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Title

Maternal pre-pregnancy weight and externalizing behavior problems in preschool children: a UK-based twin study

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Keywords

Maternal pre-pregnancy weight, twins, intrauterine environment, externalizing behavior

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Abstract

Maternal pre-pregnancy weight and externalizing problems 2

33 Background: Preschool years are a critical period for the emergence of many clinically
34 significant problem behaviors such as externalizing and internalizing disorders. Twin
35 studies have shown a substantial genetic influence and it still remains unclear what the
36 role of the intrauterine factors, such as maternal pre-pregnancy overweight, is on child
37 behavior problems. Methods: The mothers of 886 twins answered questions on pre-
38 pregnancy weight and their children's internalizing and externalizing problems. The
39 heritability of behavior problems and the association with maternal pre-pregnancy
40 weight was investigated in genetic component analysis and multivariate regressions.
41 Results: The genetic analysis suggested that genetic and common environmental factors
42 account for most of the variation in externalizing disorders; common and non-shared
43 environment explain most of the variation in internalizing disorders. Moreover, the
44 regression analysis results suggested that children of overweight mothers showed a
45 trend towards being more aggressive and exhibit externalizing behaviors compared to
46 children of normal weight mothers. Conclusion: Maternal pre-pregnancy weight may
47 play a role in children's aggressive behavior.

48
49 Strengths and limitations of this study

- 50 • In the twin genetic design maternal pre-pregnancy weight was included in the
51 genetic model, in order to investigate the influence of the intrauterine
52 environment on children's behaviour problems.
- 53 • Cut-off scores applied to the child behavior scale enabled us to clinically assess
54 the observed association.
- 55 • A number of covariates of post-natal influence were adjusted such as maternal
56 educational level and smoking after pregnancy.
- 57 • Information on other parental characteristics such as maternal psychopathology
58 and personality was not available.

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60 Keywords

61 Intrauterine environment, twins, externalizing, pre-pregnancy weight, heritability

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63 Word count: 3,155

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65 Introduction

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67 A child's preschool years are generally considered important since during this period
68 the critical emergence of many clinically significant problem behaviors occur¹.
69 Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing
70 problems (e.g., emotional problems, anxiety, depression) can often first be identified in
71 early childhood which then show considerable stability across older ages²⁻⁴. Toddlers
72 with problem behaviors are at risk for a variety of adverse developmental outcomes
73 including conflictual relationships with other peers or family, poor academic
74 performance, delinquency and later maladjustment⁵⁻⁷. Given the life course
75 implications of early onset symptomatology, it is essential to understand the underlying
76 aetiology of problem behaviors in preschool children.

77 Twin studies investigating externalizing and internalizing problems have revealed
78 substantial genetic influence, with heritabilities ranging from 40-70% in these age
79 groups⁸⁻¹⁰. The influence of the shared environment is however, more modest
80 explaining up to 40% of the variance in behavior problems⁸⁻¹¹.

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82 Since the 1990s there has been an increased interest into the research of the effect of the
83 intrauterine environmental and maternal well-being during pregnancy on later child
84 development. Epidemiological studies suggest strong links between measures of the
85 quality of the prenatal environment and the risk of cardiovascular and metabolic
86 diseases^{12 13} and more research evidence suggests that low birth weight can be linked
87 with impaired cognitive development and behavioural disorders, especially
88 hyperactivity/inattention^{14 15} while several studies have examined the association
89 between low birth weight and internalizing and externalizing behaviours¹⁶⁻¹⁸.

90 Research findings^{19 20} suggest that another intrauterine factor, maternal pre-pregnancy
91 obesity/weight is associated with reduced cognitive abilities, symptoms of inattention
92 and negative emotionality in school aged children¹⁹. These findings are of clinical
93 importance especially in the light of the increasing prevalence of obese women entering
94 pregnancy²¹. Pregnancy comes with main changes in the maternal body and a high pre-
95 pregnancy weight is more likely to make these adaptations even more difficult affecting
96 child development. With the exception of one recent study²² suggesting that fetal

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4 97 exposure to increased maternal body mass index (BMI) is associated with elevated
5 98 levels of externalizing problems in two-year old children, not much is known about the
6 99 influence of maternal pre-pregnancy weight on externalizing and internalizing
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9 100 behaviour problems in young age groups after that age and before the year of five. By
10 101 implementing a twin research design including maternal pre-pregnancy weight in the
11 102 genetic model, we set out to investigate the role of the intrauterine, common and genetic
12 103 environment on children's behaviour problems.
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16 104 17 105 Methods 18 19 106

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21 107 The Twins and Multiple Births Association Heritability Study (TAMBAHS) is a
22 108 volunteer-based study, investigating the development of twins from birth until 5 years
23 109 of age. Twins aged between 18 months and five years old at the time of the survey were
24 110 identified. In the time period between July 2008 and May 2010, 443 mothers of twins
25 111 completed the study's online questionnaire on their twins' emotional and behavioral
26 112 development. The study was approved by the ethics committee of the University of
27 113 Birmingham.
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33 114 34 115 Zygosity determination

35 116 For the determination of the twins' zygosity the previously adapted version of
36 117 Goldsmith's zygosity questionnaire was used²³. This questionnaire method of assigning
37 118 zygosity has been validated against determination by identity of polymorphic DNA
38 119 markers and has reached accuracy in verifying zygosity in 95% of the cases²⁴.
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42 120 Twin Sample

43 121 In total 443 twin pairs were included in the analyses; 186 monozygotic (MZ) male
44 122 twins, 138 monozygotic (MZ) female twins, 144 dizygotic (DZ) male twins, 158
45 123 dizygotic (DZ) female twins and 260 opposite-sex twins.
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50 124 51 125 Materials

52 126 Maternal pre-pregnancy weight and covariates

53 127 The primary exposure variable was maternal pre-pregnancy BMI, which was calculated
54 128 as the weight (kg)/ height (m²). Pre-pregnancy BMI was analysed both as a continuous
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4 129 and as a categorical variable and was classified according to the WHO standard
5 130 guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg),
6 131 overweight/obese (\geq 25kg).

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9 132 Gestational age (measured in completed weeks of gestation), educational level and
10 133 smoking (before, during, after pregnancy) were noted for mothers; age, sex and birth
11 134 weight were noted for all twins.

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15 136 The Child Behavior Checklist (CBCL/1½y-5y)

16 137 The Child Behavior Checklist for toddlers (CBCL/1½-5) ²⁵ is used to obtain
17 138 standardised parent reports of children's problem behaviors. It contains ninety-nine
18 139 problem items, split into seven subcategories: emotionally reactive, anxious/depressed,
19 140 somatic complaints, withdrawn, sleep problems, attention problems, and aggressive
20 141 behavior originally derived by factor analyses ²⁵. The broadband scale 'Internalizing' is
21 142 the sum score of items in the first four syndrome scales, whereas 'Externalizing' is the
22 143 sum score of attention problems and aggressive behaviour. 'Total problems' is the sum
23 144 score of all ninety-nine problem items. Each item is scored 0 = not true, 1 = somewhat
24 145 or sometimes true, and 2 = very true or often true, based on the preceding 2 months.
25 146 Good reliability and validity criteria have been reported for this checklist ²⁵. To identify
26 147 children that may be above the normal range for the syndrome scales, children were
27 148 categorized as being in the normal range when their T scores were below 65 (or the 93rd
28 149 percentile) and as being in the borderline/clinical range when T scores \geq 65 (or the 97th
29 150 percentile) ²⁵. For Internalizing, Externalizing, and Total Problem broadband scales the
30 151 cut-off point used for the normal range was a T score <60, and borderline/clinical \geq 60.

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43 153 Statistical analysis

44 154 Correlations

45 155 Intra-pair twin correlations were calculated by using the Pearson's (r) and the
46 156 Spearman's rho coefficient statistics as appropriate to explore the genetic and
47 157 environmental influences.

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4 161 Heritability analysis

5 162 Univariate genetic models were fit to the data in order to estimate the heritability of the
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7 163 problem scales using a maximum likelihood approach implemented in Mx²⁶. The
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9 164 estimates of the heritability are presented with 95% Confidence Intervals (CI) and
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11 165 goodness of fit statistics for several models: a full ACE model, in which the phenotypic
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13 166 variance is explained by genetic (A) common environmental factors (C) and non-shared
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15 167 (E) environmental factors. Reduced models were estimated by removing one of the
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17 168 parameters at a time and re-running the model. The goodness of fit of the reduced
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19 169 models was compared to the full model to assess whether they represented a better
20
21 170 explanation of the data using the likelihood ratio χ^2 test and the Akaike Information
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23 171 Criterion (AIC). The models were assessed by examining the decrease in the fit of the
24
25 172 model; if a parameter could be dropped without a significant decrease in fit then on the
26
27 173 grounds of parsimony the reduced model was accepted as the best fitting model. Models
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29 174 were fit both unadjusted and adjusted for pre-pregnancy maternal weight.

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32 177 Regression analyses

33 178 Effects on a continuous scale

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35 180 Standardized coefficients (Betas) are presented reflecting the change on the
36
37 181 subcategories and the broadband problem scales by change in mother's weight both
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39 182 expressed as standard deviation change.

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41 184 Effects on clinically important behavior problems

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43 186 In order to estimate the effect of the different categories of maternal weight on clinical
44
45 187 problems, logistic regression models were fitted. In these models the intercept of each
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47 188 twin pair was modelled as a function of the population intercept plus the individual
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49 189 contribution of the twin pair. Associations of maternal pre-pregnancy weight and the
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51 190 syndrome and broadband scales were explored both unadjusted and adjusted for twins'
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53 191 sex, age, birth weight, gestational age, maternal smoking (before, during and after
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4 192 pregnancy) and maternal educational level. All analyses were performed in STATA 11
5 193 ²⁷.

