

Association of poor family functioning from pregnancy onward with preadolescent behavior and subcortical brain development

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ABSTRACT

Importance: The association of poor family functioning, a potent stressor, with child behavior is potentially long term and relevant for a person's well-being later in life. Whether changes in brain development underlie the associations with preadolescent behavior and help identify periods of vulnerability is unclear.

Objective: To assess the associations of poor family functioning from pregnancy onward with cortical, white matter, and subcortical volumes, and to examine the extent to which, in particular, hippocampal volume mediates the association of prenatal parental environmental exposures with child problem behavior in preadolescence.

Design, Settings, and Participants: This population-based cohort study, conducted from April 2002 to January 2006, was embedded in Generation R, a multiethnic population-based cohort from fetal life onward. All pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 2002 and January 2006 were invited to participate. Of the 8879 pregnant women enrolled during pregnancy, 1266 mothers with no partner data and 490 with missing family functioning data were excluded, as well as 1 sibling of 32 twin pairs. After excluding an additional 657 children with poor imaging data quality or incidental findings, the final sample consisted of 2583 mother-child pairs. Data analysis was performed from March 1, 2019, to June 28, 2019.

Exposures: Mother- and father-rated poor family functioning was repeatedly measured by the General Functioning subscale of the Family Assessment Device.

Main Outcomes and Measures: Our primary hypothesis, formulated after data collection but before analysis, was that poor prenatal family functioning would be associated with smaller hippocampal and amygdala volumes in late childhood. High-resolution structural neuroimaging data of children aged 10 years were collected with a single 3-T magnetic resonance imaging system. Child emotional and behavioral problems were assessed with the Child Behavior Checklist.

Results: Data were available for 2583 children (mean [SD] age, 10.1 [0.6] years; 1315 girls [50.9%]). Data for parents included 2583 mothers (mean [SD] age, 31.1 [4.7] years; 1617 Dutch race/ethnicity [62.6%]) and 1788 fathers (mean [SD] age, 33.5 [5.3] years; 1239 Dutch race/ethnicity [69.3%]). Children exposed to prenatal maternal-reported poor family functioning had smaller hippocampal ($B = -0.08$; 95% CI, -0.13 to -0.02) and occipital lobe ($B = -0.70$; 95% CI, -1.19 to -0.21) volumes in preadolescence. There was no evidence for an association of exposure to poor family functioning at mid- or late childhood with brain morphology. Hippocampal volumes

partially mediated the association of prenatal maternal-reported poor family functioning with preadolescent problem behavior ($B=0.08$; 95% CI, 0.03-0.13), even after adjusting for prior child problems at age 1.5 years. Analyses of combined maternal and paternal family functioning ratings showed similar results, but associations were largely driven by maternal family functioning reports.

Conclusions and Relevance: In this population-based cohort study, prenatal maternal-reported poor family functioning was associated with a smaller hippocampus in preadolescents. This difference in brain structure may underlie behavioral problems and is a possible neurodevelopmental manifestation of the long-term consequences of poor family functioning for the child.

Key points

Question: To what extent is the persistent association of poor prenatal family functioning with preadolescent problem behavior mediated by subcortical brain development?

Findings: In this population-based cohort study of 2583 children with neuroimaging data, smaller hippocampal volumes were found in preadolescents exposed to prenatal maternal-reported poor family functioning. Smaller hippocampal volumes partially mediated the association of prenatal maternal-reported poor family functioning with preadolescent problem behavior.

Meaning: Subcortical brain characteristics found after more than 10 years of follow-up may help clinicians understand why poor family functioning is associated with child neurodevelopment and well-being.

