



## Early Life Exposures

# Maternal body mass index during early pregnancy, gestational weight gain, and risk of autism spectrum disorders: Results from a Swedish total population and discordant sibling study

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## Abstract

**Background:** Prenatal environmental factors such as maternal adiposity may influence the risk of offspring autism spectrum disorders (ASD), though current evidence is inconsistent. The objective of this study was to assess the relationship of parental BMI and gestational weight gain (GWG) with risk of offspring ASD in a population-based cohort study using family-based study designs.

**Methods:** The cohort was based in Stockholm County, Sweden, including 333 057 individuals born 1984–2007, of whom 6420 were diagnosed with an ASD. We evaluated maternal body mass index (BMI) at first antenatal visit, GWG and paternal BMI at the time of conscription into the Swedish military as exposures using general estimating equation (GEE) models with logit link.

**Results:** At the population level, maternal overweight/obesity was associated with increased risk of offspring ASD [odds ratio (OR)<sub>25 ≤ BMI < 30</sub> 1.31, 95% confidence interval = 1.21–1.41; OR<sub>BMI ≥ 30</sub> 1.94, 1.72–2.17], as was paternal underweight (OR<sub>BMI < 18.5</sub> 1.19, 1.06–1.33) and obesity (OR<sub>BMI ≥ 30</sub> 1.47, 1.12–1.92) in mutually adjusted models. However, in matched sibling analyses, the relationship between elevated maternal BMI

and ASD risk was not apparent. GWG had a U-shaped association with offspring ASD at the population level ( $OR_{\text{insufficient}} 1.22, 1.07\text{--}1.40$ ;  $OR_{\text{excessive}} 1.23, 1.08\text{--}1.40$ ). Matched sibling analyses were suggestive of elevated risk with excessive GWG ( $OR_{\text{insufficient}} 1.12, 0.68\text{--}1.84$ ;  $OR_{\text{excessive}} 1.48, 0.93\text{--}2.38$ ).

**Conclusions:** Whereas population-level results suggested that maternal BMI was associated with ASD, sibling analyses and paternal BMI analyses indicate that maternal BMI may also be a proxy marker for other familial risk factors. Evidence is stronger for a direct link between GWG and ASD risk.

**Key words:** Autism spectrum disorders, body mass index, pregnancy, gestational weight gain

#### Key Messages

- Maternal adiposity during pregnancy has been linked to increased risk of offspring autism spectrum disorders (ASD), but the evidence has been inconsistent.
- This study found evidence that higher maternal body mass index (BMI) at pregnancy baseline and paternal BMI at age 18 were independently associated with increased risk of ASD in offspring. However, sibling analyses suggested that the maternal BMI-ASD association may be affected by residual confounding.
- Insufficient or excessive maternal gestational weight gain both were associated with increased risk of ASD, and this was supported by sibling analyses.

## Introduction

Rates of overweight and obesity have dramatically increased in recent decades,<sup>1</sup> paralleling an increase in the prevalence of autism spectrum disorders (ASD). Some reports suggest that maternal obesity in early pregnancy<sup>2,3</sup> and excessive weight gain during pregnancy<sup>2,4</sup> increase risk of offspring ASD. Other studies have raised the possibility that associations between maternal body mass index (BMI) and ASD risk may not be causal but are instead due to familial confounding.<sup>4,5</sup> In other words, the same genetic or environmental factors shared among family members that predispose mothers to high BMI may also predispose offspring to ASD. However, all of these studies featured <1000 cases of ASD, limiting statistical power.

The aim of this study was to explore the relationship of both maternal baseline BMI and gestational weight gain (GWG) with risk of ASD in the offspring in the largest study to date. In addition, we used two family-based study designs, paternal-offspring comparisons and matched sibling comparisons,<sup>6–8</sup> to explore the potential for alternative mechanisms to explain the relationship between maternal BMI and offspring ASD risk. We used matched sibling comparisons to do the same for the relationship between GWG and offspring ASD risk.

## Methods

### Study population

Our study is nested within the Stockholm Youth Cohort (SYC), a prospective register-based cohort consisting of all individuals born 1984–2007 and resident in Stockholm County for  $\geq 4$  years.<sup>9,10</sup> Children who were adopted (7266), from a multiple birth (13 919) or born outside Sweden (60 020) were excluded. Exposure, outcome and covariate data were extracted from national and regional computerized data registers, described elsewhere.<sup>9,10</sup> This study was approved by the regional ethical review board for Karolinska Institutet (DNR 2010/1185-31/5). Informed consent was not required for the analysis of anonymized register data.

### ASD diagnosis

ASD case status as of 31 December 2011 was ascertained in a procedure covering all potential pathways to ASD care and diagnosis in Stockholm County, using ICD-9, ICD-10, and DSM-IV codes.<sup>9</sup> ASD was subtyped by absence or presence of comorbid intellectual disability (ID), defined as  $IQ < 70$ . A medical records review found that 96.0% of register-identified ASD cases were consistent with an ASD diagnosis.<sup>9</sup>