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9 195 Results

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11 197 Correlations

12 198 The means for the three broadband scales and the intra-pair twin correlations for MZ
13 199 and DZ twins were calculated (Table 1). For Externalizing problems, the MZ correlation
14 200 was $r=0.89$ and the DZ correlation was $r=0.62$. For Internalizing problems, the MZ and
15 201 DZ correlations were $r=0.81$ and $r=0.56$ respectively. For Total problems, the MZ and
16 202 DZ correlations were $r=0.92$ and $r=0.75$ respectively.
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212 Table 1 Descriptive statistics of problem broadband scales for monozygotic and dizygotic twin pairs

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Problem scales	Monozygotic twin pairs							Dizygotic twin pairs						
	Twin 1			Twin 2				r	Twin 1			Twin 2		
	N	M	SD	N	M	SD			N	M	SD	N	M	SD
Externalizing (range 0-48)	167	12.88	7.72	167	12.83	7.51	0.89	269	12.79	6.61	269	12.66	6.95	0.62
Internalizing (range 0-96)	167	5.94	4.55	167	9.13	6.98	0.81	269	5.49	4.11	269	8.24	5.89	0.56
Total problems (range 0-78)	167	31.58	16.24	167	34.66	18.90	0.92	269	30.45	14.18	269	33.06	16.10	0.75

216 N; Number of twins

217 M; Mean

218 SD; Standard deviation

219 r; Within-twin correlations

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4 224 Heritability analyses
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6 225 Variance estimates of ACE models and sub-models with their 95% Confidence Intervals
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8 226 (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For
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10 227 Externalizing problems, an ACE model was the most parsimonious with genetic factors
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12 228 explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining
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14 229 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95%
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16 230 CI: 10-16%) of the variance. For Internalizing problems a CE model was the most
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18 231 parsimonious model with common environment explaining 51% (95% CI: 44-58%) of
19
20 232 the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the
21
22 233 variance. For Total problems, an ACE model was the most parsimonious one with
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24 234 genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common
25
26 235 environment explaining 61% (95% CI: 49-70%) of the variance and non-shared
27
28 236 environment explaining 13% (95% CI: 10-17%) of the variance.
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30 237 After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the
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32 238 Externalizing problems was an ACE model with genetic factors explaining 50% (95%
33
34 239 CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared
35
36 240 environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the
37
38 241 most parsimonious model was and AE model with genetic factors explaining 59% (95%
39
40 242 CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For
41
42 243 Total problems the most parsimonious model was an ACE model with genetic factors
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44 244 explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and
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46 245 non-shared environment 13% (95% CI: .10-17%) of the variance.
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Maternal pre-pregnancy weight and externalizing problems 10

255 Table 2 Univariate genetic model-fitting for the problem scales presenting full and
 256 nested models

	A (95%CI)	C (95%CI)	E (95%CI)	$\Delta\chi^2$	P	AIC
Externalizing						
ACE*	0.46 (0.33-0.60)	0.42 (0.27-0.54)	0.13 (0.10-0.16)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	23.44	0.00	21.44
CE	(0)	0.74 (0.69-0.78)	0.26 (0.22-0.31)	49.69	0.00	47.69
Internalizing						
ACE	0.23 (0.00-0.48)	0.35 (0.12-0.54)	0.43 (0.35-0.53)	-	-	-
AE	0.60 (0.52-0.67)	(0)	0.40 (0.33-0.48)	8.84	0.00	6.84
CE*	(0)	0.51 (0.44-0.58)	0.49 (0.42-0.56)	3.15	0.07	1.15
Total Problems						
ACE*	0.26 (0.16-0.39)	0.61 (0.49-0.70)	0.13 (0.10-0.17)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	53.64	0.00	51.64
CE	(0)	0.79 (0.76-0.83)	0.21 (0.17-0.24)	22.17	0.00	20.17

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258 * Best fitting model

259 A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence

260 interval; $\Delta\chi^2$, Difference Chi-square; P, statistical significance $p < .05$; AIC, Akaike's

261 Information Criterion

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263 The mean maternal body mass index (BMI), gestational age, age and sex of the twins

264 stratified by zygosity are presented in Table 3. There were no differences in maternal

265 weight between monozygotic and dizygotic twins. However, differences were observed

266 between monozygotic and dizygotic twins with regards to their age and gestational age.

267 Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43,

268 $p < 0.001$) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the

269 monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, $p < 0.001$) compared

270 to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and

271 twin covariates and problems broadband scales are also presented in the same table.

272

273 Table 3 Means, standard deviations and Pearson/Spearman correlations with three syndrome scales for each covariate by zygosity

Maternal Characteristics	MZ twins					DZ twins				
	Mean	SD	r _a	r _b	r _c	Mean	SD	r _a	r _b	r _c
BMI	24.51	4.32	0.07	-0.03	0.02	24.76	5.10	0.10*	-0.04	0.10*
Gestational age (weeks)	35.38	2.49	-0.04	-0.12*	-0.06	36.22	2.71	-0.04	-0.15***	-0.11**
Smoking (no/yes) [^]	n	%	r_a	r_b	r_c	n	%	r_a	r_b	r_c
<i>Before</i>	133/40	76.9/23.1	0.03	-0.02	0.05	255/54	82.5/17.5	0.07	0.04	0.06
<i>During</i>	156/5	96.9/3.1	0.07	0.00	0.06	280/10	96.6/3.4	0.10	-0.03	0.05
<i>After</i>	143/18	88.2/11.2	0.04	0.04	0.10	272/29	90.4/9.6	0.09	0.00	0.06
Educational level										
<i>High School diploma or less</i>	42	25.3				53	18.1			
<i>College/professional education</i>	23	13.9	-0.02	0.04	0.00	51	17.3	-0.00	0.03	-0.03
<i>University</i>	101	60.8				191	64.6			
Twin Characteristics										
Age (years)	3.13	0.96	-0.23***	0.02	-0.15**	2.91	0.95	-0.07	0.11	-0.01
Sex	n	%	-0.05	0.18***	0.07	n	%	-0.08	-0.05	-0.09*
<i>Male</i>	206	57.22				164	26.28			
<i>Female</i>	154	42.78				174	27.88			
<i>Opposite sex</i>	-	-				286	45.84			

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275 [^]Smoking refers to smoking before, during, after pregnancy276 r_a Pearson/Spearman correlation with Externalizing scale; r_b Pearson/Spearman correlation with Internalizing scale; r_c Pearson/Spearman correlation with
277 Total Problems scale

278 *p<.05; **p<.01; ***p<.001

Maternal pre-pregnancy weight and externalizing problems 12

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4 279 Regression analyses

5 280 In the adjusted model there was a significant increase of .08 standard deviations in
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7 281 aggressive behavior with every standard deviation increase in maternal weight ($p=0.02$).

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9 282 The logistic regression analysis partly confirmed these findings. Overweight mothers
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11 283 were 1.10 times more likely to have a child with clinically aggressive behavior when
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13 284 compared to normal weight mothers and 0.78 times more likely when compared to
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15 285 underweight mothers. The individual odds ratio (OR) did not reach statistical
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17 286 significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is observed for children
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19 287 of overweight mothers to show clinically aggressive behavior.

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21 288 Similarly, there was an increase of .09 standard deviations ($p=0.02$) in externalizing
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23 289 problems with every standard deviation increase in maternal weight. An increase of the
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25 290 likelihood of externalizing (OR=1.32, 95% CI: 0.84-2.05) for children with overweight
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27 291 mothers compared to children of normal weight mothers was also apparent. No other
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29 292 statistically significant associations between maternal pre-pregnancy weight and
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31 293 behavior problems were observed.

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Table 4 Logistic and linear regression for children's behavioural problems based on maternal BMI**

	Behavioural problems*	No behavioural problems*	OR	95% CI	Beta	P	OR**	95% CI	Beta	P
Emotional reactivity	N	N			0.03	0.42			0.02	0.58
Normal weight	25	376	1.00	Reference			1.00	Reference		
Underweight	6	82	1.10	0.44-2.77			1.12	0.42-2.96		
Overweight/Obese	20	283	1.06	0.58-1.95			1.05	0.54-2.06		
Anxiety/Depression	N	N			-0.03	0.40			-0.02	0.62
Normal weight	29	372	1.00	Reference			1.00	Reference		
Underweight	5	83	0.77	0.29-2.06			0.86	.31-2.41		
Overweight/Obese	14	289	0.62	0.32-1.20			.65	.32-1.30		
Somatic Complaints	N	N			-0.01	0.11			-0.07	0.07
Normal weight	39	362	1.00	Reference			1.00	Reference		
Underweight	11	77	1.33	0.65-2.70			0.99	0.43-2.26		
Overweight/Obese	30	273	1.02	0.62-1.68			0.99	0.56-1.75		
Withdrawn behaviour	N	N			-0.06	0.09			-0.02	0.58
Normal weight	12	389	1.00	Reference			1.00	Reference		
Underweight	3	85	1.14	0.32-4.14			1.53	0.36-6.61		
Overweight/Obese	14	289	1.57	0.72-3.45			2.66	0.99-7.09		
Attention	N	N			0.06	0.08			0.03	0.39
Normal weight	30	371	1.00	Reference			1.00	Reference		
Underweight	5	83	.74	0.28-1.98			1.17	0.42-3.29		
Overweight/Obese	29	274	1.31	0.77-2.23			1.13	0.60-2.13		
Aggressive behaviour	N	N			0.08	0.02 ^a			0.07	0.07
Normal weight	23	378	1.00	Reference			1.00	Reference		
Underweight	4	84	0.78	0.26-2.32			.87	.28-2.69		
Overweight/Obese	19	284	1.10	0.58-2.06			1.04	.52-2.10		
Sleeping problems	N	N			0.06	0.11			0.03	0.48
Normal weight	14	387	1.00	Reference			1.00	Reference		
Underweight	3	85	0.98	0.27-3.47			0.97	0.20-4.66		

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	Overweight/Obese	11	292	1.04	0.47-2.33			0.92	0.37-2.29		
<i>* Categorized based on children within normal range and borderline/clinical range **Adjusted for gestational age, twins' birth weight, age and sex, mother's educational level, smoking (before, during, after pregnancy) * p<0.05</i>	Externalizing	N	N			0.09	0.02 ^a			0.07	0.08
	Normal weight	46	348	1.00	Reference			1.00	Reference		
	Underweight	6	81	0.56	0.23-1.36			.66	.26-1.66		
	Overweight/Obese	44	253	1.32	0.84-2.05			1.17	.69-1.99		
	Internalizing	N	N			-0.04	0.29			-0.03	0.44
	Normal weight	43	358	1.00	Reference			1.00	Reference		
	Underweight	16	72	1.85	0.99-3.46			1.86	.93-3.73		
	Overweight/Obese	34	269	1.05	0.65-1.70			1.01	.59-1.74		
	Total problems	N	N			0.07	0.05			0.06	0.14
	Normal weight	44	355	1.00	Reference			1.00	Reference		
	Underweight	6	82	0.59	0.24-1.43			0.59	0.23-1.50		
	Overweight/Obese	41	260	1.27	0.81-2.00			1.18	0.70-2.00		

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4 1 Discussion
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7 3 In this study the effect of maternal pre-pregnancy weight on problem behaviors and the
8 influence of genetic and environmental factors on these problems were investigated.
9 4 The heritability analysis suggested that genetic and common environmental factors
10 account for most of the variation in externalizing disorders, while common and non-
11 shared environment explain most of the variation in internalizing disorders. After
12 adjusting for mothers' weight there was a non significant decrease (of 2%) of the
13 variation in the externalizing problems that could be explained by the common
14 environment, suggesting that mothers' weight may play an important role in explaining
15 externalizing problems.
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17 12 In general, the results showed that children of overweight mothers showed a trend
18 towards being more aggressive and exhibit externalizing behaviors compared to
19 children of normal weight mothers. Aggressive behavior is considered an important
20 aspect of externalizing behavior and has developmentally been linked to antisocial
21 behavior^{28 29}. Studies with children focusing on aggression have shown that both
22 observed aggression and parental reports of externalizing behaviors are relatively stable
23 from toddlerhood to five years and beyond^{1 30}, which may highlight the role of genetic
24 influences. Consistent with this are the results of this study, which suggest that genetic
25 factors can explain a large part of the variation in externalizing and internalizing
26 behavior problems.
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28 22 The results from the logistic regression however, do not suggest a strong association
29 between maternal overweight and behavior problems. Moreover, the significantly
30 observed change in the standardized coefficients could not explain the distinction
31 between normal range and borderline/clinical range. Therefore, children appeared to be
32 more aggressive but it is not clear whether this is of clinical significance.
33

