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**Title**

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

**Authors**

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Keywords

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

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Abstract

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

Strengths and limitations of this study

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

Keywords

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

Word count: 3,155

## Introduction

A child's preschool years are generally considered important since during this period the critical emergence of many clinically significant problem behaviors occur [1]. Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing problems (e.g., emotional problems, anxiety, depression) can often first be identified in early childhood which then show considerable stability across older ages [2-4]. Toddlers with problem behaviors are at risk for a variety of adverse developmental outcomes including conflictual relationships with other peers or family, poor academic performance, delinquency and later maladjustment [5-7]. Given the life course implications of early onset symptomatology, it is essential to understand the underlying aetiology of problem behaviors in preschool children.

Twin studies investigating externalizing and internalizing problems have revealed substantial genetic influence, with heritabilities ranging from 40-70% in these age groups [8-10]. The influence of the shared environment is however, more modest explaining up to 40% of the variance in behavior problems [8-11].

Since the 1990s there has been an increased interest into the research of the effect of the intrauterine environmental and maternal well-being during pregnancy on later child development. Epidemiological studies suggest strong links between measures of the quality of the prenatal environment and the risk of cardiovascular and metabolic diseases [12 13] and more research evidence suggests that low birth weight can be linked with impaired cognitive development and behavioural disorders, especially hyperactivity/inattention [14 15] while several studies have examined the association between low birth weight and internalizing and externalizing behaviours [16-18].

Research findings [19 20] suggest that another intrauterine factor, maternal pre-pregnancy obesity/weight is associated with reduced cognitive abilities, symptoms of inattention and negative emotionality in school aged children [19]. These findings are of clinical importance especially in the light of the increasing prevalence of obese women entering pregnancy [21]. Pregnancy comes with main changes in the maternal body and a high pre-pregnancy weight is more likely to make these adaptations even more difficult affecting child development. With the exception of one recent study [22] suggesting that fetal

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

## Methods

The Twins and Multiple Births Association Heritability Study (TAMBAHS) is a volunteer-based study, investigating the development of twins from birth until 5 years of age. Twins aged between 18 months and five years old at the time of the survey were identified. In the time period between July 2008 and May 2010, 443 mothers of twins completed the study's online questionnaire on their twins' emotional and behavioral development. The study was approved by the ethics committee of the University of Birmingham.

### Zygosity determination

For the determination of the twins' zygosity the previously adapted version of Goldsmith's zygosity questionnaire was used [23]. This questionnaire method of assigning zygosity has been validated against determination by identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of the cases [24].

### Twin Sample

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

## Materials

### Maternal pre-pregnancy weight and covariates

The primary exposure variable was maternal pre-pregnancy BMI, which was calculated as the weight (kg)/ height ($m^2$). Pre-pregnancy BMI was analysed both as a continuous

and as a categorical variable and was classified according to the WHO standard guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (>=25kg).

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

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

The Child Behavior Checklist for toddlers (CBCL/1½-5) [25] is used to obtain standardised parent reports of children's problem behaviors. It contains ninety-nine problem items, split into seven subcategories: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behavior originally derived by factor analyses [25]. The broadband scale 'Internalizing' is the sum score of items in the first four syndrome scales, whereas 'Externalizing' is the sum score of attention problems and aggressive behaviour. 'Total problems' is the sum score of all ninety-nine problem items. Each item is scored 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true, based on the preceding 2 months. Good reliability and validity criteria have been reported for this checklist [25]. To identify children that may be above the normal range for the syndrome scales, children were categorized as being in the normal range when their T scores were below 65 (or the 93$^{rd}$ percentile) and as being in the borderline/clinical range when T scores ≥ 65 (or the 97$^{th}$ percentile) [25]. For Internalizing, Externalizing, and Total Problem broadband scales the cut-off point used for the normal range was a T score <60, and borderline/clinical ≥ 60.