INTRODUCTION

Poor family functioning can compromise child development; several studies in the literature refer to a range of negative exposures during childhood that are associated with mental health outcomes.¹⁻³ Poor family functioning often includes, but is not limited to, high levels of conflict and lack of cohesion, disorganization, and poor quality of communication.⁴ Prior research on child brain development has highlighted the importance and long-term developmental consequences of adverse childhood experiences, often due to poor parenting and parental stress in samples of high-risk children.⁵ Despite this evidence, it remains unclear (1) why these negative effects persist throughout childhood, (2) at what age children are most vulnerable to poor family functioning, and (3) whether this is generalizable to poor family functioning in the general population. As a potent stressor, poor family functioning interferes with children's ability to regulate stress physiology and may be associated with disruption in typical brain development.⁶

Prenatal stressful life events and maternal anxiety and depression during pregnancy increase children's risk for socioemotional and cognitive problems.^{7,8} Research has investigated the biologic correlates and mediators of these findings. These animal and human preclinical studies suggest that the hypothalamic-pituitary-adrenal axis plays a role in mediating the effects of maternal stress on the fetal brain.⁹⁻¹² Furthermore, brain imaging research suggests that maternal stress is associated with changes in the limbic and frontotemporal structures of children.¹³ There is also a large amount of literature showing that stress in adults and similarly in children induces the production of stress hormones leading to a modulation of brain function.¹⁴ Animal studies suggest that this may be accomplished, in part, by changing the structure of neurons, especially in the hippocampus, amygdala, and prefrontal cortex.¹⁵ Overall, preclinical studies during pregnancy and childhood indicate that the hippocampus is highly susceptible to early stressful experiences,^{16,17} because of its high density of glucocorticoid receptors^{18,19} and persistent postnatal neurogenesis.¹⁶

In a clinical study of monozygotic twins discordant for trauma exposure, Gilbertson et al.²⁰ showed that combat veterans with persistent posttraumatic stress disorder (PTSD) had a smaller hippocampus volume than combat veterans without PTSD. However, the non-trauma-exposed identical twins of the combat veterans with PTSD also had a smaller hippocampus. Thus, a smaller hippocampus may also indicate a preexisting familial vulnerability factor that predisposes to pathological stress reactions in the event of a traumatic exposure.

Several gaps in our understanding remain. First, the period of exposure assessment in prior studies varies, and exposures are rarely assessed repeatedly. Large follow-up studies

with repeated measures of family functioning are needed to identify whether periods of specific vulnerability exist. Second, few prospective studies in the general population have been able to demonstrate whether structural brain changes mediate the association between childhood adversities and adjustment problems.^{21,22} Finally, most studies focus on maternal reports of family functioning only, whereas adding paternal reports of family functioning may capture a different aspect of family functioning or affect children differently.^{23,24}

We conducted a neuroimaging follow-up study of the relationship between poor family functioning from pregnancy onward and preadolescent brain development. Our primary hypothesis was that poor prenatal family functioning would be associated with smaller hippocampal and amygdala volumes in late childhood. We also postulated that these subcortical volumes would mediate the association of prenatal parental environmental exposures with measures of preadolescent problem behaviors at age 10 years. In the primary analyses, we examined global brain outcome measures, ie, total brain volume, total gray and cerebral white matter volumes, and hippocampal and amygdala volumes. This represents the first step of a hierarchical approach that is followed by secondary analyses only if any associations found in the first step are further tested in substructures.

METHODS

Participants

Our research was embedded in the Generation R Study, a multi-ethnic population-based cohort from fetal life onwards.²⁵ Briefly, all pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 2002 and January 2006 were invited to participate. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants and from both parents of minors. Participants gave written informed consent for each phase of the study (fetal, preschool, childhood, and adolescence period). In accordance with Dutch law, children must sign their own consent form starting from the age of 12 years onward. Children received oral information about the study. Of the 8879 pregnant women enrolled during pregnancy, we excluded 1266 mothers with no partner data and 490 with missing family functioning data, leaving 7123 eligible mother-child pairs with 4561 actively participating fathers. We randomly excluded 1 sibling of 32 twin pairs. Data from the late-childhood assessment wave (ie, mean child age 10 years) included a research center visit, questionnaires, and a magnetic resonance imaging (MRI) assessment.²⁶ After excluding an additional 657 children with poor imaging data quality or incidental findings, our final sample consisted of 2583 mother-

child pairs (eFigure 1 in the Supplement). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Measures

