Australia to facilitate future follow-ups of their child(ren). Ninety percent of eligible women agreed to participate in the study (Newnham et al., 1993).

From the 2900 pregnancies recruited into the Raine study, 2868 live born children have been comprehensively phenotyped since the commencement of the study. The current investigation is based on data that were collected during pregnancy assessments in the second- and third-trimester and when the offspring were 19-20 years of age.

Maternal weight during pregnancy

At enrolment (16-20 weeks' pregnancy), maternal height was measured and women were asked to estimate their pre-pregnancy weight. From the measurements of height and weight, a Body Mass Index (BMI) was calculated using the formula: $(\text{weight in kilograms})/(\text{height in metres}^2)$. Pre-pregnancy BMI based on maternal self-report of weight was highly correlated with BMI values derived from objectively measured weights at enrolment ($r = .92$). For the current study, categories of BMI were defined according to World Health Organization (WHO, 2000) cut-offs, and included the following: underweight (BMI <18.5), normal range (BMI 18.5 - <25), overweight (BMI 25 - <30), obese class I (BMI 30 - <35), obese class II and above (BMI ≥35).

Autistic-like traits

At age 19-20 years, the cohort was contacted specifically for the purpose of completing the Autism Spectrum Quotient (AQ), a self-report questionnaire that provides a quantitative measure of autistic-like traits in the general population (Baron-Cohen et al., 2001). Individuals are provided with 50 statements and asked to indicate on a 4-point scale how well that statement applies to them (strongly agree, agree, disagree, strongly disagree). The statements are divided into five subscales: Social Skills, Communication, Attention to Detail, Attention Switching and Imagination. Items within each subscale are then summed to provide a quantitative measure of that particular autistic-like trait, with higher scores denoting increased symptomatology. The AQ Total Score is calculated by tallying scores from the five subscales and is intended to quantitatively represent the severity of autistic-like traits (Baron-Cohen et al., 2001). The AQ Total score has shown strong convergent validity with the Social Responsiveness Scale total score (another frequently used measure to index the severity of ASD symptoms; Constantino & Gruber, 2005) in both non-clinical (Ingersoll et al., 2011) and clinical populations (Armstrong & Iarocci, 2013). The AQ has demonstrated good test-retest reliability (ICC =

.7; Baron-Cohen et al., 2001), strong internal reliability ($\alpha = .8$; Austin, 2005), and good internal validity across cultures (Woodbury-Smith et al., 2005). The current study used the AQ Total Score threshold of 26 to identify individuals with 'high' levels of autistic-like traits (Woodbury-Smith et al., 2005). Approximately 45% of the Raine sample ($n = 1280$) completed the AQ when contacted.

Control variables

A range of control variables recorded in parental questionnaires during the second and third trimester and in the mother's medical records were included in the adjusted analyses. The control variables were selected based on their relation to three categories. (1) Obstetric risk factors for ASD: maternal cigarette smoking during pregnancy (none, 1-10 cigarettes daily, 11+ cigarettes daily), and threatened abortion (yes, no); (2) Obstetric factors known to influence other aspects of behavioral development: diabetes (none, pre-existing, gestational), hypertensive diseases of pregnancy (none, gestational hypertension, pre-eclampsia; Whitehouse et al., 2012), maternal alcohol consumption during pregnancy (none, once a week or less, several times a week or more); and (3) sociodemographic factors: maternal education (completed secondary school, did not complete secondary school), maternal age at conception (<20 years, 20-34.9 years, ≥ 35 years), paternal BMI at the time of pregnancy (<18.5, 18.5 - <25, 25 - <30, 30 - <35, ≥ 35 ; calculated using the same formula as maternal BMI), family income below the poverty line (yes, no; with reference to \$24,000AUD, which was the level of qualification for benefits or 'poverty line' defined by the Australian Government at the time of recruitment), and offspring gender (male, female) and parity (0, 1, >1) .

We also report information regarding offspring mode of delivery (vaginal without instruments, vaginal with forceps/vacuum, elective cesarean, emergency cesarean), gestational age at birth (<32 weeks, 32-37 weeks, >37 weeks), and birth weight (<2500g, 2500-4000g, >4000g) (see Table 1 and Supporting Information Table 1), however, these variables were not included in the final models as they may be better classified as potential mediators of the association between pre-pregnancy BMI and autistic-like traits (rather than confounding variables). Instead, these variables, along with paternal age at conception (of which there was only a limited number of individuals with this information) were included only to characterize the sample.