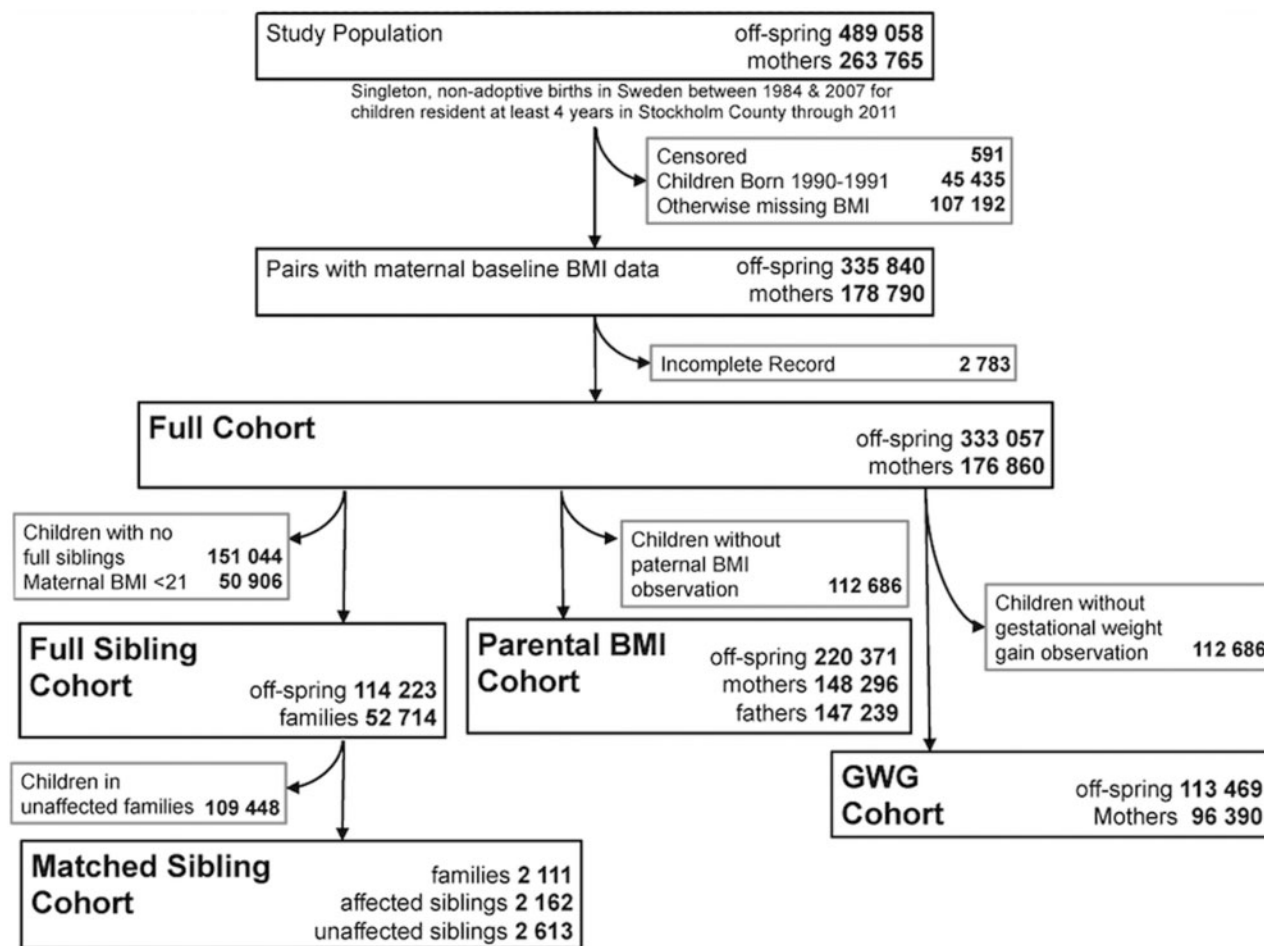


Figure 1. Derivation of the sample.

### Exposure variables: parental BMI and GWG

We used maternal weight at the first antenatal visit as a proxy for pre-pregnancy weight. The Medical Birth Register (MBR) contains objectively measured maternal weight and height data recorded by midwives at the first antenatal visit, at median 10.6 [interquartile range (IQR): 9.0–12.6] weeks.<sup>11</sup> These endpoints were not captured in 1990–91 and were available for 75.7% of the mother/child pairs otherwise (Figure 1). Weights <40 kg or >140 kg were censored, as were heights <140 cm and >200 cm. The proportion of overweight and obese mothers included in our study agrees with other longitudinal reports in Sweden during the same time period (Figure 2).<sup>12,13</sup> Maternal baseline BMI values were categorized by standard convention: underweight (BMI < 18.5), normal ( $18.5 \leq \text{BMI} < 25$ ), overweight ( $25 \leq \text{BMI} < 30$ ), and obese (BMI  $\geq 30$ ).<sup>14</sup> Maternal metabolic conditions (pre-gestational hypertension, pre-gestational diabetes, pre-eclampsia and gestational diabetes) were defined according to ICD diagnostic codes within the

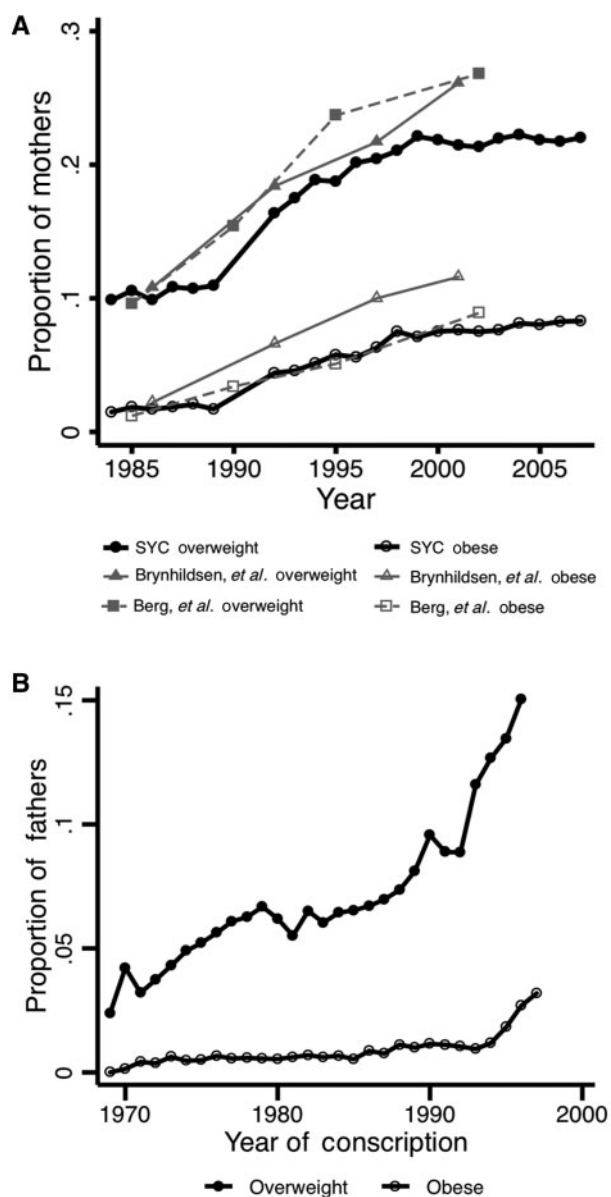
MBR or the National Patient Register (Supplementary Table 1, available as Supplementary data at *IJE* online).

Of the mother/child pairs with maternal baseline BMI data, 34% also had maternal weight at the time of delivery recorded within the MBR, similar to previous studies using MBR data.<sup>15</sup> For these mothers, GWG categories were defined as 'ideal', 'insufficient' or 'excessive' based on Institute of Medicine recommendations for each BMI category (underweight: 12.5–18 kg; normal weight 11.5–16 kg; overweight 7–11.5 kg; obese: 5–9 kg).<sup>16</sup>

Paternal BMI data from the time of conscription in the Swedish military (at age 18) were available for 66.1% of the children for whom maternal BMI data were available (Figure 1).