34 27 Two studies by Rodriguez and colleagues^{19 20} suggested a link between pre-pregnancy
35 overweight and obesity and symptoms of ADHD in children. In both studies, teacher
36 ratings of the offspring of obese mothers had increased levels of ADHD. However, in
37 the latter study, parent reports of childhood ADHD symptoms and negative
38 emotionality failed to support a link. While this could mean that the effect may be
39 small, in light of the low to moderate associations seen between parent and teacher
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Maternal pre-pregnancy weight and externalizing problems 16

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4 33 ratings of children's ADHD one cannot rule out a link. A recent study by van Lieshout
5 34 and colleagues²² showed that increased maternal pre-pregnancy BMI was associated
6 35 with higher levels of externalizing problems at age 2. However, in that study authors did
7 36 not correct for maternal or paternal psychopathology, or maternal personality traits.
8 37 Similarly in our study we may not be able to strongly establish a causal link between
9 38 pre-pregnancy weight and aggressive/externalizing problems but by applying clinically
10 39 relevant cut-off points in the measurement tool of child behavior we suggest that there
11 40 may be a trend for more aggressive behaviors from children of obese mothers. As it has
12 41 been previously suggested³¹ future studies could benefit from the utilization of
13 42 genetically sensitive designs, in this study we included pre-pregnancy weight in the
14 43 genetic model and showed that there be some intrauterine influence. Indeed, when we
15 44 added pre-pregnancy weight in the genetic model we observed a non-significant
16 45 decrease in the variation of the externalizing problems explained by the common
17 46 environment. This suggests that pre-pregnancy weight as an intrauterine factor may play
18 47 an important role in the development of externalizing problems.

19 48 A number of mechanisms could be responsible for the links between maternal obesity
20 49 and neurodevelopmental problems in young children. The potential role of nutrition on
21 50 the development of the brain has been investigated in the past. Although there is not a
22 51 clear pathway that links externalizing problems to maternal overweight, several
23 52 pathways have been proposed to explain this association and parallels between
24 53 overweight and other developmental disorders can be drawn. Leptin, which is the
25 54 protein produced by adipose cells, has been found to play multiple functions in
26 55 reproduction^{32 33}, glucose homeostasis^{34 35} as well as in brain^{36 37} and neurocognitive
27 56 development³⁸. Another possible causal pathway suggests that pre-pregnancy
28 57 overweight women may not be able to synthesize vitamin D, due to the excessive
29 58 adipose tissue, which results in deficiency in both the mother and the neonate; in turn,
30 59 vitamin D is associated with neurocognitive function³⁹. In addition, high glucose levels
31 60 pose a risk for neurobehavioral impairments⁴⁰. Moreover, epigenetic mechanisms
32 61 affecting the central nervous system dopamine signaling could be a mechanism by
33 62 which exposure to elevated maternal BMI during pregnancy might increase levels of
34 63 externalizing behavior in children²².

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4 64 However, others have emphasized the importance of the dynamics within the family
5 environment, such as the parent-child interaction and the individual differences in
6 parenting^{41 42} and their impact on the course of the developmental pathways of problem
7 behaviors. Previous research has mainly linked externalizing problems with family
8 adversity, maternal depression and low socioeconomic status^{34 41 43}.

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69 In addition, there may be other environmental factors, which weren't possible to be
70 examined in this study that could accentuate these symptoms. For instance, parents with
71 children displaying symptoms of aggressive behaviors normally report higher levels of
72 stress and frequent use of negative parenting strategies⁴⁴ while it has been found that
73 stress levels could be associated with weight gain⁴⁵. Thus, parental behavior may fuel
74 noncompliance, aggression and poor regulation of emotion, rather than providing
75 toddlers adaptive models of regulated and pro-social functioning⁴⁶.

76 The current study has a number of strengths including the use of a validated measure of
77 childhood behavior problems for children older in age than previously used in literature,
78 and utilized a genetically sensitive design to assess the influence of maternal pre-
79 pregnancy weight.

80 Moreover, compared to previous studies^{19 22} we applied cut-off scores to the child
81 behavior scale, which enabled us to clinically assess the observed association. In
82 addition, we were able to adjust for a number of covariates, which could indicate a post-
83 natal influence on externalizing behavior, such as maternal educational level and
84 smoking after pregnancy. However, we did not have information on other parental
85 characteristics such as maternal psychopathology and personality.

86 To sum up, these results suggest a possible association between aggressive/externalizing
87 problems and maternal overweight. It is important however, to keep in mind when
88 conducting research with preschoolers that the investigation of children who may be at
89 risk for externalizing problems, may pose the challenge of differentiating between age-
90 related and normative levels of this behavior from more serious early-emerging
91 problems⁴⁷.

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99 Competing Interests

100 The authors declare that they have no conflicts of interest.
101

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104

105

105 Authors' contribution

106 EA contributed to the conception and design, acquisition of the data, analysis and
107 interpretation of the data and drafting of the manuscript.

108 TF, KR, TRS, JPM and MPZ contributed to the conception, design and interpretation of
109 the data.

110 All authors critically revised the manuscript for important intellectual content, agreed to
111 be accountable for all aspects of the work and approved the final manuscript.
112

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113 Data sharing

114 No additional data available
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Title

Maternal pre-pregnancy weight and externalizing behavior problems in preschool children: a UK-based twin study

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Maternal pre-pregnancy weight, twins, intrauterine environment, externalizing behavior

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34 Intrauterine environment, twins, externalizing, pre-pregnancy weight, heritability

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38 **Abstract**

39 Objective: To estimate the heritability of child behavior problems and investigate the
40 association between maternal pre-pregnancy overweight and child behavior problems in
41 a genetically sensitive design.

42 Design: Observational cross-sectional study.

43 Setting: The Twins and Multiple Births Association Heritability Study (TAMBAHS) is
44 an online UK-wide volunteer-based study, investigating the development of twins from
45 birth until 5 years of age.

46 Participants: A total of 443 (16% of initial registered members) mothers answered
47 questions on pre-pregnancy weight and their twins' internalizing and externalizing
48 problems using the Child Behavior Checklist and correcting for important covariates
49 including gestational age, twins' birth weight, age and sex, mother's educational level
50 and smoking (before, during, after pregnancy).

51 Primary outcomes: The heritability of behavior problems and their association with
52 maternal pre-pregnancy weight.

53 Results: The genetic analysis suggested that genetic and common environmental factors
54 account for most of the variation in externalizing disorders (an ACE model was the
55 most parsimonious with genetic factors (A) explaining 46% (95 % CI: 33-60%) of the
56 variance, common environment (C) explaining 42% (95 % CI: 27-54%) and non-shared
57 environmental factors (E) explaining 13% (95% CI: 10-16%) of the variance. For
58 internalizing problems a CE model was the most parsimonious model with common
59 environment explaining 51% (95% CI: 44-58%) of the variance and non-shared
60 environment explaining 49% (95% CI: 42-56%) of the variance. Moreover, the
61 regression analysis results suggested that children of overweight mothers showed a
62 trend (OR=1.10, 95% CI: 0.58-2.06) towards being more aggressive and exhibit
63 externalizing behaviors compared to children of normal weight mothers.

64 Conclusions: Maternal pre-pregnancy weight may play a role in children's aggressive
65 behavior.

70 Strengths and limitations of this study

- 71 • In the twin genetic design maternal pre-pregnancy weight was included in the
72 genetic model, in order to investigate the influence of the intrauterine
73 environment on children's behaviour problems.
- 74 • Cut-off scores applied to the child behavior scale enabled us to clinically assess
75 the observed association.
- 76 • A number of covariates of post-natal influence were adjusted such as maternal
77 educational level and smoking after pregnancy.
- 78 • Information on other parental characteristics such as maternal psychopathology
79 and personality was not available.

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99 Introduction

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101 A child's preschool years are generally considered important since during this period
102 the critical emergence of many clinically significant problem behaviors occur^{1 2}.

103 Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing
104 problems (e.g., emotional problems, anxiety, depression) can often first be identified in
105 early childhood which then show considerable stability across older ages³⁻⁵. Toddlers
106 with problem behaviors are at risk for a variety of adverse developmental outcomes
107 including conflictual relationships with other peers or family, poor academic
108 performance, delinquency and later maladjustment⁶⁻⁸. Given the life course
109 implications of early onset symptomatology, it is essential to understand the underlying
110 aetiology of problem behaviors in preschool children.

111 Twin studies investigating externalizing and internalizing problems have revealed
112 substantial genetic influence, with heritabilities ranging from 40-70% in these age
113 groups⁹⁻¹¹. The influence of the shared environment is however, more modest
114 explaining up to 40% of the variance in behavior problems⁹⁻¹².

115

116 Since the 1990s there has been an increased interest into the research of the effect of the
117 intrauterine environmental and maternal well-being during pregnancy on later child
118 development. Epidemiological studies suggest strong links between measures of the
119 quality of the prenatal environment and the risk of cardiovascular and metabolic
120 diseases^{13 14} and more research evidence suggests that low birth weight can be linked
121 with impaired cognitive development and behavioural disorders, especially
122 hyperactivity/inattention^{15 16} while several studies have examined the association
123 between low birth weight and internalizing and externalizing behaviours¹⁷⁻¹⁹.

124 Research findings²⁰⁻²³ suggest that another intrauterine factor, maternal pre-pregnancy
125 obesity/weight is associated with reduced cognitive abilities, symptoms of inattention
126 and negative emotionality in school aged children. These findings are of clinical
127 importance especially in the light of the increasing prevalence of obese women entering

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4 128 pregnancy²⁴. Pregnancy comes with main changes in the maternal body and a high pre-
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6 129 pregnancy weight is more likely to make these adaptations even more difficult affecting
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8 130 child development. With the exception of one recent study²⁵ suggesting that fetal
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10 131 exposure to increased maternal body mass index (BMI) is associated with elevated
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12 132 levels of externalizing problems in two-year old children, not much is known about the
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14 133 influence of maternal pre-pregnancy weight on externalizing and internalizing
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16 134 behaviour problems in young age groups after that age and before the age of five. By
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18 135 implementing a twin research design including maternal pre-pregnancy weight in the
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20 136 genetic model, we set out to investigate the role of the intrauterine, common and genetic
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22 137 environment on children's behaviour problems.
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139 Methods

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141 The Twins and Multiple Births Association Heritability Study (TAMBAHS) is a
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143 volunteer-based study, investigating the development of twins from birth until 5 years
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145 of age. An invitation letter to this study was sent to all present (n=2712), at the moment
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147 of the study, twin family registered members of the Twins and Multiple Births
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149 Association (TAMBA) beginning of July 2008. TAMBA is an association, which
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151 registers and provides support to multiple birth families across the UK. Mothers of
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153 twins aged between 18 months and five years old at the time of the survey were
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155 identified. In the time period between July 2008 and May 2010, 443 (16%) mothers
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157 completed the study's online questionnaire on their twins' emotional and behavioral
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159 development. The participants show a similar and representative geographical spread of
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161 the twin families across the UK. The study was approved by the ethics committee of the
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163 University of Birmingham and all mothers consented before participating.