Statistical analysis

Correlations

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

Heritability analysis

Univariate genetic models were fit to the data in order to estimate the heritability of the problem scales using a maximum likelihood approach implemented in Mx [26]. The estimates of the heritability are presented with 95% Confidence Intervals (CI) and goodness of fit statistics for several models: a full ACE model, in which the phenotypic variance is explained by genetic (A) common environmental factors (C) and non-shared (E) environmental factors. Reduced models were estimated by removing one of the parameters at a time and re-running the model. The goodness of fit of the reduced models was compared to the full model to assess whether they represented a better explanation of the data using the likelihood ratio $\chi^2$ test and the Akaike Information Criterion (AIC). The models were assessed by examining the decrease in the fit of the model; if a parameter could be dropped without a significant decrease in fit then on the grounds of parsimony the reduced model was accepted as the best fitting model. Models were fit both unadjusted and adjusted for pre-pregnancy maternal weight.

Regression analyses

Effects on a continuous scale

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

Effects on clinically important behavior problems

In order to estimate the effect of the different categories of maternal weight on clinical problems, logistic regression models were fitted. In these models the intercept of each twin pair was modelled as a function of the population intercept plus the individual contribution of the twin pair. Associations of maternal pre-pregnancy weight and the syndrome and broadband scales were explored both unadjusted and adjusted for twins' sex, age, birth weight, gestational age, maternal smoking (before, during and after

pregnancy) and maternal educational level. All analyses were performed in STATA 11 [27].

## Results

### Correlations

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

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

| | Monozygotic twin pairs | | | | | | | Dizygotic twin pairs | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Twin 1 | | | Twin 2 | | | | Twin 1 | | | Twin 2 | | | |
| **Problem scales** | N | M | SD | N | M | SD | r | N | M | SD | N | M | SD | r |
| 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

Heritability analyses

Variance estimates of ACE models and sub-models with their 95% Confidence Intervals (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For Externalizing problems, an ACE model was the most parsimonious with genetic factors explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95% CI: 10-16%) of the variance. For Internalizing problems a CE model was the most parsimonious model with common environment explaining 51% (95% CI: 44-58%) of the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the variance. For Total problems, an ACE model was the most parsimonious one with genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common environment explaining 61% (95% CI: 49-70%) of the variance and non-shared environment explaining 13% (95% CI: 10-17%) of the variance.

After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the Externalizing problems was an ACE model with genetic factors explaining 50% (95% CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the most parsimonious model was and AE model with genetic factors explaining 59% (95% CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For Total problems the most parsimonious model was an ACE model with genetic factors explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and non-shared environment 13% (95% CI: .10-17%) of the variance.

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

| | A ( 95%CI) | C (95%CI) | E (95%CI) | $\Delta x^2$ | P | AIC |
|---|---|---|---|---|---|---|
| Externalizing | | | | | | |
| **ACE*** | 0.46 (0.33-0.60) | 0.42 (0.27-0.54) | 0.13 (0.10-.016) | - | - | - |
| **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 |

* Best fitting model

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$ , Difference Chi-square; P, statistical significance p<.05; AIC, Akaike's Information Criterion

The mean maternal body mass index (BMI), gestational age, age and sex of the twins stratified by zygosity are presented in Table 3. There were no differences in maternal weight between monozygotic and dizygotic twins. However, differences were observed between monozygotic and dizygotic twins with regards to their age and gestational age. Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43, p<0.001) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, p<0.001) compared to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and twin covariates and problems broadband scales are also presented in the same table.

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

| | MZ twins | | | | | DZ twins | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Characteristics** | **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 | | | |

^Smoking refers to smoking before, during, after pregnancy

$r_a$ Pearson/Spearman correlation with Externalizing scale; $r_b$ Pearson/Spearman correlation with Internalizing scale; $r_c$ Pearson/Spearman correlation with Total Problems scale

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

Regression analyses

In the adjusted model there was a significant increase of .08 standard deviations in aggressive behavior with every standard deviation increase in maternal weight (p=0.02). The logistic regression analysis partly confirmed these findings. Overweight mothers were 1.10 times more likely to have a child with clinically aggressive behavior when compared to normal weight mothers and 0.78 times more likely when compared to underweight mothers. The individual odds ratio (OR) did not reach statistical significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is observed for children of overweight mothers to show clinically aggressive behavior.