### Other covariates

Sociodemographic data were extracted for the year before the birth of the index child.<sup>17</sup> Disposable family income



**Figure 2.** The prevalence of overweight and obesity in Swedish women and men over time. (A) Comparison of the prevalence of overweight and obesity among mothers of children in the SYC with other reported values in Swedish women. Berg *et al.*<sup>12</sup> measured weight and height for 605 Swedish women aged 25–34 during the years 1985–2002. Brynhildsen *et al.*<sup>13</sup> collected maternal weight and height at first prenatal visit data from medical records of 4430 women delivering at Swedish hospitals. (B) Prevalence of overweight and obesity among fathers of children in the SYC, based on BMI data collected at the time of conscription into the Swedish military at the age of 18.

measurements account for all sources of income and are adjusted for family size and inflation. Parental educational achievement (highest of mother or father) was categorized as  $\leq 9$  years of schooling, 10–12 years, or  $>12$  years.<sup>17</sup> Maternal migrant status was categorized as born in Sweden or outside. Parity was categorized as primiparous or not. Parental history of psychiatric inpatient treatment before the birth of the index child (yes/no) was extracted

from the national inpatient register. Following our previous work,<sup>9,18</sup> birth year, maternal age and paternal age were centered and included in models as quadratic terms, to accommodate non-linear relationships between ASD risk and these factors. Gestational week at first antenatal visit was recorded in the MBR from mid 1995 onwards for 93–98% of women every year (Table 1).

## Statistical Analyses

### BMI and ASD

Analyses were conducted using Stata/MP 12.1 (College Station, TX). Categorical analyses used normal BMI as the referent category. Continuous analyses used restricted cubic spline models with five knots and *xbrcspline* post-estimation,<sup>19</sup> with BMI = 21 as the referent. Restricted cubic spline models flexibly fit relationships between variables that are non-linear in nature. We used general estimating equation (GEE) models with logit link clustered on maternal identification number to provide robust standard errors. Models were adjusted for sex, birth year, parity, maternal age, paternal age, maternal country of birth, parental education, income and parental psychiatric history. Covariates were chosen a priori based on reported associations with ASD.<sup>17,18,20–22</sup> Maternal and paternal BMI were considered separately and in a mutually adjusted model. We repeated these analyses stratified by ASD with and without ID. GEE models adjusted as described above were used to evaluate the relationship between maternal metabolic conditions and ASD; the analyses were repeated including adjustment for maternal BMI category.

### GWG and ASD

GWG was analysed as both a categorical and continuous variable with GEE as above. Categorical variables were analysed with ideal GWG as the referent group. For continuous analysis, we used restricted cubic spline models with five knots, with GWG = 14 kg as the referent. Models were adjusted for maternal BMI category, gestational age at birth, sex, birth year, parity, maternal age, paternal age, maternal country of birth, socioeconomic status (SES) and parental psychiatric history. Outcomes of any ASD and ASD with and without ID were considered. To distinguish the potential effects of GWG from baseline BMI, we repeated the analysis, restricting the sample to mothers who began pregnancy with a normal BMI.

### Sibling analyses

We used a sibling comparison design to assess whether observed associations of baseline BMI and GWG with ASD might be due to residual confounding by familial

**Table 1.** Characteristics of the Stockholm Youth Cohort, born 1984–2007, shown for each sub-cohort in the current analysis

	Full cohort (333 057)	Parental BMI cohort (220 371)	Full sibling cohort (114 223)	Matched sibling cohort (4775)	GWG cohort (113 822)
Prevalence, % (ASD with ID, %/ASD without ID, %)					
Full cohort	1.9% (0.5%/1.4%)	1.9% (0.4%/1.5%)	2.0% (0.5%/1.5%)	45% (11%/34%)	1.7% (0.4%/1.3%)
Prevalence by birth cohort, % (ASD with ID, %/ASD without ID, %)					
Born 1984–89 (22–27 years old)	1.5% (0.5%/1.0%)	1.4% (0.4%/1.0%)	1.5% (0.5%/1.0%)	–	1.5% (0.4%/1.1%)
Born 1992–96 (15–19 years old)	2.5% (0.7%/1.8%)	2.5% (0.6%/1.9%)	2.5% (0.7%/1.8%)	–	2.4% (0.6%/1.8%)
Born 1997–2001 (10–14 years old)	2.5% (0.6%/1.9%)	2.5% (0.4%/2.1%)	2.6% (0.7%/1.9%)	–	2.6% (0.5%/2.1%)
Born 2002–07 (4–9 years old)	1.3% (0.3%/1.0%)	1.2% (0.2%/1.0%)	1.4% (0.3%/1.1%)	–	1.5% (0.4%/1.1%)
Maternal BMI, mean (SD)	23.2 (3.8)	23.0 (3.6)	25.1 (3.5)	25.8 (4.2)	22.6 (3.6)
Paternal BMI, mean (SD)	21.5 (2.5) <sup>a</sup>	21.5 (2.5)	21.7 (2.6) <sup>a</sup>	21.6 (2.7) <sup>a</sup>	21.4 (2.5) <sup>a</sup>
Gestational weight gain (GWG), mean (SD)	13.9 (4.9) <sup>a</sup>	14.0 (4.8) <sup>a</sup>	13.9 (5.3) <sup>a</sup>	13.9 (5.5) <sup>a</sup>	13.9 (4.9)
Male, %	51.2%	51.3%	51.4%	60.1%	51.1%
Maternal age, mean (SD)	29.9 (5.1)	29.9 (4.9)	30.0 (4.9)	30.0 (5.1)	29.1 (5.2)
Paternal age, mean (SD)	32.8 (6.2)	31.9 (5.3)	32.9 (6.0)	33.0 (6.2)	32.0 (6.3)
Primiparous, %	45.4%	47.8%	37.8%	34.3%	47.3%
Mothers born outside Sweden, %	24.0%	10.5%	25.1%	23.9%	22.1%
Maternal history of inpatient psychiatric care, %	3.1%	3.0%	2.5%	4.1%	2.8%
Paternal history of inpatient psychiatric care, %	2.8%	2.5%	2.4%	3.4%	2.5%
Parents with >12 years' schooling, %	53.1%	56.0%	52.7%	49.5%	49.1%
Parental income quintile 1 (lowest), %	13.8%	7.2%	13.7%	14.4%	15.9%
Gestational week at first antenatal visit, mean (SD)	11.4 (4.5) <sup>a</sup>	11.2 (4.2) <sup>a</sup>	11.3 (4.1) <sup>a</sup>	11.2 (4.0) <sup>a</sup>	11.5 (4.6) <sup>a</sup>

<sup>a</sup>Indicates that data were not available for all members of cohort.

factors. Matched sibling comparisons were carried out using conditional logistic regression models, grouped on maternal identification number, and adjusted for sex, birth year, sibling birth order and maternal and paternal age at time of birth. Informed from earlier models, we parameterized maternal BMI as a continuous variable for BMI values  $\geq 21$ , in addition to categorization.