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165 Zygoty determination

166 For the determination of the twins' zygoty the previously adapted version of
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168 Goldsmith's zygoty questionnaire was used²⁶. This questionnaire method of assigning
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170 zygoty has been validated against determination by identity of polymorphic DNA
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172 markers and has reached accuracy in verifying zygoty in 95% of the cases²⁷.

173 Twin Sample

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4 160 In total 443 twin pairs were included in the analyses; 186 monozygotic (MZ) male
5 161 twins, 138 monozygotic (MZ) female twins, 144 dizygotic (DZ) male twins, 158
6 162 dizygotic (DZ) female twins and 260 opposite-sex twins.
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11 164 Materials

12 165 Maternal pre-pregnancy weight and covariates

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14 166 The primary exposure variable was maternal pre-pregnancy BMI. Pre-pregnancy BMI
15 167 was based on maternal self-report of weight and height and was calculated by dividing
16 168 their weight in kilograms by their self-report height in meters squared [(kg)/ height
17 169 (m²)]. Pre-pregnancy BMI was analysed both as a continuous and as a categorical
18 170 variable and was classified according to the WHO standard guidelines as: underweight
19 171 (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (≥25kg). Overweight
20 172 and obese mothers were combined in one category due to the limited number of obese
21 173 mothers.
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27 174 Gestational age (measured in completed weeks of gestation), educational level and
28 175 smoking (before, during, after pregnancy) were noted for mothers; age, sex and birth
29 176 weight were noted for all twins.
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34 178 The Child Behavior Checklist (CBCL/1½y-5y)

35 179 The Child Behavior Checklist for toddlers (CBCL/1½-5) ²⁸ is used to obtain
36 180 standardised parent reports of children's problem behaviors. It contains ninety-nine
37 181 problem items, split into seven subcategories: emotionally reactive, anxious/depressed,
38 182 somatic complaints, withdrawn, sleep problems, attention problems, and aggressive
39 183 behavior originally derived by factor analyses ²⁸. The broadband scale 'Internalizing' is
40 184 the sum score of items in the first four syndrome scales, whereas 'Externalizing' is the
41 185 sum score of attention problems and aggressive behaviour. 'Total problems' is the sum
42 186 score of all ninety-nine problem items. Each item is scored 0 = not true, 1 = somewhat
43 187 or sometimes true, and 2 = very true or often true, based on the preceding 2 months.
44 188 Good reliability and validity criteria have been reported for this checklist ²⁸. To identify
45 189 children that may be above the normal range for the syndrome scales, children were
46 190 categorized as being in the normal range when their T scores were below 65 (or the 93rd
47 191 percentile) and as being in the borderline/clinical range when T scores ≥ 65 (or the 97th
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4 192 percentile)²⁸. For Internalizing, Externalizing, and Total Problem broadband scales the
5 193 cut-off point used for the normal range was a T score <60, and borderline/clinical ≥ 60 .
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7 194 The selection of lower cut-off scores for the broadband scales was based on the notion
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9 195 that these scales encompass more numerous and diverse problems than any of the
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11 196 syndrome scales, with the latter comprising smaller, more homogeneous sets of
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13 197 problems. Therefore, higher scores are needed for the syndrome scales in order to
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15 198 conclude that a behavior is clinically deviant [Achenbach, 2000 #1392].
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17 200 Statistical analysis

18 201 Correlations

19 202 Intra-pair twin correlations were calculated by using the Pearson's (r) and the
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21 203 Spearman's rho coefficient statistics as appropriate to explore the genetic and
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23 204 environmental influences.
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25 205 Heritability analysis

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27 206 Univariate genetic models were fit to the data in order to estimate the heritability of the
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29 207 problem scales using a maximum likelihood approach implemented in Mx²⁹. The
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31 208 classical twin study design relies on studying twins raised in the same family
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33 209 environments. Monozygotic twins share all of their genes, while dizygotic twins share
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35 210 only about half of them. So, if a researcher compares the similarity between sets of
36
37 211 identical twins to the similarity between sets of fraternal twins for a particular trait, then
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39 212 any excess likeness between the identical twins should be due to genes (that is the A
40
41 213 component in a genetic model fitting) rather than environment. Researchers use this
42
43 214 method, and variations on it, to estimate the heritability of traits: The percentage of
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45 215 variance in a population due to genes. Modern twin studies also try to quantify the
46
47 216 effect of a person's common environment (family, that is the C component in a genetic
48
49 217 model fitting) and non-shared environment (the individual events that shape a life, that
50
51 218 is the E component in a genetic model fitting) on a trait.

52
53 219 The estimates of the heritability are presented with 95% Confidence Intervals (CI) and
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55 220 goodness of fit statistics for several models: a full ACE model, in which the phenotypic
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57 221 variance is explained by genetic (A) common environmental factors (C) and non-shared
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59 222 (E) environmental factors. Reduced models were estimated by removing one of the
60
61 223 parameters at a time and re-running the model. The goodness of fit of the reduced

224 models was compared to the full model to assess whether they represented a better
225 explanation of the data using the likelihood ratio χ^2 test and the Akaike Information
226 Criterion (AIC). The models were assessed by examining the decrease in the fit of the
227 model; if a parameter could be dropped without a significant decrease in fit then on the
228 grounds of parsimony the reduced model was accepted as the best fitting model. Models
229 were fit both unadjusted and adjusted for pre-pregnancy maternal weight.

231 Regression analyses

232 Effects on a continuous scale

233
234 Standardized coefficients (Betas) are presented reflecting the change on the
235 subcategories and the broadband problem scales by change in mother's weight both
236 expressed as standard deviation change.

238 Effects on clinically important behavior problems

240 In order to estimate the effect of the different categories of maternal weight on clinical
241 problems, logistic regression models were fitted. In these models the intercept of each
242 twin pair was modelled as a function of the population intercept plus the individual
243 contribution of the twin pair. Associations of maternal pre-pregnancy weight and the
244 syndrome and broadband scales were explored both unadjusted and adjusted for twins'
245 sex, age, birth weight, gestational age, maternal smoking (before, during and after
246 pregnancy) and maternal educational level. All analyses were performed in STATA 11

247 ³⁰.

249 Results

250 Correlations

251 The means for the three broadband scales and the intra-pair twin correlations for MZ
252 and DZ twins were calculated (Table 1). For Externalizing problems, the MZ correlation
253 was $r=0.89$ and the DZ correlation was $r=0.62$. For Internalizing problems, the MZ and
254 DZ correlations were $r=0.81$ and $r=0.56$ respectively. For Total problems, the MZ and
255 DZ correlations were $r=0.92$ and $r=0.75$ respectively.

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Maternal pre-pregnancy weight and externalizing problems 10

259 Table 1 Descriptive statistics of problem broadband scales for monozygotic and dizygotic twin pairs

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Problem scales	Monozygotic twin pairs							Dizygotic twin pairs						
	Twin 1			Twin 2				r	Twin 1			Twin 2		
	N	M	SD	N	M	SD			N	M	SD	N	M	SD
Externalizing (range 0-48)	167	12.88	7.72	167	12.83	7.51	0.89	269	12.79	6.61	269	12.66	6.95	0.62
Internalizing (range 0-96)	167	5.94	4.55	167	9.13	6.98	0.81	269	5.49	4.11	269	8.24	5.89	0.56
Total problems (range 0-78)	167	31.58	16.24	167	34.66	18.90	0.92	269	30.45	14.18	269	33.06	16.10	0.75

263 N; Number of twins

264 M; Mean

265 SD; Standard deviation

266 r; Within-twin correlations

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4 271 Heritability analyses
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6 272 Variance estimates of ACE models and sub-models with their 95% Confidence Intervals
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8 273 (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For
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10 274 Externalizing problems, an ACE model was the most parsimonious with genetic factors
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12 275 explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining
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14 276 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95%
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16 277 CI: 10-16%) of the variance. For Internalizing problems a CE model was the most
17
18 278 parsimonious model with common environment explaining 51% (95% CI: 44-58%) of
19
20 279 the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the
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22 280 variance. For Total problems, an ACE model was the most parsimonious one with
23
24 281 genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common
25
26 282 environment explaining 61% (95% CI: 49-70%) of the variance and non-shared
27
28 283 environment explaining 13% (95% CI: 10-17%) of the variance.
29
30 284 After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the
31
32 285 Externalizing problems was an ACE model with genetic factors explaining 50% (95%
33
34 286 CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared
35
36 287 environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the
37
38 288 most parsimonious model was and AE model with genetic factors explaining 59% (95%
39
40 289 CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For
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42 290 Total problems the most parsimonious model was an ACE model with genetic factors
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44 291 explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and
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46 292 non-shared environment 13% (95% CI: .10-17%) of the variance.
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302 Table 2 Univariate genetic model-fitting for the problem scales presenting full and
 303 nested models

	A (95%CI)	C (95%CI)	E (95%CI)	$\Delta\chi^2$	P	AIC
Externalizing						
ACE*	0.46 (0.33-0.60)	0.42 (0.27-0.54)	0.13 (0.10-0.16)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	23.44	0.00	21.44
CE	(0)	0.74 (0.69-0.78)	0.26 (0.22-0.31)	49.69	0.00	47.69
Internalizing						
ACE	0.23 (0.00-0.48)	0.35 (0.12-0.54)	0.43 (0.35-0.53)	-	-	-
AE	0.60 (0.52-0.67)	(0)	0.40 (0.33-0.48)	8.84	0.00	6.84
CE*	(0)	0.51 (0.44-0.58)	0.49 (0.42-0.56)	3.15	0.07	1.15
Total Problems						
ACE*	0.26 (0.16-0.39)	0.61 (0.49-0.70)	0.13 (0.10-0.17)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	53.64	0.00	51.64
CE	(0)	0.79 (0.76-0.83)	0.21 (0.17-0.24)	22.17	0.00	20.17

304

305 * Best fitting model

306 A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence

307 interval; $\Delta\chi^2$, Difference Chi-square; P, statistical significance $p < .05$; AIC, Akaike's

308 Information Criterion

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310 The mean maternal body mass index (BMI), gestational age, age and sex of the twins

311 stratified by zygosity are presented in Table 3. There were no differences in maternal

312 weight between monozygotic and dizygotic twins. However, differences were observed

313 between monozygotic and dizygotic twins with regards to their age and gestational age.