Statistical analysis

We first examined any group differences between individuals with an AQ Total score ≥ 26 (i.e., a 'high' level of autistic-like traits) compared to individuals with an AQ Total score <26. Mann-Whitney U tests were used to compare maternal and paternal pre-pregnancy BMI categories (i.e., <18.5, 18.5-<25, 25-<30, 30-<35, ≥ 35) between groups with an AQ Total score ≥ 26 compared to individuals with an AQ Total score <26. For all other variables, Chi-square analyses were used to compare sociodemographic characteristics between those individuals with a 'high' AQ Total score (≥ 26) compared to those with a score <26. Of note, these same analyses were applied to compare participants who did and did not contribute data in the current study (see Supporting Information Table 1). We then examined whether there was any association between pre-pregnancy BMI

categories (underweight [BMI <18.5], normal range [BMI 18.5 - <25], overweight [BMI 25 - <30], obese class I [BMI 30 - <35], obese class II and above [BMI ≥35]) and the AQ Total Score obtained from a non-clinical population using regression analyses (forced entry models with missing data deleted pairwise) in both unadjusted (linear regression) and adjusted (multiple regression) models. The adjusted model included all of the obstetric and sociodemographic variables listed in the 'Control variables' paragraph above (i.e., maternal age, education, smoking, alcohol consumption, hypertensive diseases, diabetes, threatened abortion, parity, sex, and paternal BMI at time of pregnancy). We then used binary logistic regression to examine whether pre-pregnancy obesity (i.e., BMI <30 vs BMI ≥30), specifically, was related to a 'high' level of autistic-like traits (i.e., AQ Total score ≥26) in offspring with and without the inclusion of all of the above listed 'Control' variables. The alpha level for these analyses was set at $p < .05$. All analyses were conducted using SPSS Version 25 (IBM Corp., Armonk, NY).

Results

Of the total 2868 participants from the original cohort, 41 (1.4%) had a reported diagnosis of autism, intellectual disability or Down Syndrome. These specific diagnoses were determined on the basis of parent-report as part of the larger Raine study protocol when the children were aged approximately 2, 5, 8, 10, and 14 years. For the majority of individuals with a diagnosis of autism, intellectual disability, or Down Syndrome, AQ data were intentionally not collected ($n = 29$); for 12 individuals with a diagnosis of autism, however, AQ data were inadvertently collected though excluded from the current study, consistent with the current study's focus on a non-clinical population. Of the remaining sample, 1559 individuals were excluded due to not having completed the AQ when contacted at 19-20 years of age. An additional 30 cases were excluded due to missing maternal pre-pregnancy BMI data. The final, included sample comprised 1238 (43.2%) of the possible 2868 participants from the original cohort (see Figure 1). The AQ Total score for the final included sample ranged from 1 – 47 with a median of 15 (Interquartile range = 6).

(Insert Figure 1 about here)

Table 1 provides a summary of participant characteristics for individuals with a 'high' AQ score (i.e., ≥26) versus those with an AQ score below 26 for those participants included in the current study. The groups differed on only two variables; mothers of offspring with a 'high' AQ Total score were more likely to have a BMI in the 'obese' range (i.e., ≥30) at the time of pregnancy and were more likely to experience a threatened abortion during their pregnancy compared to mothers of offspring with an AQ Total score <26. Supporting Information Table 1 summarizes and compares characteristics of those individuals that contributed data to the current study from the Raine sample versus those that did not contribute data. In summary, participants that contributed data to the current study were less likely to have mothers that were under 20 years of age at the time of conception and more likely to have mothers who were 35 years or older at the time of conception. Participants in the current study were

also more likely to be born to mothers that had completed secondary education, and less likely to be born to mothers who were obese. Included mothers were less likely to smoke cigarettes during pregnancy and less likely to have gestational hypertension or preeclampsia, though were more likely to have one drink per week (or less) during their pregnancy. The fathers of the offspring in the current study were more likely to have a BMI in the normal weight range at the time of pregnancy. The offspring in the current study were less likely to have a birth weight of less than 2500g, and were more likely to be female and be born into a family with an income above the poverty line.