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

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 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Overweight/Obese | 11 | 292 | 1.04 | 0.47-2.33 | | | 0.92 | 0.37-2.29 | | |
| **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 | | |

** 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)* [a] p<0.05

## Discussion

In this study the effect of maternal pre-pregnancy weight on problem behaviors and the influence of genetic and environmental factors on these problems were investigated. The heritability analysis suggested that genetic and common environmental factors account for most of the variation in externalizing disorders, while common and non-shared environment explain most of the variation in internalizing disorders. After adjusting for mothers' weight there was a non significant decrease (of 2%) of the variation in the externalizing problems that could be explained by the common environment, suggesting that mothers' weight may play an important role in explaining externalizing problems.

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

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

Two studies by Rodriguez and colleagues [19 20] 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 colleagues [22] 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 suggested [31] 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 [32 33], glucose homoeostasis [34 35] as well as in brain [36 37] and neurocognitive development [38]. 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, vitamin D is associated with neurocognitive function [39]. In addition, high glucose levels pose a risk for neurobehavioral impairments [40]. Moreover, epigenetic mechanisms affecting the central nervous system dopamine signaling could be a mechanism by which exposure to elevated maternal BMI during pregnancy might increase levels of externalizing behavior in children [22].

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

In addition, there may be other environmental factors, which weren't possible to be examined in this study that could accentuate these symptoms. For instance, parents with children displaying symptoms of aggressive behaviors normally report higher levels of stress and frequent use of negative parenting strategies [44] while it has been found that stress levels could be associated with weight gain [45]. Thus, parental behavior may fuel noncompliance, aggression and poor regulation of emotion, rather than providing toddlers adaptive models of regulated and pro-social functioning [46].

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

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

To sum up, these results suggest a possible association between aggressive/externalizing problems and maternal overweight. It is important however, to keep in mind when conducting research with preschoolers that the investigation of children who may be at risk for externalizing problems, may pose the challenge of differentiating between age-related and normative levels of this behavior from more serious early-emerging problems [47].

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Competing Interests

The authors declare that they have no conflicts of interest.

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Authors' contribution

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

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

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

Data sharing

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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**Abstract**

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

Design: Observational cross-sectional study.

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

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

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

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

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

Strengths and limitations of this study

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

## Introduction

A child's preschool years are generally considered important since during this period the critical emergence of many clinically significant problem behaviors occur [1 2]. Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing problems (e.g., emotional problems, anxiety, depression) can often first be identified in early childhood which then show considerable stability across older ages [3-5]. Toddlers with problem behaviors are at risk for a variety of adverse developmental outcomes including conflictual relationships with other peers or family, poor academic performance, delinquency and later maladjustment [6-8]. Given the life course implications of early onset symptomatology, it is essential to understand the underlying aetiology of problem behaviors in preschool children.

Twin studies investigating externalizing and internalizing problems have revealed substantial genetic influence, with heritabilities ranging from 40-70% in these age groups [9-11]. The influence of the shared environment is however, more modest explaining up to 40% of the variance in behavior problems [9-12].

Since the 1990s there has been an increased interest into the research of the effect of the intrauterine environmental and maternal well-being during pregnancy on later child development. Epidemiological studies suggest strong links between measures of the quality of the prenatal environment and the risk of cardiovascular and metabolic diseases [13 14] and more research evidence suggests that low birth weight can be linked with impaired cognitive development and behavioural disorders, especially hyperactivity/inattention [15 16] while several studies have examined the association between low birth weight and internalizing and externalizing behaviours [17-19].

Research findings [20-23] suggest that another intrauterine factor, maternal pre-pregnancy obesity/weight is associated with reduced cognitive abilities, symptoms of inattention and negative emotionality in school aged children. These findings are of clinical importance especially in the light of the increasing prevalence of obese women entering

pregnancy [24]. Pregnancy comes with main changes in the maternal body and a high pre-pregnancy weight is more likely to make these adaptations even more difficult affecting child development. With the exception of one recent study[25] suggesting that fetal exposure to increased maternal body mass index (BMI) is associated with elevated levels of externalizing problems in two-year old children, not much is known about the influence of maternal pre-pregnancy weight on externalizing and internalizing behaviour problems in young age groups after that age and before the age of five. By implementing a twin research design including maternal pre-pregnancy weight in the genetic model, we set out to investigate the role of the intrauterine, common and genetic environment on children's behaviour problems.