314 Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43,

315 $p < 0.001$) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the

316 monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, $p < 0.001$) compared

317 to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and

318 twin covariates and problems broadband scales are also presented in the same table.

319

320 Table 3 Means, standard deviations and Pearson/Spearman correlations with three syndrome scales for each covariate by zygosity

Maternal Characteristics	MZ twins					DZ twins				
	Mean	SD	r _a	r _b	r _c	Mean	SD	r _a	r _b	r _c
BMI	24.51	4.32	0.07	-0.03	0.02	24.76	5.10	0.10*	-0.04	0.10*
Gestational age (weeks)	35.38	2.49	-0.04	-0.12*	-0.06	36.22	2.71	-0.04	-0.15***	-0.11**
Smoking (no/yes) [^]	n	%	r_a	r_b	r_c	n	%	r_a	r_b	r_c
<i>Before</i>	133/40	76.9/23.1	0.03	-0.02	0.05	255/54	82.5/17.5	0.07	0.04	0.06
<i>During</i>	156/5	96.9/3.1	0.07	0.00	0.06	280/10	96.6/3.4	0.10	-0.03	0.05
<i>After</i>	143/18	88.2/11.2	0.04	0.04	0.10	272/29	90.4/9.6	0.09	0.00	0.06
Educational level										
<i>High School diploma or less</i>	42	25.3				53	18.1			
<i>College/professional education</i>	23	13.9	-0.02	0.04	0.00	51	17.3	-0.00	0.03	-0.03
<i>University</i>	101	60.8				191	64.6			
Twin Characteristics										
Age (years)	3.13	0.96	-0.23***	0.02	-0.15**	2.91	0.95	-0.07	0.11	-0.01
Sex	n	%	-0.05	0.18***	0.07	n	%	-0.08	-0.05	-0.09*
<i>Male</i>	206	57.22				164	26.28			
<i>Female</i>	154	42.78				174	27.88			
<i>Opposite sex</i>	-	-				286	45.84			

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322 [^]Smoking refers to smoking before, during, after pregnancy
323 r_a Pearson/Spearman correlation with Externalizing scale; r_b Pearson/Spearman correlation with Internalizing scale; r_c Pearson/Spearman correlation with
324 Total Problems scale
325 *p<.05; **p<.01; ***p<.001

Maternal pre-pregnancy weight and externalizing problems 14

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4 326 Regression analyses

5 327 In the adjusted model (Table 4) there was a significant increase of .08 standard
6 deviations in aggressive behavior with every standard deviation increase in maternal
7 weight (p=0.02). The logistic regression analysis partly confirmed these findings.
8
9 329 Overweight mothers were 1.10 times more likely to have a child with clinically
10 aggressive behavior when compared to normal weight mothers and 0.78 times more
11 likely when compared to underweight mothers. The individual odds ratio (OR) did not
12 reach statistical significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is
13 observed for children of overweight mothers to show clinically aggressive behavior.
14
15 335 Similarly, there was an increase of .09 standard deviations (p=0.02) in externalizing
16 problems with every standard deviation increase in maternal weight. An increase of the
17 likelihood of externalizing (OR=1.32, 95% CI: 0.84-2.05) for children with overweight
18 mothers compared to children of normal weight mothers was also apparent. No other
19 statistically significant associations between maternal pre-pregnancy weight and
20 behavior problems were observed.
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Table 4 Logistic and linear regression for children's behavioural problems based on maternal BMI**

	Behavioural problems*	No behavioural problems*	OR	95% CI	Beta	P	OR**	95% CI	Beta	P
Emotional reactivity	N	N			0.03	0.42			0.02	0.58
Underweight	6	82	1.10	0.44-2.77			1.12	0.42-2.96		
Normal weight	25	376	1.00	Reference			1.00	Reference		
Overweight/Obese	20	283	1.06	0.58-1.95			1.05	0.54-2.06		
Anxiety/Depression	N	N			-0.03	0.40			-0.02	0.62
Underweight	5	83	0.77	0.29-2.06			0.86	.31-2.41		
Normal weight	29	372	1.00	Reference			1.00	Reference		
Overweight/Obese	14	289	0.62	0.32-1.20			.65	.32-1.30		
Somatic Complaints	N	N			-0.01	0.11			-0.07	0.07
Underweight	11	77	1.33	0.65-2.70			0.99	0.43-2.26		
Normal weight	39	362	1.00	Reference			1.00	Reference		
Overweight/Obese	30	273	1.02	0.62-1.68			0.99	0.56-1.75		
Withdrawn behaviour	N	N			-0.06	0.09			-0.02	0.58
Underweight	3	85	1.14	0.32-4.14			1.53	0.36-6.61		
Normal weight	12	389	1.00	Reference			1.00	Reference		
Overweight/Obese	14	289	1.57	0.72-3.45			2.66	0.99-7.09		
Attention	N	N			0.06	0.08			0.03	0.39
Underweight	5	83	.74	0.28-1.98			1.17	0.42-3.29		
Normal weight	30	371	1.00	Reference			1.00	Reference		
Overweight/Obese	29	274	1.31	0.77-2.23			1.13	0.60-2.13		
Aggressive behaviour	N	N			0.08	0.02 ^a			0.07	0.07
Underweight	4	84	0.78	0.26-2.32			.87	.28-2.69		
Normal weight	23	378	1.00	Reference			1.00	Reference		
Overweight/Obese	19	284	1.10	0.58-2.06			1.04	.52-2.10		
Sleeping problems	N	N			0.06	0.11			0.03	0.48
Normal weight	14	387	1.00	Reference			1.00	Reference		
Underweight	3	85	0.98	0.27-3.47			0.97	0.20-4.66		

Maternal pre-pregnancy weight and externalizing problems 16

	Overweight/Obese	11	292	1.04	0.47-2.33			0.92	0.37-2.29		
* Categorized based on children within normal range and borderline/clinical range **Adjusted for gestational age, twins' birth weight, age and sex, mother's educational level, smoking (before, during, after pregnancy) * p<0.05	Externalizing	N	N			0.09	0.02 ^a			0.07	0.08
	Underweight	6	81	0.56	0.23-1.36			.66	.26-1.66		
	Normal weight	46	348	1.00	Reference			1.00	Reference		
	Overweight/Obese	44	253	1.32	0.84-2.05			1.17	.69-1.99		
	Internalizing	N	N			-0.04	0.29			-0.03	0.44
	Underweight	16	72	1.85	0.99-3.46			1.86	.93-3.73		
	Normal weight	43	358	1.00	Reference			1.00	Reference		
	Overweight/Obese	34	269	1.05	0.65-1.70			1.01	.59-1.74		
	Total problems	N	N			0.07	0.05			0.06	0.14
	Underweight	6	82	0.59	0.24-1.43			0.59	0.23-1.50		
	Normal weight	44	355	1.00	Reference			1.00	Reference		
	Overweight/Obese	41	260	1.27	0.81-2.00			1.18	0.70-2.00		

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4 1 Discussion
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7 3 In this study the effect of maternal pre-pregnancy weight on problem behaviors and the
8 4 influence of genetic and environmental factors on these problems were investigated.
9 5 The heritability analysis suggested that genetic and common environmental factors
10 6 account for most of the variation in externalizing disorders, while common and non-
11 7 shared environment explain most of the variation in internalizing disorders. After
12 8 adjusting for mothers' weight there was a non significant decrease (of 2%) of the
13 9 variation in the externalizing problems that could be explained by the common
14 10 environment, suggesting that mothers' weight may play an important role in explaining
15 11 externalizing problems. In order to further explore the role of overweight in
16 12 externalizing problems we repeated the analysis by comparing the twins of overweight
17 13 to the twins of normal weight mothers. The results suggested that 50% (CI's: 29%-67%)
18 14 of the variation in externalizing problem behavior in twins of overweight mothers could
19 15 be explained by common environment compared to 35% (CI's: 10%-53%) in twins of
20 16 normal weight mothers.

21 17 In general, the results showed that children of overweight mothers showed a trend
22 18 towards being more aggressive and exhibit externalizing behaviors compared to
23 19 children of normal weight mothers. Aggressive behavior is considered an important
24 20 aspect of externalizing behavior and has developmentally been linked to antisocial
25 21 behavior^{31 32}. Studies with children focusing on aggression have shown that both
26 22 observed aggression and parental reports of externalizing behaviors are relatively stable
27 23 from toddlerhood to five years and beyond^{1 33}, which may highlight the role of genetic
28 24 influences. Consistent with this are the results of this study, which suggest that genetic
29 25 factors can explain a large part of the variation in externalizing and internalizing
30 26 behavior problems.

31 27 The results from the logistic regression however, do not suggest a strong association
32 28 between maternal overweight and behavior problems. Moreover, the significantly
33 29 observed change in the standardized coefficients could not explain the distinction
34 30 between normal range and borderline/clinical range. Therefore, children appeared to be
35 31 more aggressive but it is not clear whether this is of clinical significance.

Maternal pre-pregnancy weight and externalizing problems 18

Two studies by Rodriguez and colleagues^{20 21} suggested a link between pre-pregnancy overweight and obesity and symptoms of ADHD in children. In both studies, teacher ratings of the offspring of obese mothers had increased levels of ADHD. However, in the latter study, parent reports of childhood ADHD symptoms and negative emotionality failed to support a link. While this could mean that the effect may be small, in light of the low to moderate associations seen between parent and teacher ratings of children's ADHD one cannot rule out a link. A recent study by van Lieshout and colleagues³⁴ showed that increased maternal pre-pregnancy BMI was associated with higher levels of externalizing problems at age 2. However, in that study authors did not correct for maternal or paternal psychopathology, or maternal personality traits. Similarly in our study we may not be able to strongly establish a causal link between pre-pregnancy weight and aggressive/externalizing problems but by applying clinically relevant cut-off points in the measurement tool of child behavior we suggest that there may be a trend for more aggressive behaviors from children of obese mothers. As it has been previously suggested²³ future studies could benefit from the utilization of genetically sensitive designs, in this study we included pre-pregnancy weight in the genetic model and showed that there be some intrauterine influence. Indeed, when we added pre-pregnancy weight in the genetic model we observed a non-significant decrease in the variation of the externalizing problems explained by the common environment. This suggests that pre-pregnancy weight as an intrauterine factor may play an important role in the development of externalizing problems.

A number of mechanisms could be responsible for the links between maternal obesity and neurodevelopmental problems in young children. The potential role of nutrition on the development of the brain has been investigated in the past. Although there is not a clear pathway that links externalizing problems to maternal overweight, several pathways have been proposed to explain this association and parallels between overweight and other developmental disorders can be drawn. Leptin, which is the protein produced by adipose cells, has been found to play multiple functions in reproduction^{35 36}, glucose homeostasis^{37 38} as well as in brain^{39 40} and neurocognitive development⁴¹. Another possible causal pathway suggests that pre-pregnancy overweight women may not be able to synthesize vitamin D, due to the excessive adipose tissue, which results in deficiency in both the mother and the neonate; in turn,

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4 64 vitamin D is associated with neurocognitive function⁴². In addition, high glucose levels
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6 65 pose a risk for neurobehavioral impairments⁴³. Moreover, epigenetic mechanisms
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8 66 affecting the central nervous system dopamine signaling could be a mechanism by
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10 67 which exposure to elevated maternal BMI during pregnancy might increase levels of
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12 68 externalizing behavior in children⁴⁴.