(Insert Table 1 about here)

Regression Analyses: Pre-pregnancy BMI Categories (Underweight, Normal Weight, Overweight, Obesity Class I, Obesity Class II+) and AQ Total Scores

Linear regression (unadjusted model): We first examined the association between pre-pregnancy BMI and AQ Total Score without the inclusion of any control variables (i.e., in an unadjusted model). The model was significant, $F(1, 1236) = 7.86, p < .01, R^2 = .01$, with pre-pregnancy BMI being a significant predictor of offspring AQ Total score ($B = .63, \beta = .08, t = 2.80, p < .01$). This suggests that a higher pre-pregnancy BMI category is associated with a higher AQ Total score in offspring.

(Insert Figure 2 about here)

Multiple regression (adjusted model): After adjusting for the control variables (maternal age, education, income, smoking, alcohol consumption, hypertensive diseases, diabetes, threatened abortion, parity, sex, and paternal BMI at conception), the overall multiple regression model remained significant, $F(12, 992) = 2.95, p < .01, R^2 = .03$. Increasing maternal pre-pregnancy BMI category ($t = 2.41, p = .02, 95\% \text{ CI: } 0.11, 1.11$) was associated with higher AQ Total Scores. This pattern of results is reflected in Figure 2. In addition, male offspring gender ($t = 2.73, p = .01, 95\% \text{ CI: } 0.27, 1.63$), and a household income below the poverty line at the time of pregnancy ($t = 2.37, p = .02, 95\% \text{ CI: } 0.16, 1.67$) were also predictors of higher AQ Total Scores in the model. No other variables (i.e., paternal BMI category, hypertensive diseases of pregnancy, maternal cigarette smoking during pregnancy, maternal alcohol consumption during pregnancy, parity, maternal education, maternal age at conception, threatened abortion, or maternal diabetes) were independently associated with AQ Total Scores (all p -values $> .05$). Of note, multicollinearity was not a concern in the model, with Tolerance values ranging from .83 – .99 and a variance inflation factor range of 1.0 – 1.2 across all variables. In addition, all pairwise combinations of covariates (as indexed using Spearman rank correlations) were below 0.3. Table 2 presents the findings from the adjusted multiple regression model.

(Insert Table 2 about here)

Binary Logistic Regression: Pre-pregnancy Obesity (BMI <30 vs BMI ≥30) and High AQ Total Scores

After establishing an association between maternal pre-pregnancy BMI category and AQ Total Scores, we were particularly interested in the effects of obesity on elevated AQ scores considering the previously described links between obesity and increased ASD risk. To investigate this, we used a binary logistic regression to examine whether pre-pregnancy obesity (BMI ≥ 30) is associated with 'high' scores on the AQ as compared to women with a pre-pregnancy BMI of <30 . A 'high' score threshold referred to an AQ Total Score of ≥ 26 . Just over 5% of the women in the current study had a pre-pregnancy BMI of 30 or greater ($n = 67/1238$, 5.4%) and were thus classed as obese.

The unadjusted logistic regression model revealed that offspring of mothers with pre-pregnancy obesity were significantly more likely to demonstrate high levels of autism-associated behaviors compared to mothers who were not obese at the time of pregnancy (odds ratio = 2.79, 95% CI: 1.20, 6.43, $p = .02$). This association remained after accounting for the control variables (maternal age, education, smoking, alcohol consumption, hypertensive diseases, diabetes, threatened abortion, parity, sex, paternal obesity [BMI ≥ 30 vs <30] at conception), with an odds ratio of 2.80 (95% CI: 1.06, 7.43, $p = .04$). Supporting Information Table 2 presents the summary of results from this unadjusted and adjusted logistic regression models.

Discussion

We observed a statistically significant association between increasing maternal pre-pregnancy BMI and higher autism-associated traits in offspring without an autism diagnosis in young adulthood (i.e., in a non-clinical population). This association remained after adjusting for a range of sociodemographic and obstetric variables, with the effect most apparent in the higher BMI categories (i.e., 'obese' and 'obese class II'). Follow-up analyses specifically focused on the association between pre-pregnancy maternal obesity and AQ Total Scores found that the offspring of mothers with a pre-pregnancy BMI of 30 or above (i.e., obese) were more likely to demonstrate 'high' levels of autism-associated behaviors, even after taking control variables into account, with an odds ratio exceeding 2.0. These findings are in line with an increasing body of evidence from clinical populations demonstrating links between maternal pre-pregnancy obesity and increased ASD risk amongst offspring (e.g., Dodds et al., 2011; Getz et al., 2016; Li et al., 2016a, 2016b; Wang et al., 2016).