Family Assessment Device

Family functioning was assessed using the General Functioning subscale of the Family Assessment Device (FAD), a validated self-report measure of family health and pathology consisting of 12 items (resulting scores range from 1 = not at all to 4 = poor family functioning), with higher scores indicating poor family functioning.^{27,28} Both mothers and fathers completed this measure at 20 weeks of pregnancy (18-25 weeks' gestational age) and when their child was aged 10 years (late childhood). In addition, mothers completed the questionnaire when their child was aged 6 years (midchildhood). The FAD uses the Dutch term *gezin*, which refers only to the nuclear family (ie, siblings and parents). However, even if a pregnant woman already has a child, the wording of the FAD items makes it likely that parents would primarily have their partner in mind (eMethods in the Supplement).

Child Problem Behavior

The Child Behavior Checklist for Ages 1.5 to 5²⁹ and the Child Behavior Checklist for Ages 6 to 18³⁰ were used to obtain standardized parent reports of children's emotional and behavioral problems. We used the continuous Total Problems score (the sum of ratings on all problem items; scores range from 0 [not true] to 1 [somewhat or sometimes true] or 2 [very true or often true], with higher scores indicating more emotional and behavior problems) for children aged 10 years as our outcome measure (eMethods in the Supplement).

Image Acquisition

All images were acquired using the same sequence on the same 3-T 750w Discovery scanner (GE Healthcare) when children were aged 10 years.²⁶ High-resolution, T1-weighted structural MRI data were acquired using a coronal inversion recovery fast spoiled gradient recalled sequence. Structural MRI data were processed through the FreeSurfer analysis suite, version 6.0³¹ (Athinoula A. Martinos Center for Biomedical Imaging) (eMethods in the Supplement).

Covariates

Child age at MRI (based on date of birth) and sex were obtained from birth records. Maternal and paternal age were assessed at intake. Parental race/ethnicity, education, smoking, alcohol consumption, parity, marital status, and parental psychopathology (using the total score of the Brief Symptom Inventory^{32,33} were assessed prenatally using

self-report questionnaires. Harsh parenting was assessed when the child was aged 3 years using the Parent-Child Conflict Tactics Scale,³⁴ a self-report questionnaire completed by the mother and father (eMethods in the Supplement).

Statistical Analysis

Statistical analyses of the data were performed from March 1, 2019, to June 28, 2019. First, we computed descriptive statistics and the correlations between mother- and father-reported poor family functioning scores at different time points (eTable 10 in the Supplement). Then, the prospective associations between maternal and paternal family functioning as assessed at each time point and child brain morphology were determined with separate linear regressions. We ran all models adjusting for all baseline previously mentioned confounders including maternal and paternal psychopathology. The interaction between child sex and poor family functioning was entered into the model in a separate step. In addition, we used structural equation modeling to test prenatal parental family functioning with a latent construct in relation to preadolescent brain morphology. Similarly, a latent construct based on child problem behavior reported by mothers and fathers was constructed (eMethods in the Supplement).

We used a stepwise hierarchical approach to limit the number of comparisons. Total brain volume, cerebral white and gray matter volumes, and amygdala and hippocampus volumes were examined in relation to poor family functioning. If we observed an association with any of these brain measures, subsequent analyses of substructures were conducted to facilitate interpretation of results obtained with the primary outcome measures (eMethods in the Supplement). A visualization of primary and secondary brain measures is presented in eFigure 3 in the Supplement. False discovery rate was applied to adjust for multiple comparisons.³⁵ We adjusted for multiple hypothesis testing of 5 outcomes—ie, total brain volume, total gray and cerebral white matter volumes, hippocampal and amygdala volumes—and the 2 relevant exposure periods (prenatal and early childhood) in the multiple testing correction (10 comparisons). Furthermore, we tested for potential periods of heightened susceptibility to adversity using repeated measures of poor family functioning measures in relation to brain outcomes^{36,37} (eMethods in the Supplement).