## Methods

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

### Zygosity determination

For the determination of the twins' zygosity the previously adapted version of Goldsmith's zygosity questionnaire was used [26]. This questionnaire method of assigning zygosity has been validated against determination by identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of the cases [27].

### Twin Sample

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

## Materials

### Maternal pre-pregnancy weight and covariates

The primary exposure variable was maternal pre-pregnancy BMI. Pre-pregnancy BMI was based on maternal self-report of weight and height and was calculated by dividing their weight in kilograms by their self-report height in meters squared [(kg)/ height ($m^2$)]. Pre-pregnancy BMI was analysed both as a continuous and as a categorical variable and was classified according to the WHO standard guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (>=25kg). Overweight and obese mothers were combined in one category due to the limited number of obese mothers.

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

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

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

percentile) [28]. For Internalizing, Externalizing, and Total Problem broadband scales the cut-off point used for the normal range was a T score <60, and borderline/clinical ≥ 60. The selection of lower cut-off scores for the broadband scales was based on the notion that these scales encompass more numerous and diverse problems than any of the syndrome scales, with the latter comprising smaller, more homogeneous sets of problems. Therefore, higher scores are needed for the syndrome scales in order to conclude that a behavior is clinically deviant [Achenbach, 2000 #1392].

Statistical analysis

Correlations

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

Heritability analysis

Univariate genetic models were fit to the data in order to estimate the heritability of the problem scales using a maximum likelihood approach implemented in Mx [29]. The classical twin study design relies on studying twins raised in the same family environments. Monozygotic twins share all of their genes, while dizygotic twins share only about half of them. So, if a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a particular trait, then any excess likeness between the identical twins should be due to genes (that is the A component in a genetic model fitting) rather than environment. Researchers use this method, and variations on it, to estimate the heritability of traits: The percentage of variance in a population due to genes. Modern twin studies also try to quantify the effect of a person's common environment (family, that is the C component in a genetic model fitting) and non-shared environment (the individual events that shape a life, that is the E component in a genetic model fitting) on a trait.

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

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

Regression analyses

Effects on a continuous scale

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

Effects on clinically important behavior problems

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

Results

Correlations

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

256

257

258

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

| | Monozygotic twin pairs | | | | | | | Dizygotic twin pairs | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Twin 1 | | | Twin 2 | | | | Twin 1 | | | Twin 2 | | | |
| **Problem scales** | N | M | SD | N | M | SD | r | N | M | SD | N | M | SD | r |
| 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

Heritability analyses

Variance estimates of ACE models and sub-models with their 95% Confidence Intervals (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For Externalizing problems, an ACE model was the most parsimonious with genetic factors explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95% CI: 10-16%) of the variance. For Internalizing problems a CE model was the most parsimonious model with common environment explaining 51% (95% CI: 44-58%) of the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the variance. For Total problems, an ACE model was the most parsimonious one with genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common environment explaining 61% (95% CI: 49-70%) of the variance and non-shared environment explaining 13% (95% CI: 10-17%) of the variance.

After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the Externalizing problems was an ACE model with genetic factors explaining 50% (95% CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the most parsimonious model was and AE model with genetic factors explaining 59% (95% CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For Total problems the most parsimonious model was an ACE model with genetic factors explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and non-shared environment 13% (95% CI: .10-17%) of the variance.

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

| | A ( 95%CI) | C (95%CI) | E (95%CI) | $\Delta x^2$ | P | AIC |
|---|---|---|---|---|---|---|
| Externalizing | | | | | | |
| **ACE*** | 0.46 (0.33-0.60) | 0.42 (0.27-0.54) | 0.13 (0.10-.016) | - | - | - |
| **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 |

* Best fitting model

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$ , Difference Chi-square; P, statistical significance p<.05; AIC, Akaike's Information Criterion

The mean maternal body mass index (BMI), gestational age, age and sex of the twins stratified by zygosity are presented in Table 3. There were no differences in maternal weight between monozygotic and dizygotic twins. However, differences were observed between monozygotic and dizygotic twins with regards to their age and gestational age. Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43, p<0.001) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, p<0.001) compared to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and twin covariates and problems broadband scales are also presented in the same table.