While the specific mechanistic pathways linking maternal obesity and ASD have not yet been delineated, several biological mechanisms have been proposed. Obesity has been linked to a state of chronic inflammation, with an increased expression of pro-inflammatory cytokines (Cancello & Clement, 2006; van der Burg et al., 2016); this state may contribute to an inflammatory in utero environment for the fetus, the sequelae of which exerts adverse effects on the developing central nervous system (van der Burg et al., 2016). Indeed, studies have reported the presence of markers of neuro- and systemic inflammation in children with ASD (Onore et al., 2012). There is also evidence from animal models suggesting maternal obesity is associated with synaptic alterations in offspring

(Hatanaka et al., 2017). Both animal and human research has suggested that some manifestations of ASD may be underlain by disturbances in synaptic function and structure (Zoghbi & Bear, 2012).

Another pathway that has been proposed is through the link between obesity and maternal diabetes, and the potential consequences of diabetes (e.g., hyperglycemia) on the developing fetus (e.g., hypoxia, increased oxidative stress; Biri et al., 2006; Chen & Scholl, 2005). While the association between maternal diabetes and ASD risk has been established (Modabbernia et al., 2017), in our study, maternal diabetes was not a significant predictor of autism-associated behaviors, and the association between pre-pregnancy BMI, and pre-pregnancy obesity, with AQ Total Scores remained even after taking maternal diabetes into account. However, our study was not designed, nor well-positioned, to address the association between maternal diabetes and autism-associated behaviors as few obese mothers in our sample had diabetes prior to, or during, their pregnancy ($n = 7$). Indeed, the incidence of gestational diabetes in our sample was significantly lower than current population estimates (1.8% versus 6%; AIHW, 2014), reflective of the lower incidence of gestational diabetes three decades ago, when the current sample were recruited (Chamberlain et al., 2014). This low rate of diabetes in our sample also precluded our capacity to examine any combined effects of maternal weight and diabetes on autism-associated behaviors in a non-clinical sample. This is an avenue that warrants examination in lieu of recent evidence from clinical populations reporting that children of mothers with a combination of diabetes and obesity are at the highest risk of ASD (e.g., Li et al., 2016b). Given the biological complexity of obesity and ASD, it is most likely that multiple biological, genetic, and environmental factors are implicated in the link between maternal obesity and offspring ASD-related traits and behaviors.

Strengths of this study include the large sample size and prospectively collected data spanning two decades. However, we note that none of the offspring in the current study had a clinical diagnosis of ASD. Nevertheless, the relatively large general population sample (even after sample attrition) meant that a moderate number of individuals exceeding the Woodbury-Smith et al. (2005) threshold for 'high' levels of autistic-like traits were identified ($n = 67$). Although, we acknowledge that for the logistic regression analysis in the current study, our sample size was limited when individuals were stratified by 'high' AQ scores and weight category and thus, the results should be interpreted within this context. In addition, while the current study identified an association between increasing pre-pregnancy BMI category and increasing AQ Total scores in offspring, the pattern of results suggested a particularly prominent increase in AQ scores within the 'Obese Class II' category (i.e., $\text{BMI} \geq 35$). While the sample size was small in this category (relative to the other categories), this pattern of results suggests that further investigation and stratification across the 'obese' range of BMI categories may be of high utility to better understand the relationship (and eventual biological mechanisms) between obesity and autistic-like traits. The relationship between autistic-like traits in the general population and clinical ASD is still debated, but there is mounting evidence (supported by the findings in the current study) that the latter may be at the extreme end of the same etiological continuum (Lundström et al., 2012; Robinson et al., 2011, 2016).