Next, we tested whether any subcortical brain structures mediated the association between prenatal maternal-reported poor family functioning and preadolescent problem behavior factor at age 10 years. To this aim, we used a mediation analysis framework providing estimates of the natural direct effect size, the natural indirect effect size, and the total effect size.³⁸ All models were adjusted for baseline confounders and child problem behavior when the child was aged 1.5 years.

Inverse probability weights³⁹ were tested to correct for any participants lost to follow-up (eMethods in the Supplement). In sensitivity analyses, all microstructural left and right hemispheres were used for their respective volumes (eTables 8 and 9 in the Supplement).

The unstandardized β coefficients (B) and 95% CIs were calculated. All missing values (maximum percentage, maternal psychopathology = 10.8%) of the potential confounding factors were imputed using multiple imputations.⁴⁰ Statistical significance was set at a 2-sided P value of less than .05. All analyses were performed using SAS software, version 9.4 (SAS Institute).

RESULTS

The descriptive sample characteristics regarding parental socioeconomic factors, parental psychopathology, and child age at the time of MRI scanning are shown in Table 1. Data were available for 2583 children (mean [SD] age, 10.1 [0.6] years; 1315 [50.9%] girls). Data for parents included 2583 mothers (mean [SD] age, 31.1 [4.7] years; 1617 [62.6%] Dutch race/ethnicity) and 1788 fathers (mean [SD] age, 33.5 [5.3] years; 1239 [69.3%] Dutch race/ethnicity).

As shown in Table 2, prenatal maternal-reported poor family functioning was associated with a decreased total brain volume, cerebral white matter volume, and total gray volume in late childhood (model 1, $B = -26.8$ [95% CI, -34.6 to -18.9]; $B = -9.76$ [95% CI, -13.3 to -6.20]; $B = -16.7$ [95% CI, -21.3 to -12.2], respectively; $P < .001$), but these associations did not survive correction for multiple testing. Poor prenatal family functioning was associated with a smaller hippocampal volume after adjusting for intracranial volume, an association that remained after correction for multiple testing ($B = -0.08$; 95% CI, -0.13 to -0.02). Adjusting for harsh parenting also did not meaningfully change this association. We observed no association between poor family functioning and amygdala volume (model 1, $B = -0.01$ [95% CI, -0.03 to 0.02]; $P = .59$).

We observed no associations between mid- or late-childhood poor family functioning scores and any measure of brain morphology in fully adjusted models. Concurrent associations between late-childhood family functioning and brain outcomes are depicted in eTable 1 in the Supplement. Windows of susceptibility results showed the associations of repeated maternal-reported family functioning with hippocampal volume (poor family functioning \times exposure period interaction $P = .01$), but no other brain outcomes varied by the timing of family functioning measurement (eResults in the Supplement).