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

| | MZ twins | | | | | DZ twins | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Characteristics** | **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 | | | |

^Smoking refers to smoking before, during, after pregnancy

r $_a$ Pearson/Spearman correlation with Externalizing scale; r $_b$ Pearson/Spearman correlation with Internalizing scale; r $_c$ Pearson/Spearman correlation with Total Problems scale

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

Regression analyses

In the adjusted model (Table 4) there was a significant increase of .08 standard deviations in aggressive behavior with every standard deviation increase in maternal weight (p=0.02). The logistic regression analysis partly confirmed these findings. Overweight mothers were 1.10 times more likely to have a child with clinically aggressive behavior when compared to normal weight mothers and 0.78 times more likely when compared to underweight mothers. The individual odds ratio (OR) did not reach statistical significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is observed for children of overweight mothers to show clinically aggressive behavior.

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

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 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Overweight/Obese | 11 | 292 | 1.04 | 0.47-2.33 | | | 0.92 | 0.37-2.29 | | |
| **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 | | |

** 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)* [a] p<0.05

## Discussion

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

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

The results from the logistic regression however, do not suggest a strong association between maternal overweight and behavior problems. Moreover, the significantly observed change in the standardized coefficients could not explain the distinction between normal range and borderline/clinical range. Therefore, children appeared to be 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 colleagues[34] 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 suggested [23] 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 homoeostasis [37 38] as well as in brain [39 40] and neurocognitive development [41]. 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,

vitamin D is associated with neurocognitive function [42]. In addition, high glucose levels pose a risk for neurobehavioral impairments [43]. Moreover, epigenetic mechanisms affecting the central nervous system dopamine signaling could be a mechanism by which exposure to elevated maternal BMI during pregnancy might increase levels of externalizing behavior in children [44].

However, others have emphasized the importance of the dynamics within the family environment, such as the parent-child interaction and the individual differences in parenting [45 46] and their impact on the course of the developmental pathways of problem behaviors. Previous research has mainly linked externalizing problems with family adversity, maternal depression and low socioeconomic status [37 45 47].

In addition, there may be other environmental factors, which weren't possible to be examined in this study that could accentuate these symptoms. For instance, parents with children displaying symptoms of aggressive behaviors normally report higher levels of stress and frequent use of negative parenting strategies [48] while it has been found that stress levels could be associated with weight gain [49]. Thus, parental behavior may fuel noncompliance, aggression and poor regulation of emotion, rather than providing toddlers adaptive models of regulated and pro-social functioning [50].

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

Moreover, compared to previous studies [20 44] we applied cut-off scores to the child behavior scale, which enabled us to clinically assess the observed association. In addition, we were able to adjust for a number of covariates, which could indicate a post-natal influence on externalizing behavior, such as maternal educational level and smoking after pregnancy. However, we did not have information on other parental characteristics such as maternal psychopathology and personality.

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

related and normative levels of this behavior from more serious early-emerging problems [51].

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**Competing Interests**

The authors declare that they have no conflicts of interest.

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**Authors' contribution**

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

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

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

**Data sharing**

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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Keywords

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

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Abstract

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

Design: Observational cross-sectional study.

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

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

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

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

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

Strengths and limitations of this study

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

Keywords

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

Word count: 3,155

## Introduction

A child's preschool years are generally considered important since during this period the critical emergence of many clinically significant problem behaviors occur [1 2]. Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing problems (e.g., emotional problems, anxiety, depression) can often first be identified in early childhood which then show considerable stability across older ages [3-5]. Toddlers with problem behaviors are at risk for a variety of adverse developmental outcomes including conflictual relationships with other peers or family, poor academic performance, delinquency and later maladjustment [6-8]. Given the life course implications of early onset symptomatology, it is essential to understand the underlying aetiology of problem behaviors in preschool children.

Twin studies investigating externalizing and internalizing problems have revealed substantial genetic influence, with heritabilities ranging from 40-70% in these age groups [9-11]. The influence of the shared environment is however, more modest explaining up to 40% of the variance in behavior problems [9-12].

Since the 1990s there has been an increased interest into the research of the effect of the intrauterine environmental and maternal well-being during pregnancy on later child development. Epidemiological studies suggest strong links between measures of the quality of the prenatal environment and the risk of cardiovascular and metabolic diseases [13 14] and more research evidence suggests that low birth weight can be linked with impaired cognitive development and behavioural disorders, especially hyperactivity/inattention [15 16] while several studies have examined the association between low birth weight and internalizing and externalizing behaviours [17-19].