A limitation of the current study was the considerable sample attrition, with only 43.2% of the original Raine cohort providing data at both time points. Participants in the current study were less likely to have been born to mothers with pre-pregnancy obesity and hypertensive diseases. This pattern of attrition raises the possibility that our findings may have been influenced by systematic bias, and potentially, may have underestimated the strength of the association between pre-pregnancy BMI and autistic-like traits (considering both of these variables have been associated with increased rates of atypical development [Getz et al., 2016; Li et al., 2016b; Whitehouse et al., 2012]). Participants in the current study were also less likely to come from a socially-disadvantaged background. We attempted to minimize this possibility by statistically adjusting our regression analyses for indices of social disadvantage, such as maternal education and household income, as well as previously identified risk factors for ASD. It is also relevant to note that the women for the Raine Study were recruited from a tertiary maternity hospital, and the original sample is known to have over-represented socially disadvantaged women (Whitehouse et al., 2010). However, despite these considerations, we note caution in generalizing our findings to the general population prior to replication in an independent sample. There is also the possibility that the association between maternal pre-pregnancy BMI and autistic-like traits could be influenced by factors that were not measured in the current study, such as maternal stress during pregnancy (e.g., Varcin et al., 2017), offspring IQ (Nishiyama et al., 2009), or the presence of other developmental disorders that were not ascertained as part of the current study (e.g., epilepsy).

The current study identified an association between maternal pre-pregnancy weight and offspring autistic-like traits in young adulthood, which remained after accounting for a range of sociodemographic and obstetric variables. Specifically, we found that pre-pregnancy obesity is associated with a higher likelihood of offspring showing 'high' levels of autism-associated behaviors in early adulthood. This finding converges with evidence from cohort studies of clinical populations demonstrating links between maternal pre-pregnancy obesity and ASD risk. These epidemiological studies pave the way for future studies to examine the possible biological bases for this relationship, which may provide insights into mechanistic pathways linked to the development of ASD.

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Figure Legends

Figure 1. Flowchart of individuals included and excluded in the current study.

Figure 2. Box-and-whisker plots (with 5-95th percentile bars and triangles representing data points outside of these bounds) of offspring AQ scores by maternal pre-pregnancy BMI category.

Live births in Raine study

N = 2868

Valid AQ data

N = 1268

Pre-pregnancy BMI data

N = 1238

1559 Missing AQ data

29 AQ not collected due to a diagnosis of a neurodevelopmental disorder

12 AQ excluded due to a diagnosis of a neurodevelopmental disorder

30 Missing pre-pregnancy BMI data

Table 1. Characteristics of participants with AQ Total scores ≥ 26 (i.e., 'high' autistic-like traits) compared to participants with AQ Total scores < 26 .

	AQ Total Score ≥26		AQ Total Score <26		p value
	N = 54		N = 1184		
	N	n (%)	N	n (%)	
Maternal pre-pregnancy body mass index	54		1184		.12
<18.5		5 (9.3)		131 (11.1)	
18.5 - <25		36 (66.7)		866 (73.1)	
25 - <30		6 (11.1)		127 (10.7)	
30 - <35		4 (7.4)		45 (3.8)	
≥ 35		3 (5.6)		15 (1.3)	
Paternal body mass index at time of pregnancy	47		1014		.29
<18.5		0 (0.0)		15 (1.5)	
18.5 - <25		35 (74.5)		641 (63.2)	
25 - <30		9 (19.1)		302 (29.8)	
30 - <35		2 (4.3)		49 (4.8)	
≥ 35		1 (2.1)		7 (0.7)	
Maternal pre-pregnancy body mass index (Non-obese vs obese)	54		1184		.01
< 30		47 (87.0)		1124 (94.9)	
≥ 30		7 (13.0)		60 (5.1)	
Paternal body mass index at time of pregnancy (Non-obese vs obese)	47		1014		.92
< 30		44 (93.6)		953 (94.0)	
≥ 30		3 (6.4)		61 (6.0)	
Maternal age at conception	54		1178		.56
< 20 years		2 (3.7)		69 (5.9)	
20–34.9 years		45 (83.3)		909 (77.2)	
≥35		7 (13.0)		200 (17.0)	
Paternal age at conception	31		724		.56
< 20 years		1 (3.2)		12 (1.7)	
20–34.9 years		24 (77.4)		519 (71.7)	
≥35		6 (19.4)		193 (26.7)	
Maternal education at pregnancy	54		1184		.79
Completed secondary school		27 (50.0)		570 (48.1)	
Did not complete secondary school		27 (50.0)		614 (51.9)	
Family income below poverty line	53		1143		.13