Table 1. Baseline characteristics

| | Mother (<i>n</i> =2,583) | Father (<i>n</i> =1,788) |
|--|------------------------------|------------------------------|
| Age, M (SD) | 31.1 (4.7) | 33.5 (5.3) |
| Ethnicity | | |
| Dutch, (%) | 62.6 | 69.3 |
| Other Western, (%) | 9.3 | 5.8 |
| Non Western, (%) | 28.1 | 24.9 |
| Education level | | |
| High, (%) | 52.4 | 56.4 |
| Middle, (%) | 28.9 | 27.4 |
| Low, (%) | 18.7 | 16.2 |
| Alcohol use during pregnancy | | |
| No consumption during pregnancy, (%) | 37.4 | |
| Until pregnancy recognized, (%) | 13.8 | |
| Continued occasionally, (%) | 38.4 | |
| Continued frequently, (%) | 10.4 | |
| Smoking during pregnancy | | |
| No smoking during pregnancy, (%) | 79.8 | |
| Until pregnancy recognized, (%) | 12.5 | |
| Continued during pregnancy, (%) | 7.6 | |
| Parental psychopathology score, M (SD) | 0.26 (0.3) | 0.13(0.2) |
| Marital status, prenatal, Yes (%) | 90.6 | |
| Child age at the MRI scan, years, M (SD) | 10.1 (0.6) | |
| Gender, (% boy) | 49.1 | |
| Harsh parenting score, M (SD) | 1.73 (1.57) | 1.74 (1.57) |
| Poor family functioning - FAD | | |
| Poor family functioning (FAD-score) prenatal, M (SD) | 1.48 (0.4) | 1.49 (0.4) |
| Poor family functioning (FAD-score) at age 5, M (SD) | 1.50 (0.4) | |
| Poor family functioning (FAD-score) at age 9, M (SD) | 1.51 (0.4) | 1.48 (0.4) |
| Child problem behavior | | |
| CBCL total problems score at age 10, M (SD) | 17.2 (15.0) | 17.3 (14.9) |

Note: Numbers denotes children included in one or more analyses. Values are frequencies for categorical and means and standard deviations (M ±SD) for continuous measures.

Exposure to prenatal maternal-reported poor family functioning was associated with smaller occipital lobe volume ($B = -0.70$; 95% CI, -1.19 to -0.21). We further explored the nominally significant anatomical findings and present the results of the relation between poor family functioning and the occipital lobe stratified by regions (eTable 3 in the Supplement). These post hoc analyses suggest that children prenatally exposed to poor family adjustment have a smaller lateral occipital lobe ($B = -0.47$ [95% CI, -0.61 to -0.09]; $P = .01$). In contrast, we observed no associations between any family

Table 2. Associations of poor family functioning with brain morphology.

| Mother reported poor family functioning | Brain morphology (N = 2,583) | | | | | | | | | | | | | |
|---|---------------------------------------|---|---------------------------------------|-------------------------------------|--|---------------------------|------------------------------------|------|--------------------------|------------|------|---------------------------|------|------|
| | Global brain measures | | | | | | Specific brain volumetric measures | | | | | | | |
| | Total brain volume (cm ³) | Cerebral white matter, (cm ³) | Total gray volume, (cm ³) | Amygdala volume, (cm ³) | Hippocampus volume, (cm ³) | | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | | |
| Poor prenatal family functioning (FAD), per score | | | | | | | | | | | | | | |
| Model 1 | -26.8 (-34.6 to -18.9) | <.001 | -9.76 (-13.3 to -6.20) | <.001 | .002 | -16.7 (-21.3 to -12.2) | <.001 | .002 | -0.01 (-0.03 to 0.02) | .593 | .741 | -0.04 (-0.09 to 0.04) | .075 | .107 |
| Model 2 | .025 | .035 | .087 | .035 | .087 | -5.24 (-10.1 to -0.41) | .034 | .088 | -0.02 (-0.05 to 0.06) | .129 | .258 | -0.08 (-0.13 to -0.02) | .004 | .040 |
| Model 3 | .033 | .041 | .105 | .041 | .105 | -4.05 (-7.92 to -0.18) | .042 | .105 | -0.02 (-0.04 to 0.09) | .192 | .384 | -0.07 (-0.13 to -0.03) | .003 | .030 |
| Age 5 poor family functioning (FAD), per score | | | | | | | | | | | | | | |
| Model 1 | -15.6 (-24.9 to -6.36) | .001 | -5.05 (-9.27 to -0.83) | .019 | .031 | -10.4 (-15.8 to -4.97) | <.001 | .002 | 0.01 (-0.02 to 0.03) | .938 | .938 | -0.01 (-0.06 to 0.05) | .872 | .938 |
| Model 2 | .248 | .400 | .500 | .400 | .500 | -1.86 (-6.14 to 2.45) | .199 | .574 | -0.01 (-0.03 to 0.03) | .823 | .823 | -0.02 (-0.07 to 0.04) | .528 | .586 |
| Model 3 | .311 | .499 | .586 | .499 | .586 | -1.47 (-5.80 to 2.83) | .292 | .444 | -0.01 (-0.03 to 0.03) | .933 | .933 | -0.02 (-0.08, to 0.04) | .528 | .586 |
| p Homogeneity for age of FAD assessment* | .234 | .011 | | .180 | .133 | | | | | | | | .001 | |