### Sensitivity analyses

Given differences in ASD risk factors (such as income distribution and maternal migration status) between those with a paternal BMI measurement and those without (see [Table 1](#)), we repeated the analysis of maternal BMI in the full cohort with maternal baseline BMI measures. Gestational week at first antenatal visit might influence both maternal weight and offspring health; therefore, we repeated analysis of maternal BMI including gestational week at the time of the first visit. To explore residual confounding by parental psychiatric illness, we examined a more inclusive indicator of parental psychiatric service use including both inpatient and outpatient psychiatric history. To examine cohort effects, we stratified the sample on the median birth year (1997). For paternal BMI measures, sensitivity analyses included additional adjustment for

paternal IQ measured at the time of conscription, adjustment for any parental psychiatric service use (inpatient or outpatient), and stratification on the median birth year (1997).

## Results

### Study sample

The final sample included 333 057 individuals, born to 176 850 mothers; 6420 offspring had an ASD.

Characteristics of each sub-cohort in this analysis are presented in [Table 1](#). Compared with children with maternal BMI data but lacking paternal BMI data, children with paternal BMI data were more likely to be born to a mother who was born in Sweden and less likely to have low socioeconomic status ([Table 1](#)). Otherwise, the sub-cohorts were generally similar, except where differences were expected (e.g. a higher prevalence of ASD and a lower proportion of primiparous mothers in the matched sibling cohort). Mother/child pairs with BMI data were similar to those without BMI data ([Supplementary Table 2](#), available as [Supplementary data](#) at *IJE* online). Mother/child pairs with GWG data were similar to those without GWG data,

except for birth year of the index child (Supplementary Table 3, available as Supplementary data at *IJE* online). GWG data capture varied year-to-year and tended to be better in earlier part of the cohort.

### Maternal BMI and ASD risk

In categorical analysis, risk of ASD was elevated for overweight and obese mothers compared with normal weight mothers (Table 2). In continuous analysis, we observed a dose-response relationship between maternal BMI > 21 and increasing risk for ASD (Figure 3). Adjusting for paternal BMI did not alter the relationship between maternal BMI and ASD (Table 2). Similar risk patterns were observed after stratifying ASD with and without intellectual disability (Figure 3; Table 2). The observed dose-response relationship between maternal BMI > 21 and ASD was unchanged in sensitivity analyses (Supplementary Figure 1, available as Supplementary data at *IJE* online).

Pre-eclampsia, pre-gestational diabetes and gestational diabetes were associated with an increased risk of ASD in the offspring (Supplementary Table 4, available as Supplementary data at *IJE* online), though these relationships were largely attenuated when maternal BMI was included in the adjusted model. Estimates for maternal overweight and obesity were stable in models including maternal metabolic conditions (Supplementary Table 4).

In matched sibling analyses, there was no relationship between maternal BMI > 21 and ASD, nor was there risk associated with maternal overweight or obesity in categorical analyses (Table 3; Supplementary Figure 2, available as Supplementary data at *IJE* online). The mean change in BMI between pregnancies for mothers included in the matched sibling cohort was 0.92 kg/m<sup>2</sup> (5th–95th percentile: –1.1–5.1; see Supplementary Table 5 and Supplementary Figure 3, available as Supplementary data at *IJE* online) and for women in the full sibling cohort was 0.77 kg/m<sup>2</sup> (–1.1–4.3).

### Paternal BMI and ASD risk

Maternal baseline BMI and paternal BMI at age 18 were weakly correlated ( $P = 0.07$ ). Risk of ASD was elevated for underweight and obese fathers (Table 2). In continuous analysis, risk of ASD was increased for fathers with BMI below 20 and for fathers with BMI above 23 (Figure 3). Adjusting for maternal BMI somewhat attenuated the relationships between paternal BMI and ASD (Table 2). Similar risk patterns were observed after stratifying for ASD with and without intellectual disability (Figure 3; Table 2).

The relationship between paternal BMI and ASD was unchanged in sensitivity analyses with the exception that the risk of ASD without ID associated with elevated paternal BMI was only apparent in those born before 1998 (Supplementary Figure 1).

### GWG and ASD risk

GWG was inversely associated with maternal baseline BMI ( $P = -0.06$ ). However, the proportions of overweight (63.7%) and obese (53.8%) women who exceeded guidelines for GWG were greater compared with the proportions of normal weight (28.0%) and underweight (13.2%) women who exceeded guidelines.

Even in models accounting for baseline BMI, both insufficient and excessive GWG were independently associated with ASD (Table 4; Figure 4). The risk pattern was similar, with somewhat higher ORs, when restricted to women with a normal baseline BMI (Table 4; Figure 4). A similar risk pattern for ASD with and without ID was observed in these models (Table 4).

In matched sibling analysis, the ORs for risk of ASD associated with too much GWG among mothers with normal baseline BMI were higher compared with standard analysis (Table 4; Figure 4), although confidence intervals were wide. The mean change in GWG between pregnancies for mothers within the matched sibling cohort was –0.81 kg (5th–95th percentile: –11–8) and for women in the full sibling cohort was –0.51 kg (–8–5).

## Discussion

In population-level analysis, increasing maternal baseline BMI above 21 was associated with greater ASD risk. Surprisingly, the results of the paternal BMI analysis and the sibling analysis suggest that these results are affected by residual confounding and that maternal BMI may be a proxy for other risk factors shared among members of the same family, such as genetics. On the other hand, excess risk of ASD was observed for both too little GWG as well as too much GWG, and these relationships were of consistent magnitude across all analyses.