Yes		23 (43.4)	382 (33.4)	
No		30 (56.6)	761 (66.6)	
Maternal alcohol intake during pregnancy	51		1110	.23
None		32 (62.7)	647 (58.3)	
Once a week or less		19 (37.3)	402 (36.2)	
Several times a week or more		0 (0.0)	61 (5.5)	
Maternal smoking in pregnancy	51		1110	.84
None		43 (84.3)	899 (81.0)	
1–10 cigarettes daily		4 (7.8)	105 (9.5)	
11+ cigarettes daily		4 (7.8)	106 (9.5)	
Threatened abortion	54		1183	.02
No		46 (85.2)	1107 (93.6)	
Yes		8 (14.8)	76 (6.4)	
Maternal hypertensive diseases of pregnancy	54		1184	.49
None		42 (77.8)	938 (79.2)	
Gestational hypertension		12 (22.2)	222 (18.8)	
Pre-eclampsia		0 (0.0)	24 (2.0)	
Mode of delivery	53		1134	.75
Vaginal without instruments		30 (56.6)	720 (63.5)	
Vaginal with forceps and /or vacuum		11 (20.8)	181 (16.0)	
Elective Caesarean		7 (13.2)	132 (11.6)	
Emergency Caesarean		5 (9.4)	101 (8.9)	
Gestational age at birth	54		1181	.84
< 32 weeks		1 (1.9)	13 (1.1)	
32 – 37 weeks		10 (18.5)	202 (17.1)	
> 37 weeks		43 (79.6)	966 (81.8)	
Birthweight	54		1181	.76
< 2500g		5 (9.3)	80 (6.8)	
2500g – 4000g		44 (81.5)	1000 (84.7)	
> 4000 g		5 (9.3)	101 (8.6)	
Sex	54		1184	.22
Males		30 (55.6)	557 (47.0)	
Females		24 (44.4)	627 (53.0)	
Parity	54		1184	.45
0		21 (38.9)	565 (47.7)	
1		19 (35.2)	357 (30.2)	
>1		14 (25.9)	262 (22.1)	
Maternal diabetes	54		1184	.55

None	51 (94.4)	1144 (96.6)
Pre-existing	1 (1.9)	20 (1.7)
Gestational	2 (3.7)	20 (1.7)

Table 2. Multiple regression (adjusted) model showing the association between maternal pre-pregnancy BMI and scores on the Autism Quotient (AQ) Total Score among offspring (n = 1238).

	Autism Quotient (AQ) Total Score				
	β	B	SE B	<i>p</i>	95% CI
Maternal pre-pregnancy body mass index	.08	.61	.25	.02	0.11, 1.11
Paternal body mass index at time of pregnancy	-.05	-.39	.28	.16	-0.93, 0.15
Maternal age at conception	-.00	-.05	.41	.91	-0.85, 0.76
Maternal education at pregnancy	.05	.58	.36	.11	-0.14, 1.29
Family income below poverty line	.08	.91	.39	.02	0.16, 1.67
Maternal alcohol intake during pregnancy	-.06	-.51	.30	.09	-1.10, 0.08
Maternal smoking in pregnancy	.03	.22	.29	.40	-0.34, 0.79
Threatened abortion	.05	1.02	.69	.14	-0.34, 2.37
Maternal hypertensive diseases of pregnancy	-.01	-.11	.38	.78	-0.85, 0.64
Sex	.09	.95	.35	.01	0.27, 1.63
Parity	.04	.28	.23	.32	-0.17, 0.73
Maternal diabetes	-.01	-.12	.60	.77	-1.30, 1.06

Supporting Information Table 1. Characteristics of participants in the Raine cohort who were and were not included in the current study.