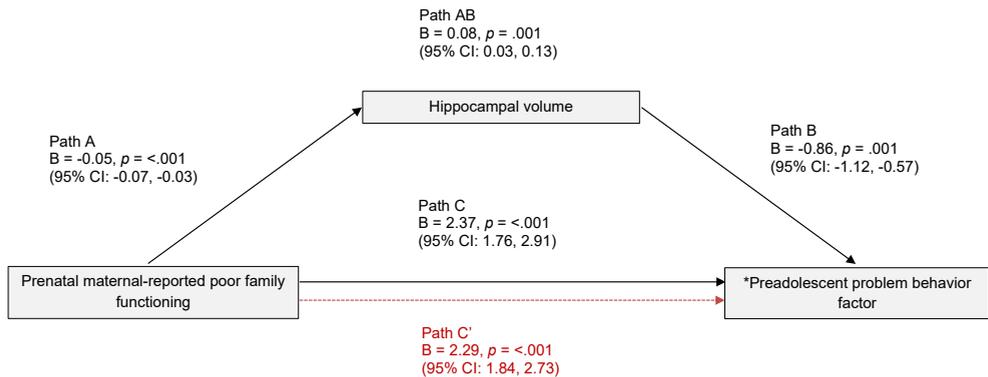
Note: Linear regression analysis of FAD and brain morphology outcome. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, marital status, parity, maternal psychopathology, smoking and alcohol consumption. Model 3 is additionally adjusted for harsh parenting assessed when child was 3 years old. Global brain measures are not adjusted for total ICV. *p_{FDR} = false discovery rate correction for multiple testing. Number of tests = 5 outcomes and the 2 relevant exposure periods (prenatal and early childhood). Critical value for FDR = 0.05. * p Homogeneity for age of FAD assessment = Multiple-partial test used to test whether exposure from different time points of poor family functioning relates in the same manner to brain morphology outcome.

functioning score and temporal, frontal, and parietal lobar volumes (eTable 2 in the Supplement). Similarly, no associations were found between family functioning and thalamus, accumbens, caudate, and putamen volumes (eTable 4 in the Supplement).

After adjusting for socioeconomic factors and paternal psychopathology, we observed no associations between paternal-reported family functioning at either time point and brain morphology (eTables 5 and 6 in the Supplement). We found no interaction by child sex in the association between family functioning and any brain measure. The results using the prenatal parental family functioning factor reflect the common variance in the associations of maternal and paternal family functioning with preadolescent brain outcomes. These results were very similar to those of the unique prenatal maternal-reported associations (eFigure 2 in the Supplement).

As the Figure illustrates, hippocampal volume partially mediated the association of prenatal maternal-reported poor family functioning with preadolescent problem behavior

Figure 1. Hippocampal volume as mediator of the association between prenatal maternal-reported poor family functioning and preadolescent problem behavior.



Mediation analysis of hippocampal volumes at age 10 years in association with maternal-reported poor family functioning per FAD score during pregnancy with preadolescent problem behavior factor at age 10. B statistics are averaged from 10 imputed data sets. Model is adjusted for child age at brain MRI scan, child sex, total ICV, maternal age, race/ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption, and prior child problem behavior when child was aged 1.5 years and harsh parenting when child was aged 3 years. FAD indicates Family Assessment Device; ICV, intracranial volume; MRI, magnetic resonance imaging.