### Strengths and weaknesses

This is the largest study to date to examine the relationships of maternal BMI and GWG with ASD risk. We used multiple family-based study designs to assess the evidence for causality, as recommended for studies of the effects of developmental overnutrition on offspring health.<sup>6</sup> Case-ascertainment bias was minimized by our validated case-finding approach. The prevalence of ASDs in our cohort is

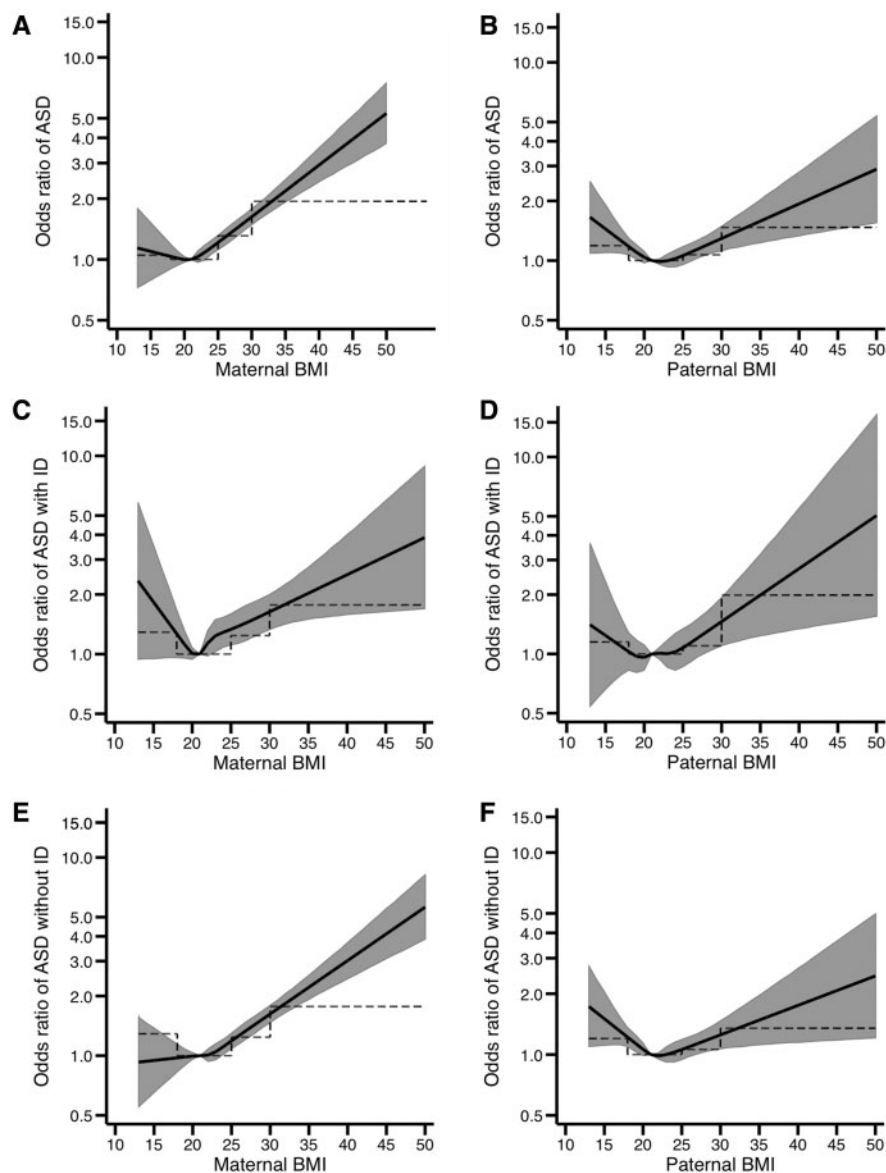
**Table 2.** Odds ratios and 95% confidence intervals for the association between parental BMI and autism spectrum disorders (all ASD, and with or without comorbid intellectual disability) for children born in Sweden 1984–2007 with BMI data available for both parents (220 317 from 148 296 mothers)

	Maternal BMI at first antenatal visit			Paternal BMI at conscription (age ~18)				
	No. cases/non-cases	OR <sup>a</sup>	Adjusted OR <sup>b</sup>	Mutually adjusted OR <sup>c</sup>	No. cases/non-cases	OR <sup>a</sup>	Adjusted OR <sup>b</sup>	Mutually adjusted OR <sup>c</sup>
Normal (BMI ≥ 18.5 & < 25), reference category								
ASD	2755/159 480	-	-	-	3381/182 393	-	-	-
ASD with ID	548/159 480	-	-	-	660/182 393	-	-	-
ASD without ID	2207/159 480	-	-	-	2721/182 393	-	-	-
Underweight (BMI < 18.5)								
ASD	165/9024	1.10 (0.94–1.29)	1.05 (0.90–1.24)	1.05 (0.90–1.24)	382/16 557	1.23 (1.10–1.38)	1.20 (1.08–1.34)	1.19 (1.06–1.33)
ASD with ID	43/9024	1.33 (0.97–1.82)	1.28 (0.94–1.76)	1.29 (0.94–1.76)	74/16 557	1.19 (0.93–1.52)	1.16 (0.90–1.48)	1.15 (0.90–1.47)
ASD without ID	122/9024	1.04 (0.86–1.25)	0.99 (0.82–1.19)	0.99 (0.82, 1.19)	308/16 557	1.25 (1.10–1.41)	1.21 (1.07–1.37)	1.20 (1.06–1.36)
Overweight (BMI ≥ 25 & < 30)								
ASD	864/37 227	1.34 (1.24–1.45)	1.31 (1.21–1.42)	1.31 (1.21–1.41)	334/15 429	1.18 (1.05–1.33)	1.13 (1.00–1.27)	1.07 (0.95–1.20)
ASD with ID	159/37 227	1.29 (1.08–1.55)	1.25 (1.04–1.50)	1.24 (1.03–1.48)	66/15 429	1.20 (0.93–1.56)	1.15 (0.89–1.49)	1.10 (0.85–1.43)
ASD without ID	705/37 227	1.35 (1.24–1.48)	1.33 (1.22–1.45)	1.32 (1.21–1.45)	268/15 429	1.18 (1.04–1.35)	1.12 (0.9–1.28)	1.06 (0.93–1.21)
Obese (BMI ≥ 30)								
ASD	375/10 481	2.07 (1.85–2.32)	1.97 (1.76–2.21)	1.94 (1.72–2.17)	62/1833	1.90 (1.46–2.48)	1.69 (1.30–2.20)	1.47 (1.12–1.92)
ASD with ID	65/10 481	1.97 (1.51–2.55)	1.83 (1.40–2.38)	1.77 (1.35–2.31)	15/1833	2.49 (1.48–4.19)	2.23 (1.33–3.78)	1.99 (1.18–3.36)
ASD without ID	310/10 481	2.11 (1.86–2.39)	2.01 (1.77–2.28)	1.98 (1.74–2.25)	47/1833	1.77 (1.31–2.40)	1.57 (1.16–2.12)	1.35 (1.0–1.83)

<sup>a</sup>GEE model including maternal or paternal BMI as appropriate, adjusted only for sex and birth year of the child.