	Included		Not included		<i>p</i> value
	N = 1238		N = 1630		
	N	n (%)	N	n (%)	
Maternal pre-pregnancy body mass index	1238		1563		.11
<18.5		136 (11.0)		185 (11.8)	
18.5 - <25		902 (72.9)		1066 (68.2)	
25 - <30		133 (10.7)		195 (12.5)	
30 - <35		49 (4.0)		79 (5.1)	
≥ 35		18 (1.4)		38 (2.4)	
Paternal body mass index at time of pregnancy	1061		1145		.04
<18.5		15 (1.4)		21 (1.8)	
18.5 - <25		676 (63.7)		671 (58.6)	
25 - <30		311 (29.3)		383 (33.4)	
30 - <35		51 (4.8)		61 (5.3)	
≥ 35		8 (0.8)		9 (0.8)	
Maternal pre-pregnancy body mass index (Non-obese vs obese)	1238		1563		.03
< 30		1171 (94.6)		1446 (92.5)	
≥ 30		67 (5.4)		117 (7.5)	
Paternal body mass index at time of pregnancy (Non-obese vs obese)	1061		1145		.74
< 30		997 (94.0)		1072 (93.6)	
≥ 30		64 (6.0)		73 (6.4)	
Maternal age at conception	1232		1535		< .01
< 20 years		71 (5.8)		202 (13.2)	
20–34.9 years		954 (77.4)		1182 (77.0)	
≥35		207 (16.8)		151 (9.8)	
Maternal education at pregnancy	1238		1567		< .01
Completed secondary school		597 (48.2)		500 (31.9)	
Did not complete secondary school		641 (51.8)		1067 (68.0)	
Family income below poverty line	1196		1507		< .01
Yes		405 (33.9)		777 (51.6)	
No		791 (66.1)		730 (48.4)	
Maternal alcohol intake during pregnancy	1161		1437		< .01
None		679 (58.5)		946 (65.8)	

Once a week or less	421 (36.3)	415 (28.9)	
Several times a week or more	61 (5.3)	76 (5.3)	
Maternal smoking in pregnancy	1161	1437	< .01
None	942 (81.1)	999 (69.5)	
1–10 cigarettes daily	109 (9.4)	223 (15.5)	
11+ cigarettes daily	110 (9.5)	215 (15.0)	
Threatened abortion	1237	1564	.85
No	1153 (93.2)	1455 (93.0)	
Yes	84 (6.8)	109 (7.0)	
Maternal hypertensive diseases of pregnancy	1238	1545	.04
None	980 (79.2)	1189 (77.0)	
Gestational hypertension	234 (18.9)	302 (19.5)	
Pre-eclampsia	24 (1.9)	54 (3.5)	
Mode of delivery	1187	1564	.93
Vaginal without instruments	750 (63.2)	969 (62.0)	
Vaginal with forceps and /or vacuum	192 (16.2)	264 (16.9)	
Elective Caesarean	139 (11.7)	187 (12.0)	
Emergency Caesarean	106 (8.9)	144 (9.2)	
Gestational age at birth	1235	1534	.39
< 32 weeks	14 (1.1)	27 (1.8)	
32 – 37 weeks	212 (17.2)	256 (16.7)	
> 37 weeks	1009 (81.7)	1251 (81.5)	
Birthweight	1235	1621	< .01
< 2500g	85 (6.9)	167 (10.3)	
2500g – 4000g	1044 (84.5)	1307 (80.6)	
> 4000 g	106 (8.6)	147 (9.1)	
Sex	1238	1630	< .01
Males	587 (47.4)	868 (53.3)	
Females	651 (52.6)	762 (46.7)	
Parity	1238	1628	.19
0	586 (47.3)	787 (48.3)	
1	376 (30.4)	447 (27.5)	
>1	276 (22.3)	394 (24.2)	
Maternal diabetes	1238	1630	.68
None	1195 (96.5)	1563 (95.9)	
Pre-existing	21 (1.7)	33 (2.0)	
Gestational	22 (1.8)	34 (2.1)	

Supporting Information Table 2. Logistic regression models (unadjusted and adjusted) showing the association between maternal pre-pregnancy obesity (BMI ≥ 30) and 'high' scores (≥ 26) on the Autism Quotient (AQ) Total Score among offspring (n = 1238).

	Unadjusted				Adjusted*			
	B	SE B	p	OR (95% CI)	B	SE B	p	OR (95% CI)
Total AQ Score	1.03	.43	.02	2.79 (1.21, 6.43)	1.03	.50	.04	2.80 (1.06, 7.43)

*Adjusted for paternal obesity at time of pregnancy (BMI ≥ 30 versus BMI <30), maternal age at conception, maternal smoking during pregnancy, alcohol consumption during pregnancy, maternal hypertensive diseases of pregnancy, maternal education, family income at time of pregnancy, threatened abortion, offspring gender, diabetes, and parity.