^aPath A is the association of prenatal maternal-reported poor family functioning with hippocampal volume at age 10, and path B is for the association of hippocampal volume with preadolescent problem behavior factor. Path C (in black) is the total association between poor prenatal family functioning and preadolescent problem behavior with hippocampal volume not in the model. Path C' (in red) is the direct association between prenatal maternal-reported poor family functioning and preadolescent problem behavior factor with hippocampal volume in the model.

^bThe latent construct of maternal- and paternal-reported child problems. Preadolescent problem behavior factor captures covariation across raters, or the extent to which a given dimension is reflected across parents (ie, a between-rater dimension factor).

factor at age 10 years ($B = 0.08$; 95% CI, 0.03-0.13). The observed indirect association suggests that lower hippocampal volumes account for a portion of the observed pre-adolescent problem behavior in late childhood. When we adjusted for preexisting child problem behavior at age 1.5 years, we found no meaningful change in mediation results.

Last, in order to ascertain whether selection bias substantially altered any associations, we weighted complete cases by the inverse of their probability of being a complete case to address a possible source of bias due to selection. Results were essentially unchanged (eTable 7 in the Supplement).

DISCUSSION

This cohort study of children from fetal life onward suggests that poor maternal-reported prenatal family functioning is associated with brain development in late childhood. In particular, we observed smaller hippocampal volumes in children exposed to poor family functioning occurring prenatally but not in mid- or late childhood. The association remained when we accounted for parental psychopathology and harsh parenting, indicating a unique association of poor prenatal family functioning with differences in preadolescent brain development. The contribution of prenatal maternal-reported poor family functioning to preadolescent problem behavior was partially mediated by hippocampal volumes. Interestingly, prenatal maternal-reported poor family functioning was associated with smaller occipital lobe volumes. Associations between poor family functioning and brain outcomes did not differ by child sex.

The vulnerability of the hippocampus to prenatal family functioning is consistent with previous studies reporting that the hippocampus matures rapidly and is functional very early in childhood.^{41,42} That the association between poor family functioning and hippocampal volumes was observed only from prenatal maternal-reported family functioning and not from mid- or late-childhood family functioning may reflect a sensitive period, which occurs early in life.⁴³ Other research supports this inference. For example, higher levels of early-life maternal support have been linked to increased volume of the hippocampus.^{44,45} Our key finding, namely the interaction of poor family functioning with child age, suggests that pregnancy is a vulnerable period when development in response to parental care disruptions is maximally dynamic.⁴⁶

In contrast to our hypothesis, we were not able to demonstrate an association between poor family functioning and amygdala volumes. The lack of a discernible sensitive period to family functioning for amygdala development is consistent with previous studies

of children exposed to adversity, which have found no difference in amygdala volume in adults.⁴⁷

The present findings provide evidence for a smaller occipital lobe in children exposed to prenatal maternal-reported poor family functioning. This observation, which was not expected a priori, should be interpreted with caution until it is replicated. However, the face-processing systems relating to occipital regions, in particular the lateral occipital lobe, were found to be particularly vulnerable to early-life adversities.⁴⁸⁻⁵⁰

Furthermore, we found that the association between prenatal maternal-reported poor family functioning and preadolescent problem behavior was partially mediated by hippocampal volumes. This may suggest that brain morphologic changes precede or may even contribute to behavioral changes. Our results are consistent with the extant literature, showing that smaller hippocampal volumes partially mediated the contribution of early-life stress to higher levels of behavioral problems.²¹ However, it is likely that the associations in the mediation model are more complex, and they may well be bidirectional. A sample with multiple repeated measures of imaging data starting early in childhood would be necessary to test the directionality between behavior and brain development. Indeed, a twin study in veterans with PTSD showed that a smaller hippocampus may reflect a preexisting vulnerability to stress and thus reverse causality.²⁰ Alternatively, the difference in hippocampal volume could be explained by genetic variation. Recently, a genome-wide association meta-analysis identified a few genetic loci associated with hippocampal volume,⁵¹ which could be (indirectly) associated with poor family functioning.