<sup>b</sup>GEE model including maternal or paternal BMI as appropriate, adjusted for sex, birth year of the child, parity, maternal age at the time of birth, paternal age at the time of birth, maternal country of birth, SES factors and parental history of psychiatric treatment.

<sup>c</sup>GEE model including both maternal BMI and paternal BMI, and covariates from the previous model.



**Figure 3.** Risk of ASD in relation to maternal baseline BMI and paternal BMI (age 18). The solid line indicates odds ratios estimated using a restricted cubic spline model with five knots. BMI of 21 was used as the referent value. The 95% confidence interval is represented as grey bands. The model was adjusted for sex, birth year of the child, parity, maternal age, paternal age, maternal country of birth, SES factors and parental history of psychiatric treatment. OR estimates from a categorical model, similarly adjusted, are shown for comparison (dotted line; see Table 2). Results are shown for all ASD cases (A, B), for ASD cases with ID (C, D), and for ASD cases without ID (E, F).

higher compared with ~1–1.5% prevalence found elsewhere,<sup>23,24</sup> though our recent analysis found that the prevalence of ASD in the SYC is highly comparable to prevalences reported by population monitoring programmes when comparing similar birth cohorts.<sup>10</sup> We observed comparable relationships between maternal BMI and risk of ASD when comparing children born earlier and later in the cohort in our sensitivity analyses, suggesting that the changing prevalence over time does not explain our results.

Weaknesses include the limited availability of parental BMI and GWG data, potentially limiting the

generalizability of the study. However, there were no notable differences between women with and without BMI data in the eligible population. This pattern of missing data is not likely to be due to differences in antenatal care-seeking patterns, as fewer than 5% of women in Sweden register at an antenatal clinic after 15 weeks or have fewer than three visits before delivery.<sup>25</sup> There were no notable differences, other than year of birth of the index child, between mother/child pairs with GWG data and those without. Although fathers with BMI data (i.e. conscripted) were less likely to be immigrants and less likely to have a child with an immigrant than men not conscripted, these



**Table 3.** Odds ratios and 95% confidence intervals for the association between maternal BMI and autism spectrum disorders in the full sibling (114 223 children born to 52 714 mothers) and matched sibling (4775 children born to 2066 mothers) cohorts, born in Sweden 1984–2007

	Full siblings <sup>a</sup>			Matched siblings <sup>b</sup>		
	OR, 1 unit increase in maternal BMI <sup>c</sup>	OR, overweight vs normal <sup>d</sup>	OR, obese vs normal <sup>d</sup>	OR, 1 unit increase in maternal BMI <sup>c</sup>	OR, overweight vs normal <sup>d</sup>	OR, obese vs normal <sup>d</sup>
ASD	1.05 (1.04 – 1.07)	1.24 (1.14 – 1.36)	1.80 (1.59 – 2.04)	0.99 (0.95 – 1.03)	1.03 (0.84 – 1.25)	1.06 (0.75 – 1.50)
ASD with ID	1.06 (1.03 – 1.08)	1.15 (0.96 – 1.37)	1.76 (1.38 – 2.23)	0.96 (0.89 – 1.04)	0.72 (0.49 – 1.07)	0.78 (0.40 – 1.53)
ASD without ID	1.05 (1.04 – 1.07)	1.28 (1.16 – 1.42)	1.83 (1.59 – 2.11)	0.99 (0.95 – 1.04)	1.13 (0.90 – 1.43)	1.15 (0.76 – 1.73)

<sup>a</sup>Cohort of full siblings included in the study population, evaluated using GEE models adjusted for sex, birth year of the child, sibling birth order, maternal age at the time of birth, paternal age at the time of birth, maternal country of birth, SES factors and parental history of psychiatric treatment. Results of the matched sibling comparison analyses are compared with results for all 114 223 full siblings in the cohort (those families that could potentially contribute to a matched sibling analysis) in order to guard against bias possibly introduced by excluding single children and half-siblings.

<sup>b</sup>Cohort of ASD cases and their unaffected siblings, evaluated using conditional logistic regression models adjusted for sex, birth year of the child, sibling birth order, maternal age at the time of birth, paternal age at the time of birth.

<sup>c</sup>Models investigating maternal BMI  $\geq 21$ , parameterized as a continuous variable.

<sup>d</sup>Models investigating maternal BMI, categorized according to WHO standards (Overweight: BMI  $\geq 25$  &  $<30$ ; Obese: BMI  $\geq 30$ ) compared with normal maternal BMI (BMI  $\geq 18.5$  &  $<25$ ) as the referent.

social factors were included in all adjusted models and not likely to influence internal validity of findings.

Another weakness of our study is that we have not considered developmental outcomes other than ASD. Several studies have found an inverse relationship between maternal BMI and children's cognitive development,<sup>26–28</sup> and one study showed that risk associated with maternal obesity was slightly higher for non-ASD developmental delays as for ASDs.<sup>3</sup>

We used maternal weight measured at the first antenatal visit as a proxy measure of pre-pregnancy BMI. Weight gain within the first trimester is on average low (0–2 kg).<sup>29,30</sup> Additionally, the proportions of overweight and obesity within our study population agree well with reports among Swedish women in the general population during the same time period.<sup>12</sup> Taken together, this indicates that BMI at first antenatal visit is a reasonable proxy for maternal BMI at the start of pregnancy.

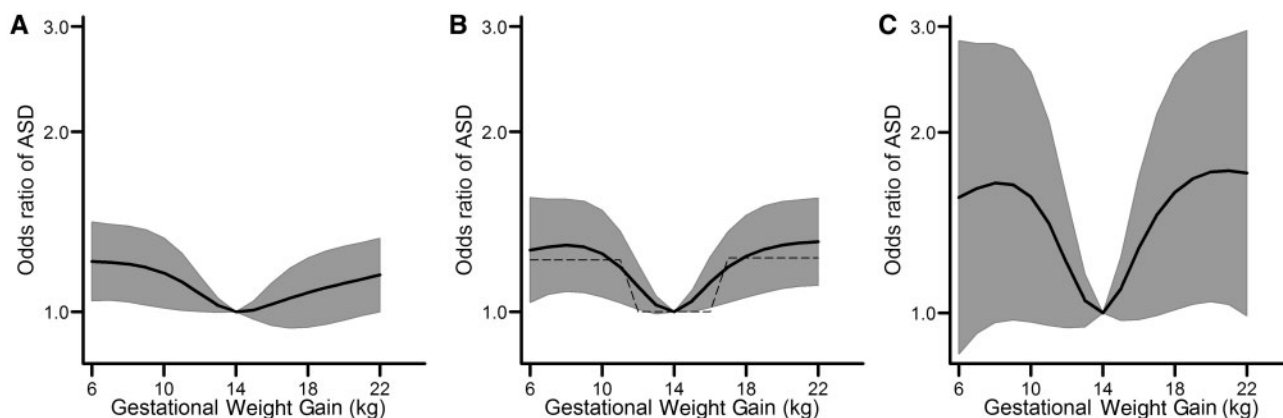
We used paternal BMI data collected at the time of conscription to the Swedish military to determine whether maternal BMI associations with ASD were independent of other familial factors.<sup>7,8</sup> Follow-up data on paternal BMI proximal to the birth of the index child were not available. On average, about 15 years elapsed between the time of paternal BMI measurements and the birth of the index child. However, paternal conscription BMI data are of high quality.<sup>31</sup> These data also have the advantage that, in testing the hypothesis that genetic factors may explain some risk attributable to parental BMI, some potential confounding in modelling the relationship between paternal BMI and ASD risk was avoided, given that both BMI and offspring risk of ASD increase with parental age.<sup>18,32,33</sup>