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14 69 However, others have emphasized the importance of the dynamics within the family
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16 70 environment, such as the parent-child interaction and the individual differences in
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18 71 parenting^{45 46} and their impact on the course of the developmental pathways of problem
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20 72 behaviors. Previous research has mainly linked externalizing problems with family
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22 73 adversity, maternal depression and low socioeconomic status^{37 45 47}.

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24 74 In addition, there may be other environmental factors, which weren't possible to be
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26 75 examined in this study that could accentuate these symptoms. For instance, parents with
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28 76 children displaying symptoms of aggressive behaviors normally report higher levels of
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30 77 stress and frequent use of negative parenting strategies⁴⁸ while it has been found that
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32 78 stress levels could be associated with weight gain⁴⁹. Thus, parental behavior may fuel
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34 79 noncompliance, aggression and poor regulation of emotion, rather than providing
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36 80 toddlers adaptive models of regulated and pro-social functioning⁵⁰.

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38 81 The current study has a number of strengths including the use of a validated measure of
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40 82 childhood behavior problems for children older in age than previously used in literature,
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42 83 and utilized a genetically sensitive design to assess the influence of maternal pre-
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44 84 pregnancy weight.

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46 85 Moreover, compared to previous studies^{20 44} we applied cut-off scores to the child
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48 86 behavior scale, which enabled us to clinically assess the observed association. In
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50 87 addition, we were able to adjust for a number of covariates, which could indicate a post-
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52 88 natal influence on externalizing behavior, such as maternal educational level and
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54 89 smoking after pregnancy. However, we did not have information on other parental
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56 90 characteristics such as maternal psychopathology and personality.

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58 91 To sum up, these results suggest a possible association between aggressive/externalizing
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60 92 problems and maternal overweight. It is important however, to keep in mind when
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94 93 conducting research with preschoolers that the investigation of children who may be at
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96 94 risk for externalizing problems, may pose the challenge of differentiating between age-

Maternal pre-pregnancy weight and externalizing problems 20

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4 95 related and normative levels of this behavior from more serious early-emerging
5 96 problems⁵¹.
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11 131 **Competing Interests**

12 132 The authors declare that they have no conflicts of interest.
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14 133

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16
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19 136

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21 137 **Authors' contribution**

22 138 EA contributed to the conception and design, acquisition of the data, analysis and
23 139 interpretation of the data and drafting of the manuscript.

24 140 TF, KR, TRS, JPM and MPZ contributed to the conception, design and interpretation of
25 141 the data.

26 142 All authors critically revised the manuscript for important intellectual content, agreed to
27 143 be accountable for all aspects of the work and approved the final manuscript.
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31 145 **Data sharing**

32 146 No additional data available
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Maternal pre-pregnancy weight and externalizing problems 24

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Title

Maternal pre-pregnancy weight and externalizing behavior problems in preschool children: a UK-based twin study

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Keywords

Maternal pre-pregnancy weight, twins, intrauterine environment, externalizing behavior

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33 Abstract

34 **Objective:** To estimate the heritability of child behavior problems and investigate the
35 association between maternal pre-pregnancy overweight and child behavior problems in
36 a genetically sensitive design.

37 **Design:** Observational cross-sectional study.

38 **Setting:** The Twins and Multiple Births Association Heritability Study (TAMBAHS) is
39 an online UK-wide volunteer-based study, investigating the development of twins from
40 birth until 5 years of age.

41 **Participants:** A total of 443 (16% of initial registered members) mothers answered
42 questions on pre-pregnancy weight and their twins' internalizing and externalizing
43 problems using the Child Behavior Checklist and correcting for important covariates
44 including gestational age, twins' birth weight, age and sex, mother's educational level
45 and smoking (before, during, after pregnancy).

46 **Primary outcomes:** The heritability of behavior problems and their association with
47 maternal pre-pregnancy weight.

48 **Results:** The genetic analysis suggested that genetic and common environmental factors
49 account for most of the variation in externalizing disorders (an ACE model was the
50 most parsimonious with genetic factors (A) explaining 46% (95 % CI: 33-60%) of the
51 variance, common environment (C) explaining 42% (95 % CI: 27-54%) and non-shared
52 environmental factors (E) explaining 13% (95% CI: 10-16%) of the variance. For
53 internalizing problems a CE model was the most parsimonious model with common
54 environment explaining 51% (95% CI: 44-58%) of the variance and non-shared
55 environment explaining 49% (95% CI: 42-56%) of the variance. Moreover, the
56 regression analysis results suggested that children of overweight mothers showed a
57 trend (OR=1.10, 95% CI: 0.58-2.06, p=0.08) towards being more aggressive and exhibit
58 externalizing behaviors compared to children of normal weight mothers.

59 **Conclusions:** Maternal pre-pregnancy weight may play a role in children's aggressive
60 behavior.

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4 65 Strengths and limitations of this study

- 5 66
- 6 67 • In the twin genetic design maternal pre-pregnancy weight was included in the
7 68 genetic models, in order to investigate the influence of the intrauterine
8 69 environment on children's behaviour problems.
9 70
 - 10 71 • Cut-off scores applied to the child behavior scale enabled us to clinically assess
11 72 the observed association.
12 73
 - 13 74 • A number of covariates of post-natal influence were adjusted such as maternal
14 75 educational level and smoking after pregnancy.
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 - 16 77 • Information on other parental characteristics such as maternal psychopathology
17 78 and personality was not available.
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65 Keywords

66 Intrauterine environment, twins, externalizing, pre-pregnancy weight, heritability

67 Word count: 3,155

97 Introduction

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99 A child's preschool years are generally considered important since during this period
100 the critical emergence of many clinically significant problem behaviors occur^{1 2}.
101 Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing
102 problems (e.g., emotional problems, anxiety, depression) can often first be identified in
103 early childhood which then show considerable stability across older ages³⁻⁵. Toddlers
104 with problem behaviors are at risk for a variety of adverse developmental outcomes
105 including conflictual relationships with other peers or family, poor academic
106 performance, delinquency and later maladjustment⁶⁻⁸. Given the life course
107 implications of early onset symptomatology, it is essential to understand the underlying
108 aetiology of problem behaviors in preschool children.

109 Twin studies investigating externalizing and internalizing problems have revealed
110 substantial genetic influence, with heritabilities ranging from 40-70% in these age
111 groups⁹⁻¹¹. The influence of the shared environment is however, more modest
112 explaining up to 40% of the variance in behavior problems⁹⁻¹².

113

114 Since the 1990s there has been an increased interest into the research of the effect of the
115 intrauterine environmental and maternal well-being during pregnancy on later child
116 development. Epidemiological studies suggest strong links between measures of the
117 quality of the prenatal environment and the risk of cardiovascular and metabolic
118 diseases^{13 14} and more research evidence suggests that low birth weight can be linked
119 with impaired cognitive development and behavioural disorders, especially
120 hyperactivity/inattention^{15 16} while several studies have examined the association
121 between low birth weight and internalizing and externalizing behaviours¹⁷⁻¹⁹.

122 Research findings²⁰⁻²³ suggest that another intrauterine factor, maternal pre-pregnancy
123 obesity/weight is associated with reduced cognitive abilities, symptoms of inattention
124 and negative emotionality in school aged children. These findings are of clinical
125 importance especially in the light of the increasing prevalence of obese women entering
126 pregnancy²⁴. Pregnancy comes with main changes in the maternal body and a high pre-
127 pregnancy weight is more likely to make these adaptations even more difficult affecting
128 child development. With the exception of one recent study²⁵ suggesting that fetal

129 exposure to increased maternal body mass index (BMI) is associated with elevated
130 levels of externalizing problems in two-year old children, not much is known about the
131 influence of maternal pre-pregnancy weight on externalizing and internalizing
132 behaviour problems in young age groups after that age and before the **age** of five. By
133 implementing a twin research design including maternal pre-pregnancy weight in the
134 genetic model, we set out to investigate the role of the intrauterine, common and genetic
135 environment on children's behaviour problems.

137 Methods

139 The Twins and Multiple Births Association Heritability Study (TAMBAHS) is a
140 volunteer-based study, investigating the development of twins from birth until 5 years
141 of age. **An invitation letter to this study was sent to all present (n=2712), at the moment**
142 **of the study, twin family registered members of the Twins and Multiple Births**
143 **Association (TAMBA) beginning of July 2008. TAMBA is an association, which**
144 **registers and provides support to multiple birth families across the UK.** Mothers of
145 twins aged between 18 months and five years old at the time of the survey were
146 identified. **In the time period between July 2008 and May 2010, 443 (16%) mothers**
147 **completed the study's online questionnaire on their twins' emotional and behavioral**
148 **development. The participants show a similar and representative geographical spread of**
149 **the twin families across the UK.** The study was approved by the ethics committee of the
150 University of Birmingham **and all mothers consented before participating.**

152 Zygosity determination

153 For the determination of the twins' zygosity the previously adapted version of
154 Goldsmith's zygosity questionnaire was used²⁶. This questionnaire method of assigning
155 zygosity has been validated against determination by identity of polymorphic DNA
156 markers and has reached accuracy in verifying zygosity in 95% of the cases²⁷.

157 Twin Sample

158 In total 443 twin pairs were included in the analyses; 186 monozygotic (MZ) male
159 twins, 138 monozygotic (MZ) female twins, 144 dizygotic (DZ) male twins, 158
160 dizygotic (DZ) female twins and 260 opposite-sex twins.

161

162 Materials

163 Maternal pre-pregnancy weight and covariates

164 The primary exposure variable was maternal pre-pregnancy BMI. **Pre-pregnancy BMI**
165 **was based on maternal self-report of weight and height and was calculated by dividing**
166 **their weight in kilograms by their self-report height in meters squared [(kg)/ height**
167 **(m²)].** Pre-pregnancy BMI was analysed both as a continuous and as a categorical
168 variable and was classified according to the WHO standard guidelines as: underweight
169 (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (≥25kg). **Overweight**
170 **and obese mothers were combined in one category due to the limited number of obese**
171 **mothers.**

172 Gestational age (measured in completed weeks of gestation), educational level and
173 smoking (before, during, after pregnancy) were noted for mothers; age, sex and birth
174 weight were noted for all twins.