Research findings [20-23] suggest that another intrauterine factor, maternal pre-pregnancy obesity/weight is associated with reduced cognitive abilities, symptoms of inattention and negative emotionality in school aged children. These findings are of clinical importance especially in the light of the increasing prevalence of obese women entering pregnancy [24]. Pregnancy comes with main changes in the maternal body and a high pre-pregnancy weight is more likely to make these adaptations even more difficult affecting child development. With the exception of one recent study[25] suggesting that fetal

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

## Methods

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

### Zygosity determination

For the determination of the twins' zygosity the previously adapted version of Goldsmith's zygosity questionnaire was used [26]. This questionnaire method of assigning zygosity has been validated against determination by identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of the cases [27].

### Twin Sample

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

## Materials

### Maternal pre-pregnancy weight and covariates

The primary exposure variable was maternal pre-pregnancy BMI. Pre-pregnancy BMI was based on maternal self-report of weight and height and was calculated by dividing their weight in kilograms by their self-report height in meters squared [(kg)/ height ($m^2$)]. Pre-pregnancy BMI was analysed both as a continuous and as a categorical variable and was classified according to the WHO standard guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (>=25kg). Overweight and obese mothers were combined in one category due to the limited number of obese mothers.

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

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

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

that these scales encompass more numerous and diverse problems than any of the syndrome scales, with the latter comprising smaller, more homogeneous sets of problems. Therefore, higher scores are needed for the syndrome scales in order to conclude that a behavior is clinically deviant [Achenbach, 2000 #1392].

Statistical analysis

Correlations

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

Heritability analysis

Univariate genetic models were fit to the data in order to estimate the heritability of the problem scales using a maximum likelihood approach implemented in Mx [29]. The classical twin study design relies on studying twins raised in the same family environments. Monozygotic twins share all of their genes, while dizygotic twins share only about half of them. So, if a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a particular trait, then any excess likeness between the identical twins should be due to genes (that is the A component in a genetic model fitting) rather than environment. Researchers use this method, and variations on it, to estimate the heritability of traits: The percentage of variance in a population due to genes. Modern twin studies also try to quantify the effect of a person's common environment (family, that is the C component in a genetic model fitting) and non-shared environment (the individual events that shape a life, that is the E component in a genetic model fitting) on a trait.

The estimates of the heritability are presented with 95% Confidence Intervals (CI) and goodness of fit statistics for several models: a full ACE model, in which the phenotypic variance is explained by genetic (A) common environmental factors (C) and non-shared (E) environmental factors. Reduced models were estimated by removing one of the parameters at a time and re-running the model. The goodness of fit of the reduced models was compared to the full model to assess whether they represented a better explanation of the data using the likelihood ratio $\chi^2$ test and the Akaike Information Criterion (AIC). The models were assessed by examining the decrease in the fit of the

model; if a parameter could be dropped without a significant decrease in fit then on the grounds of parsimony the reduced model was accepted as the best fitting model. Models were fit both unadjusted and adjusted for pre-pregnancy maternal weight.

Regression analyses

Effects on a continuous scale

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

Effects on clinically important behavior problems

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

Results

Correlations

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

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

| | Monozygotic twin pairs | | | | | | | Dizygotic twin pairs | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Twin 1 | | | Twin 2 | | | | Twin 1 | | | Twin 2 | | | |
| **Problem scales** | N | M | SD | N | M | SD | r | N | M | SD | N | M | SD | r |
| 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

Heritability analyses

Variance estimates of ACE models and sub-models with their 95% Confidence Intervals (CI) are presented in Table 2, in which the most parsimonious model is highlighted. For Externalizing problems, an ACE model was the most parsimonious with genetic factors explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95% CI: 10-16%) of the variance. For Internalizing problems a CE model was the most parsimonious model with common environment explaining 51% (95% CI: 44-58%) of the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the variance. For Total problems, an ACE model was the most parsimonious one with genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common environment explaining 61% (95% CI: 49-70%) of the variance and non-shared environment explaining 13% (95% CI: 10-17%) of the variance.