Although the use of sibling comparison design can account for unmeasured familial confounders that standard

adjustment may miss, there are limitations to this study design.<sup>34</sup> Sibling comparison estimates, although less susceptible to shared confounding, are more severely biased by non-shared confounders than standard comparisons. Only those mother/child groups with a change in the exposure of interest between pregnancies will contribute to the risk estimate, and thus it is possible that there are residual non-shared confounders amongst offspring of women who substantially modify their weight between pregnancies. Use of a sibling comparison design by default limits the population included in the analysis, possibly affecting the power of the study. In our study, the power may be particularly limited for the categorical analyses for which only discordant matched sibling sets can contribute to the risk estimate; the continuous analyses are better powered and more reliable. It is also important to remember that ASDs are spectrum disorders and that subclinical autistic traits are more common among siblings of affected children.<sup>35</sup> Such similarities between affected and unaffected siblings would be expected to attenuate within-family associations.

### Comparison with previous studies

Previous studies have reported an association between maternal pre-pregnancy obesity and offspring ASD risk.<sup>2,3</sup> Dodds *et al.* compared mothers who weighed  $\geq 90$  kg at start of pregnancy with those who weighed less, in a population-based cohort study.<sup>2</sup> Krakowiak *et al.* reported an association between maternal obesity, in the presence or absence of three other metabolic conditions, and ASD, in a population-based case-control study.<sup>3</sup> Lyall *et al.* reported an association between maternal obesity at age 18 and ASD risk, but not with maternal BMI more proximal to



**Figure 4.** Risk of offspring ASD associated with maternal gestational weight gain. The solid line indicates odds ratios estimated using a restricted cubic spline model with five knots. A gestational weight gain of 14 kg was used as the referent value. The 95% confidence interval is represented as grey bands. Due to the relatively small sample size for spline analysis, results are shown for weight gain between 7 and 22 kg, representing the 5th to 95th percentiles of the population distribution. (A) Association of maternal GWG with ASD risk among 113 469 mother/child pairs, including adjustment for maternal baseline BMI category. (B) Association of maternal GWG with ASD risk among 84 655 mother/child pairs with normal baseline BMI. OR estimates from a categorical model, similarly adjusted, are shown for comparison (dotted line; see Table 4). (C) Association of maternal GWG with ASD risk among 1156 children in the matched sibling comparison cohort.

the birth of the index child, in the National Nurses Health Study II.<sup>36</sup> Among these studies, the risk attributable to maternal obesity is similar (on the order of 1.5–2 fold), and these agree with the results of our traditional analysis of maternal obesity.

On the other hand, Bilder *et al.* reported no association between maternal BMI in a population-based cohort or within a separate sibling comparison cohort.<sup>4</sup> However, these were relatively small cohorts (128 and 228 cases, respectively), where adequate power may be lacking particularly for continuous analysis of BMI. Outside this study,<sup>4</sup> no other study has previously employed a sibling comparison design to evaluate ASD risks associated with maternal BMI.

Surén *et al.* reported an association between maternal obesity and certain ASDs in a Norwegian cohort of the same order as the aforementioned studies, although this association was attenuated after adjustment for paternal BMI.<sup>5</sup> The study may be limited in terms of the ability to detect an association by use of self-reported height and weight measures for both parents, use of ASD subtype diagnoses which have shown poor clinical reliability,<sup>37</sup> and substantial case under-ascertainment. ASDs were diagnosed in only 0.45% of children (compared with ~1–1.5% prevalence found elsewhere<sup>23,24</sup>).

In line with previous reports,<sup>3,38</sup> we note that some maternal metabolic conditions were associated with offspring ASD risk. However, these associations were largely attenuated when maternal BMI was also considered in the model. The focus of our analysis is on the exploration of the relationship between maternal baseline BMI and GWG with offspring ASD risk. Elevated BMI is a core feature defining the metabolic syndrome and is strongly associated with

development of metabolic pregnancy complications such as pre-eclampsia and gestational diabetes.<sup>39–41</sup> Studying the full range of BMI and GWG values among mothers might better capture undiagnosed or subclinical metabolic dysfunction if an underlying metabolic dysfunction were driving the risk.

Consistent with previous reports,<sup>24</sup> we found that excess maternal GWG was associated with increased ASD risk. We observed a similar risk pattern in our sibling comparison cohort with regard to excessive weight gain. The risk attributable to every 2.3 kg (5 lb) of weight gained during pregnancy was smaller in our study compared with Bilder *et al.* who reported a 17% increased odds for every 2.3 kg (5 lb) of weight gain. A novel finding here is evidence of elevated risk associated with too little weight gain. Whereas the confidence intervals in the sibling comparison were wide, the ORs were of similar magnitude in both study designs, providing the first evidence that maternal undernutrition during the time of pregnancy may also contribute to ASD risk.