175

176 The Child Behavior Checklist (CBCL/1½y-5y)

177 The Child Behavior Checklist for toddlers (CBCL/1½-5) ²⁸ is used to obtain
178 standardised parent reports of children's problem behaviors. It contains ninety-nine
179 problem items, split into seven subcategories: emotionally reactive, anxious/depressed,
180 somatic complaints, withdrawn, sleep problems, attention problems, and aggressive
181 behavior originally derived by factor analyses ²⁸. The broadband scale 'Internalizing' is
182 the sum score of items in the first four syndrome scales, whereas 'Externalizing' is the
183 sum score of attention problems and aggressive behaviour. 'Total problems' is the sum
184 score of all ninety-nine problem items. Each item is scored 0 = not true, 1 = somewhat
185 or sometimes true, and 2 = very true or often true, based on the preceding 2 months.
186 Good reliability and validity criteria have been reported for this checklist ²⁸. To identify
187 children that may be above the normal range for the syndrome scales, children were
188 categorized as being in the normal range when their T scores were below 65 (or the 93rd
189 percentile) and as being in the borderline/clinical range when T scores ≥ 65 (or the 97th
190 percentile) ²⁸. For Internalizing, Externalizing, and Total Problem broadband scales the
191 cut-off point used for the normal range was a T score <60, and borderline/clinical ≥ 60.

192 **The selection of lower cut-off scores for the broadband scales was based on the notion**

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4 193 that these scales encompass more numerous and diverse problems than any of the
5 194 syndrome scales, with the latter comprising smaller, more homogeneous sets of
6 195 problems. Therefore, higher scores are needed for the syndrome scales in order to
7 196 conclude that a behavior is clinically deviant [Achenbach, 2000 #1392].
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12 198 Statistical analysis

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14 199 Correlations

15 200 Intra-pair twin correlations were calculated by using the Pearson's (r) and the
16 201 Spearman's rho coefficient statistics as appropriate to explore the genetic and
17 202 environmental influences.

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19 203 Heritability analysis

20 204 Univariate genetic models were fit to the data in order to estimate the heritability of the
21 205 problem scales using a maximum likelihood approach implemented in Mx²⁹. The
22 206 classical twin study design relies on studying twins raised in the same family
23 207 environments. Monozygotic twins share all of their genes, while dizygotic twins share
24 208 only about half of them. So, if a researcher compares the similarity between sets of
25 209 identical twins to the similarity between sets of fraternal twins for a particular trait, then
26 210 any excess likeness between the identical twins should be due to genes (that is the A
27 211 component in a genetic model fitting) rather than environment. Researchers use this
28 212 method, and variations on it, to estimate the heritability of traits: The percentage of
29 213 variance in a population due to genes. Modern twin studies also try to quantify the
30 214 effect of a person's common environment (family, that is the C component in a genetic
31 215 model fitting) and non-shared environment (the individual events that shape a life, that
32 216 is the E component in a genetic model fitting) on a trait.

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34 217 The estimates of the heritability are presented with 95% Confidence Intervals (CI) and
35 218 goodness of fit statistics for several models: a full ACE model, in which the phenotypic
36 219 variance is explained by genetic (A) common environmental factors (C) and non-shared
37 220 (E) environmental factors. Reduced models were estimated by removing one of the
38 221 parameters at a time and re-running the model. The goodness of fit of the reduced
39 222 models was compared to the full model to assess whether they represented a better
40 223 explanation of the data using the likelihood ratio χ^2 test and the Akaike Information
41 224 Criterion (AIC). The models were assessed by examining the decrease in the fit of the
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225 model; if a parameter could be dropped without a significant decrease in fit then on the
226 grounds of parsimony the reduced model was accepted as the best fitting model. Models
227 were fit both unadjusted and adjusted for pre-pregnancy maternal weight.

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229 Regression analyses

230 Effects on a continuous scale

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232 Standardized coefficients (Betas) are presented reflecting the change on the
233 subcategories and the broadband problem scales by change in mother's weight both
234 expressed as standard deviation change.

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236 Effects on clinically important behavior problems

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238 In order to estimate the effect of the different categories of maternal weight on clinical
239 problems, logistic regression models were fitted. In these models the intercept of each
240 twin pair was modelled as a function of the population intercept plus the individual
241 contribution of the twin pair. Associations of maternal pre-pregnancy weight and the
242 syndrome and broadband scales were explored both unadjusted and adjusted for twins'
243 sex, age, birth weight, gestational age, maternal smoking (before, during and after
244 pregnancy) and maternal educational level. All analyses were performed in STATA 11
245 ³⁰.

246

247 Results

248 Correlations

249 The means for the three broadband scales and the intra-pair twin correlations for MZ
250 and DZ twins were calculated (Table 1). For Externalizing problems, the MZ correlation
251 was $r=0.89$ and the DZ correlation was $r=0.62$. For Internalizing problems, the MZ and
252 DZ correlations were $r=0.81$ and $r=0.56$ respectively. For Total problems, the MZ and
253 DZ correlations were $r=0.92$ and $r=0.75$ respectively.

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Table 1 Descriptive statistics of problem broadband scales for monozygotic and dizygotic twin pairs

Problem scales	Monozygotic twin pairs							Dizygotic twin pairs						
	Twin 1			Twin 2				r	Twin 1			Twin 2		
	N	M	SD	N	M	SD			N	M	SD	N	M	SD
Externalizing (range 0-48)	167	12.88	7.72	167	12.83	7.51	0.89	269	12.79	6.61	269	12.66	6.95	0.62
Internalizing (range 0-96)	167	5.94	4.55	167	9.13	6.98	0.81	269	5.49	4.11	269	8.24	5.89	0.56
Total problems (range 0-78)	167	31.58	16.24	167	34.66	18.90	0.92	269	30.45	14.18	269	33.06	16.10	0.75

N; Number of twins

M; Mean

SD; Standard deviation

r; Within-twin correlations

Maternal pre-pregnancy weight and externalizing problems 10

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4 269 Heritability analyses
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6 270 Variance estimates of ACE models and sub-models with their 95% Confidence Intervals
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8 271 (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For
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10 272 Externalizing problems, an ACE model was the most parsimonious with genetic factors
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12 273 explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining
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14 274 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95%
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16 275 CI: 10-16%) of the variance. For Internalizing problems a CE model was the most
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18 276 parsimonious model with common environment explaining 51% (95% CI: 44-58%) of
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20 277 the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the
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22 278 variance. For Total problems, an ACE model was the most parsimonious one with
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24 279 genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common
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26 280 environment explaining 61% (95% CI: 49-70%) of the variance and non-shared
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28 281 environment explaining 13% (95% CI: 10-17%) of the variance.
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30 282 After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the
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32 283 Externalizing problems was an ACE model with genetic factors explaining 50% (95%
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34 284 CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared
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36 285 environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the
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38 286 most parsimonious model was and AE model with genetic factors explaining 59% (95%
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40 287 CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For
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42 288 Total problems the most parsimonious model was an ACE model with genetic factors
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44 289 explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and
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46 290 non-shared environment 13% (95% CI: .10-17%) of the variance.
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300 Table 2 Univariate genetic model-fitting for the problem scales presenting full and
 301 nested models

	A (95%CI)	C (95%CI)	E (95%CI)	$\Delta\chi^2$	P	AIC
Externalizing						
ACE*	0.46 (0.33-0.60)	0.42 (0.27-0.54)	0.13 (0.10-0.16)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	23.44	0.00	21.44
CE	(0)	0.74 (0.69-0.78)	0.26 (0.22-0.31)	49.69	0.00	47.69
Internalizing						
ACE	0.23 (0.00-0.48)	0.35 (0.12-0.54)	0.43 (0.35-0.53)	-	-	-
AE	0.60 (0.52-0.67)	(0)	0.40 (0.33-0.48)	8.84	0.00	6.84
CE*	(0)	0.51 (0.44-0.58)	0.49 (0.42-0.56)	3.15	0.07	1.15
Total Problems						
ACE*	0.26 (0.16-0.39)	0.61 (0.49-0.70)	0.13 (0.10-0.17)	-	-	-
AE	0.87 (0.84-0.90)	(0)	0.13 (0.10-0.16)	53.64	0.00	51.64
CE	(0)	0.79 (0.76-0.83)	0.21 (0.17-0.24)	22.17	0.00	20.17

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303 * Best fitting model

304 A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence

305 interval; $\Delta\chi^2$, Difference Chi-square; P, statistical significance $p < .05$; AIC, Akaike's

306 Information Criterion

307

308 The mean maternal body mass index (BMI), gestational age, age and sex of the twins

309 stratified by zygosity are presented in Table 3. There were no differences in maternal

310 weight between monozygotic and dizygotic twins. However, differences were observed

311 between monozygotic and dizygotic twins with regards to their age and gestational age.

312 Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43,

313 $p < 0.001$) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the

314 monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, $p < 0.001$) compared

315 to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and

316 twin covariates and problems broadband scales are also presented in the same table.

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Maternal pre-pregnancy weight and externalizing problems 12

318 Table 3 Means, standard deviations and Pearson/Spearman correlations with three syndrome scales for each covariate by zygosity

Maternal Characteristics	MZ twins					DZ twins				
	Mean	SD	r _a	r _b	r _c	Mean	SD	r _a	r _b	r _c
BMI	24.51	4.32	0.07	-0.03	0.02	24.76	5.10	0.10*	-0.04	0.10*
Gestational age (weeks)	35.38	2.49	-0.04	-0.12*	-0.06	36.22	2.71	-0.04	-0.15***	-0.11**
Smoking (no/yes) [^]	n	%	r_a	r_b	r_c	n	%	r_a	r_b	r_c
Before	133/40	76.9/23.1	0.03	-0.02	0.05	255/54	82.5/17.5	0.07	0.04	0.06
During	156/5	96.9/3.1	0.07	0.00	0.06	280/10	96.6/3.4	0.10	-0.03	0.05
After	143/18	88.2/11.2	0.04	0.04	0.10	272/29	90.4/9.6	0.09	0.00	0.06
Educational level										
High School diploma or less	42	25.3				53	18.1			
College/professional education	23	13.9	-0.02	0.04	0.00	51	17.3	-0.00	0.03	-0.03
University	101	60.8				191	64.6			
Twin Characteristics										
Age (years)	3.13	0.96	-0.23***	0.02	-0.15**	2.91	0.95	-0.07	0.11	-0.01
Sex	n	%	-0.05	0.18***	0.07	n	%	-0.08	-0.05	-0.09*
Male	206	57.22				164	26.28			
Female	154	42.78				174	27.88			
Opposite sex	-	-				286	45.84			

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 320 [^]Smoking refers to smoking before, during, after pregnancy
 321 r_a Pearson/Spearman correlation with Externalizing scale; r_b Pearson/Spearman correlation with Internalizing scale; r_c Pearson/Spearman correlation with
 322 Total Problems scale
 323 *p<.05; **p<.01; ***p<.001

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4 324 Regression analyses

5 325 In the adjusted model (Table 4) there was a significant increase of .08 standard
6 326 deviations in aggressive behavior with every standard deviation increase in maternal
7 327 weight ($p=0.02$). The logistic regression analysis partly confirmed these findings.
8
9 328 Overweight mothers were 1.10 times more likely to have a child with clinically
10 329 aggressive behavior when compared to normal weight mothers and 0.78 times more
11 330 likely when compared to underweight mothers. The individual odds ratio (OR) did not
12 331 reach statistical significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is
13 332 observed for children of overweight mothers to show clinically aggressive behavior.
14 333 Similarly, there was an increase of .09 standard deviations ($p=0.02$) in externalizing
15 334 problems with every standard deviation increase in maternal weight. An increase of the
16 335 likelihood of externalizing (OR=1.32, 95% CI: 0.84-2.05) for children with overweight
17 336 mothers compared to children of normal weight mothers was also apparent. No other
18 337 statistically significant associations between maternal pre-pregnancy weight and
19 338 behavior problems were observed.