After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the Externalizing problems was an ACE model with genetic factors explaining 50% (95% CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the most parsimonious model was and AE model with genetic factors explaining 59% (95% CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For Total problems the most parsimonious model was an ACE model with genetic factors explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and non-shared environment 13% (95% CI: .10-17%) of the variance.

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

| | A ( 95%CI) | C (95%CI) | E (95%CI) | $\Delta x^2$ | P | AIC |
|---|---|---|---|---|---|---|
| Externalizing | | | | | | |
| **ACE*** | 0.46 (0.33-0.60) | 0.42 (0.27-0.54) | 0.13 (0.10-.016) | - | - | - |
| **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 |

* Best fitting model

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$ , Difference Chi-square; P, statistical significance p<.05; AIC, Akaike's Information Criterion

The mean maternal body mass index (BMI), gestational age, age and sex of the twins stratified by zygosity are presented in Table 3. There were no differences in maternal weight between monozygotic and dizygotic twins. However, differences were observed between monozygotic and dizygotic twins with regards to their age and gestational age. Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43, p<0.001) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, p<0.001) compared to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98). Correlations of maternal and twin covariates and problems broadband scales are also presented in the same table.

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

| | MZ twins | | | | | DZ twins | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Characteristics** | **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 | | | |

^Smoking refers to smoking before, during, after pregnancy

r $_a$ Pearson/Spearman correlation with Externalizing scale; r $_b$ Pearson/Spearman correlation with Internalizing scale; r $_c$ Pearson/Spearman correlation with Total Problems scale

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

Regression analyses

In the adjusted model (Table 4) there was a significant increase of .08 standard deviations in aggressive behavior with every standard deviation increase in maternal weight (p=0.02). The logistic regression analysis partly confirmed these findings. Overweight mothers were 1.10 times more likely to have a child with clinically aggressive behavior when compared to normal weight mothers and 0.78 times more likely when compared to underweight mothers. The individual odds ratio (OR) did not reach statistical significance; a trend, however, (OR=1.10, 95% CI: 0.58-2.06) is observed for children of overweight mothers to show clinically aggressive behavior.

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

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 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Overweight/Obese | 11 | 292 | 1.04 | 0.47-2.33 | | | 0.92 | 0.37-2.29 | | |
| **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 | | |

** 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)* [a] p<0.05

## Discussion

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

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

The results from the logistic regression however, do not suggest a strong association between maternal overweight and behavior problems. Moreover, the significantly observed change in the standardized coefficients could not explain the distinction between normal range and borderline/clinical range. Therefore, children appeared to be 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 colleagues[34] 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 suggested [23] 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 homoeostasis [37 38] as well as in brain [39 40] and neurocognitive development [41]. 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,

vitamin D is associated with neurocognitive function [42]. In addition, high glucose levels pose a risk for neurobehavioral impairments [43]. Moreover, epigenetic mechanisms affecting the central nervous system dopamine signaling could be a mechanism by which exposure to elevated maternal BMI during pregnancy might increase levels of externalizing behavior in children [44].

However, others have emphasized the importance of the dynamics within the family environment, such as the parent-child interaction and the individual differences in parenting [45 46] and their impact on the course of the developmental pathways of problem behaviors. Previous research has mainly linked externalizing problems with family adversity, maternal depression and low socioeconomic status [37 45 47].

In addition, there may be other environmental factors, which weren't possible to be examined in this study that could accentuate these symptoms. For instance, parents with children displaying symptoms of aggressive behaviors normally report higher levels of stress and frequent use of negative parenting strategies [48] while it has been found that stress levels could be associated with weight gain [49]. Thus, parental behavior may fuel noncompliance, aggression and poor regulation of emotion, rather than providing toddlers adaptive models of regulated and pro-social functioning [50].

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

Moreover, compared to previous studies [20 44] we applied cut-off scores to the child behavior scale, which enabled us to clinically assess the observed association. In addition, we were able to adjust for a number of covariates, which could indicate a post-natal influence on externalizing behavior, such as maternal educational level and smoking after pregnancy. However, we did not have information on other parental characteristics such as maternal psychopathology and personality.

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

related and normative levels of this behavior from more serious early-emerging problems [51].

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Competing Interests

The authors declare that they have no conflicts of interest.

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Authors' contribution

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

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

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

Data sharing

No additional data available

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