### Interpretation and potential mechanisms

Previous studies have posited intra-uterine mechanisms mediated by circulating signalling molecules produced by maternal adipose tissue, such as leptin, sex hormones or pro-inflammatory cytokines, to explain the association between adiposity and ASD risk. The mechanisms underlying potential neurodevelopmental effects of maternal pre-pregnancy obesity and GWG need not be the same, and further investigation into such mechanisms is warranted. However, each of the proposed signalling imbalances would be more strongly influenced by the existing

**Table 4.** Odds ratios and 95% confidence intervals for the association between gestational weight gain (GWG) categories and ASD risk for children born in Sweden 1984–2007. Gestational weight gain categories were set using guidelines provided by the US Institute of Medicine and were conditioned on maternal baseline BMI

	Ideal GWG (reference) No. cases/ non-cases	Insufficient GWG No. cases/ non-cases	OR	Excessive GWG No. cases/ non-cases	OR	Every 2.3 kg (5 lb) increase <sup>d</sup> OR
All mother/child pairs <sup>a</sup>						
ASD	737/47 250	502/27 331	1.17 (1.04 – 1.31)	708/37 314	1.12 (1.01 – 1.25)	1.03 (1.00 – 1.06)
ASD with ID	195/47 250	139/27 331	1.19 (0.96 – 1.48)	182/37 314	1.16 (0.93 – 1.43)	
ASD without ID	542/47 250	363/27 311	1.16 (1.01 – 1.32)	526/37 314	1.11 (0.98 – 1.26)	
All mother/child pairs with normal maternal baseline BMI <sup>b</sup>						
ASD	139/37 111	118/22 917	1.22 (1.07 – 1.40)	417/23 284	1.23 (1.08 – 1.40)	1.05 (1.02 – 1.09)
ASD with ID	527/37 111	409/22 917	1.31 (1.02 – 1.68)	108/23 284	1.24 (0.96 – 1.60)	
ASD without ID	388/37 111	291/22 917	1.19 (1.02 – 1.39)	309/23 284	1.22 (1.05 – 1.42)	
Sibling cohort <sup>c</sup>						
ASD	167/225	106/136	1.21 (0.81 – 1.82)	239/283	1.22 (0.85 – 1.76)	1.04 (0.93 – 1.16)
Sibling cohort with normal maternal baseline BMI						
ASD	113/160	83/100	1.12 (0.68 – 1.84)	114/123	1.48 (0.93 – 2.38)	1.09 (0.90 – 1.31)

<sup>a</sup>Analysis conducted in all mother/child pairs with gestational weight gain data (113 469 children born to 96 390 mothers). GEE models, clustered on maternal ID, were adjusted for maternal BMI, gestational age at birth, sex, birth year of the child, parity, maternal age, paternal age, maternal country of birth, SES factors and parental history of psychiatric treatment.

<sup>b</sup>Analysis restricted to mother/child pairs with normal baseline BMI values ( $18.5 \leq \text{BMI} < 25$ ; 84 655 children born to 73 501 mothers).

<sup>c</sup>Analysis conducted in matched sibling pairs (1156 children born to 550 mothers). Conditional logistic regression models were adjusted for sex, birth year of the child, maternal age, paternal age and sibling birth order.

<sup>d</sup>To allow comparison with a previous report,<sup>4</sup> we calculated the risk of any ASD associated with every 5 lb (2.27 kg) of weight gained among women who met at least minimum GWG recommendations (i.e. those in the Ideal or Excessive GWG categories).

maternal adipose mass compared with the adipose tissue gained during the course of pregnancy,<sup>42–44</sup> and are thus unlikely to fully explain the findings of this study regarding maternal baseline BMI.

The sibling analyses suggest that shared familial factors, such as socioeconomic status or genetic background, confound the association between maternal BMI and ASD risk at the population level. Given the quality of socioeconomic data, the small impact that inclusion of these factors made on risk estimates and the consistent results of the sensitivity analyses, these factors are not likely to explain the difference in results between the traditional analysis and the sibling analysis of maternal BMI. Both the sibling study and the study of paternal BMI suggest that confounding by genetic background potentially explains some of the risk attributable to maternal BMI in the traditional analysis. Genome-wide association studies (GWAS) implicate genetic variation in a number of neurogenesis and neuronal differentiation pathways as determinants of BMI.<sup>45</sup> Some,<sup>46,47</sup> but not all,<sup>48</sup> studies have reported increased prevalence of overweight and obesity among children and adolescents with ASD compared with unaffected peers. Overlap in genetic determinants of BMI with genetic risk factors for ASD should be specifically examined in future studies. Additional mechanisms may exist to explain the perplexing difference in the results of the

traditional analysis compared with the sibling analysis. For example, maternal BMI and diet during pregnancy affect the epigenome of the offspring.<sup>49–52</sup> Differences in DNA methylation patterns have been detected between ASD cases and controls.<sup>53,54</sup> Since many epigenetic markers are dependent on the underlying genetic code,<sup>55</sup> risks mediated by such a mechanism could be difficult to detect using a matched sibling design. Finally, it is possible that if the relationship between maternal BMI and ASD risk were mediated by a molecular factor that is responsive to an obesogenic environment, and thus correlated to maternal BMI in general, but was also influenced by genetic factors, and thus much more strongly correlated within a woman over time compared with differences between women, that we might observe a similar attenuation of the results of the traditional analysis in the matched sibling analysis. The role of such mediating factors in sibling analyses remains to be explored.

The association of offspring ASD with paternal BMI at age 18 also suggests the influence of genetic factors in terms of the association of parental BMI and ASD status. Elevated BMI in males has been associated with increased DNA fragmentation,<sup>56,57</sup> thus leading to the possibility that obese fathers may be more likely to pass on *de novo* mutations that confer risk for ASD.<sup>58</sup> Evidence from animal and human studies shows that paternal diet, BMI and

preconceptional stress experiences can also affect the methylation pattern of the offspring epigenome.<sup>50,59–61</sup>

This is the largest study to date exploring the relationship of maternal BMI, paternal BMI and GWG with risk of offspring ASD. Our results indicate that maternal weight gain during pregnancy is consequential for offspring ASD risk. However, the underlying mechanisms connecting GWG with ASD remain unclear, so that specific public health recommendations beyond focusing on healthy GWG are not yet possible. Our results also indicate that maternal and paternal BMI may also be proxy markers for other familial risk factors, including potentially a shared genetic risk. In order to move forward and understand the mechanisms underlying the associations between parental BMI, GWG and offspring ASD risk, future studies employing analysis of biological samples for nutritional, genetic and epigenetic markers, as well as including measures of parental BMI and GWG, are necessary.

## Supplementary Data

Supplementary data are available at *IJE* online.

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## Author contributions

R.G., C.D. and B.K.L. had the research idea, and D.R., C.M., T.F., H.K. and S.I. helped with its development. R.G. conducted the statistical analysis and wrote the first and subsequent drafts of the paper with important intellectual input from all co-authors. All authors had full access to the data and the statistical reports and tables arising from the data analysis, and take responsibility for the integrity of the data and accuracy of the data analysis. All authors have approved the final version of the manuscript submitted for publication. R.G. and C.D. act as guarantors, and assure that this manuscript is an honest, accurate and transparent account of the analysis undertaken.

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