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Table 4 Logistic and linear regression for children's behavioural problems based on maternal BMI**

	Behavioural problems*	No behavioural problems*	OR	95% CI	Beta	P	OR**	95% CI	Beta	P
Emotional reactivity	N	N			0.03	0.42			0.02	0.58
Underweight	6	82	1.10	0.44-2.77			1.12	0.42-2.96		
Normal weight	25	376	1.00	Reference			1.00	Reference		
Overweight/Obese	20	283	1.06	0.58-1.95			1.05	0.54-2.06		
Anxiety/Depression	N	N			-0.03	0.40			-0.02	0.62
Underweight	5	83	0.77	0.29-2.06			0.86	.31-2.41		
Normal weight	29	372	1.00	Reference			1.00	Reference		
Overweight/Obese	14	289	0.62	0.32-1.20			.65	.32-1.30		
Somatic Complaints	N	N			-0.01	0.11			-0.07	0.07
Underweight	11	77	1.33	0.65-2.70			0.99	0.43-2.26		
Normal weight	39	362	1.00	Reference			1.00	Reference		
Overweight/Obese	30	273	1.02	0.62-1.68			0.99	0.56-1.75		
Withdrawn behaviour	N	N			-0.06	0.09			-0.02	0.58
Underweight	3	85	1.14	0.32-4.14			1.53	0.36-6.61		
Normal weight	12	389	1.00	Reference			1.00	Reference		
Overweight/Obese	14	289	1.57	0.72-3.45			2.66	0.99-7.09		
Attention	N	N			0.06	0.08			0.03	0.39
Underweight	5	83	.74	0.28-1.98			1.17	0.42-3.29		
Normal weight	30	371	1.00	Reference			1.00	Reference		
Overweight/Obese	29	274	1.31	0.77-2.23			1.13	0.60-2.13		
Aggressive behaviour	N	N			0.08	0.02 ^a			0.07	0.07
Underweight	4	84	0.78	0.26-2.32			.87	.28-2.69		
Normal weight	23	378	1.00	Reference			1.00	Reference		
Overweight/Obese	19	284	1.10	0.58-2.06			1.04	.52-2.10		
Sleeping problems	N	N			0.06	0.11			0.03	0.48
Normal weight	14	387	1.00	Reference			1.00	Reference		
Underweight	3	85	0.98	0.27-3.47			0.97	0.20-4.66		

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	Overweight/Obese	11	292	1.04	0.47-2.33			0.92	0.37-2.29		
<i>* Categorized based on children within normal range and borderline/clinical range **Adjusted for gestational age, twins' birth weight, age and sex, mother's educational level, smoking (before, during, after pregnancy) * p<0.05</i>	Externalizing	N	N			0.09	0.02 ^a			0.07	0.08
	Underweight	6	81	0.56	0.23-1.36			.66	.26-1.66		
	Normal weight	46	348	1.00	Reference			1.00	Reference		
	Overweight/Obese	44	253	1.32	0.84-2.05			1.17	.69-1.99		
	Internalizing	N	N			-0.04	0.29			-0.03	0.44
	Underweight	16	72	1.85	0.99-3.46			1.86	.93-3.73		
	Normal weight	43	358	1.00	Reference			1.00	Reference		
	Overweight/Obese	34	269	1.05	0.65-1.70			1.01	.59-1.74		
	Total problems	N	N			0.07	0.05			0.06	0.14
	Underweight	6	82	0.59	0.24-1.43			0.59	0.23-1.50		
	Normal weight	44	355	1.00	Reference			1.00	Reference		
	Overweight/Obese	41	260	1.27	0.81-2.00			1.18	0.70-2.00		

1 Discussion

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3 In this study the effect of maternal pre-pregnancy weight on problem behaviors and the
4 influence of genetic and environmental factors on these problems were investigated.
5 The heritability analysis suggested that genetic and common environmental factors
6 account for most of the variation in externalizing disorders, while common and non-
7 shared environment explain most of the variation in internalizing disorders. After
8 adjusting for mothers' weight there was a non significant decrease (of 2%) of the
9 variation in the externalizing problems that could be explained by the common
10 environment, suggesting that mothers' weight may play an important role in explaining
11 externalizing problems. **In order to further explore the role of overweight in
12 externalizing problems we repeated the analysis by comparing the twins of overweight
13 to the twins of normal weight mothers. The results suggested that 50% (CI's: 29%-67%)
14 of the variation in externalizing problem behavior in twins of overweight mothers could
15 be explained by common environment compared to 35% (CI's: 10%-53%) in twins of
16 normal weight mothers.**

17 In general, the results showed that children of overweight mothers showed a trend
18 towards being more aggressive and exhibit externalizing behaviors compared to
19 children of normal weight mothers. Aggressive behavior is considered an important
20 aspect of externalizing behavior and has developmentally been linked to antisocial
21 behavior^{31 32}. Studies with children focusing on aggression have shown that both
22 observed aggression and parental reports of externalizing behaviors are relatively stable
23 from toddlerhood to five years and beyond^{1 33}, which may highlight the role of genetic
24 influences. Consistent with this are the results of this study, which suggest that genetic
25 factors can explain a large part of the variation in externalizing and internalizing
26 behavior problems.

27 The results from the logistic regression however, do not suggest a strong association
28 between maternal overweight and behavior problems. Moreover, the significantly
29 observed change in the standardized coefficients could not explain the distinction
30 between normal range and borderline/clinical range. Therefore, children appeared to be
31 more aggressive but it is not clear whether this is of clinical significance.

Two studies by Rodriguez and colleagues^{20 21} suggested a link between pre-pregnancy overweight and obesity and symptoms of ADHD in children. In both studies, teacher ratings of the offspring of obese mothers had increased levels of ADHD. However, in the latter study, parent reports of childhood ADHD symptoms and negative emotionality failed to support a link. While this could mean that the effect may be small, in light of the low to moderate associations seen between parent and teacher ratings of children's ADHD one cannot rule out a link. A recent study by van Lieshout and colleagues³⁴ showed that increased maternal pre-pregnancy BMI was associated with higher levels of externalizing problems at age 2. However, in that study authors did not correct for maternal or paternal psychopathology, or maternal personality traits. Similarly in our study we may not be able to strongly establish a causal link between pre-pregnancy weight and aggressive/externalizing problems but by applying clinically relevant cut-off points in the measurement tool of child behavior we suggest that there may be a trend for more aggressive behaviors from children of obese mothers. As it has been previously suggested²³ future studies could benefit from the utilization of genetically sensitive designs, in this study we included pre-pregnancy weight in the genetic model and showed that there be some intrauterine influence. Indeed, when we added pre-pregnancy weight in the genetic model we observed a non-significant decrease in the variation of the externalizing problems explained by the common environment. This suggests that pre-pregnancy weight as an intrauterine factor may play an important role in the development of externalizing problems.

A number of mechanisms could be responsible for the links between maternal obesity and neurodevelopmental problems in young children. The potential role of nutrition on the development of the brain has been investigated in the past. Although there is not a clear pathway that links externalizing problems to maternal overweight, several pathways have been proposed to explain this association and parallels between overweight and other developmental disorders can be drawn. Leptin, which is the protein produced by adipose cells, has been found to play multiple functions in reproduction^{35 36}, glucose homeostasis^{37 38} as well as in brain^{39 40} and neurocognitive development⁴¹. Another possible causal pathway suggests that pre-pregnancy overweight women may not be able to synthesize vitamin D, due to the excessive adipose tissue, which results in deficiency in both the mother and the neonate; in turn,

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4 64 vitamin D is associated with neurocognitive function⁴². In addition, high glucose levels
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6 65 pose a risk for neurobehavioral impairments⁴³. Moreover, epigenetic mechanisms
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8 66 affecting the central nervous system dopamine signaling could be a mechanism by
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10 67 which exposure to elevated maternal BMI during pregnancy might increase levels of
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12 68 externalizing behavior in children⁴⁴.

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14 69 However, others have emphasized the importance of the dynamics within the family
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16 70 environment, such as the parent-child interaction and the individual differences in
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18 71 parenting^{45 46} and their impact on the course of the developmental pathways of problem
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20 72 behaviors. Previous research has mainly linked externalizing problems with family
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22 73 adversity, maternal depression and low socioeconomic status^{37 45 47}.

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24 74 In addition, there may be other environmental factors, which weren't possible to be
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26 75 examined in this study that could accentuate these symptoms. For instance, parents with
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28 76 children displaying symptoms of aggressive behaviors normally report higher levels of
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30 77 stress and frequent use of negative parenting strategies⁴⁸ while it has been found that
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32 78 stress levels could be associated with weight gain⁴⁹. Thus, parental behavior may fuel
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34 79 noncompliance, aggression and poor regulation of emotion, rather than providing
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36 80 toddlers adaptive models of regulated and pro-social functioning⁵⁰.

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38 81 The current study has a number of strengths including the use of a validated measure of
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40 82 childhood behavior problems for children older in age than previously used in literature,
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42 83 and utilized a genetically sensitive design to assess the influence of maternal pre-
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44 84 pregnancy weight.

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46 85 Moreover, compared to previous studies^{20 44} we applied cut-off scores to the child
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48 86 behavior scale, which enabled us to clinically assess the observed association. In
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50 87 addition, we were able to adjust for a number of covariates, which could indicate a post-
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52 88 natal influence on externalizing behavior, such as maternal educational level and
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54 89 smoking after pregnancy. However, we did not have information on other parental
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56 90 characteristics such as maternal psychopathology and personality.

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58 91 To sum up, these results suggest a possible association between aggressive/externalizing
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60 92 problems and maternal overweight. It is important however, to keep in mind when
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94 93 conducting research with preschoolers that the investigation of children who may be at
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96 94 risk for externalizing problems, may pose the challenge of differentiating between age-

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95 related and normative levels of this behavior from more serious early-emerging
96 problems⁵¹.

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103

104 Competing Interests

105 The authors declare that they have no conflicts of interest.

106

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109

110 Authors' contribution

111 EA contributed to the conception and design, acquisition of the data, analysis and
112 interpretation of the data and drafting of the manuscript.

113 TF, KR, TRS, JPM and MPZ contributed to the conception, design and interpretation of
114 the data.

115 All authors critically revised the manuscript for important intellectual content, agreed to
116 be accountable for all aspects of the work and approved the final manuscript.

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118 Data sharing

119 No additional data available

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