In addition, associations between paternal-reported family functioning and brain structural measures did not remain after adjustment for sociodemographic factors and paternal psychopathology. Although prenatal parental family functioning factors reflect a common variance across mother- and father-reported family functioning, their association with brain structural measures was largely driven by the maternal report. Thus, the clear association found using maternal-reported functioning during pregnancy suggests that direct maternal physiological changes may underlie the findings. This is consistent with the developmental origins hypothesis that the prenatal or early postnatal environment can be associated with negative health outcomes later in life. Maternal psychological distress may lead to a suboptimal intrauterine environment with long-term consequences for the growth and health of the child.⁵²⁻⁵⁴ Intrauterine stress exposure may affect child development via dysregulation of the hypothalamic-pituitary-adrenal axis, but it may also affect brain development through inflammatory responses and changes in the balance of the autonomic nervous system.⁵⁵ Another potential mechanism is dietary behavior and poor nutrition by which a variation in maternal

nutrition (either a surplus or paucity of maternal nutrition) plays multiple roles in the health outcomes of children⁵⁶ However, postnatal experiences cannot be ruled out as a mechanism underlying our findings, because the prenatal period could be a marker of exposures in the early postnatal period, such as poor parenting.⁵⁷ Thus, children of parents with poor family functioning may be more likely to experience a less optimal environment, which underlies the relation with brain developmental differences.

Parental psychopathology remains another important mechanism potentially underlying our observations. However, when we adjusted for parental psychopathology, we found that the association between poor prenatal family functioning and hippocampal volumes was, if anything, stronger. Thus, our results suggest that poor family functioning and parental psychopathology are closely associated and may predispose each other,¹³ but higher levels of parental psychopathology did not account for the association of poor family functioning with hippocampal volume.

The current study has several limitations. First, this study has a population-based design, but the relative homogeneity of the population limits its generalizability. Second, we found an association between poor prenatal family functioning and preadolescent brain morphology among children aged 10 years. Although we assessed prenatal family functioning, we cannot establish whether these associations result from strictly prenatal exposures or whether our measure indexes childhood exposure during the period up to age 6 years when parents were reassessed. Third, because poor family functioning was associated with brain findings in children aged 10 years, it is possible that the associations of family functioning reported prenatally had their effects in utero. However, because no scans were obtained before age 10, this cannot be determined. Furthermore, we were unable to examine whether the parental hippocampus is a marker of vulnerability that increases the likelihood of poor family adjustment and whether this propensity is transmitted genetically to the children. Strengths of the present study are the large number of participants and broad spectrum of measured covariates, which enabled us to adjust for multiple confounders. Because of our longitudinal design, we were able to look at possible sensitive periods by leveraging baseline and repeated assessments of poor family functioning reported by both mothers and fathers.

In summary, the findings of this cohort study suggest that prenatal maternal-reported poor family functioning is associated with smaller hippocampal and occipital lobe volumes in preadolescents. Importantly, no such association was found for poor family functioning reported later in childhood, ie, at ages 6 and 10 years, suggesting that there is a sensitive period for the associations of poor family functioning during pregnancy with hippocampal and occipital lobe development. The association of maternal-reported poor family functioning during pregnancy with preadolescent problem behavior was

partially mediated by hippocampal volume. That the associations between poor prenatal family functioning and hippocampal volumes were found after more than 10 years of follow-up may help clarify why poor family functioning is associated with child neurodevelopment and well-being. This study increases our understanding of how poor family functioning shapes brain and behavioral development and underscores the need to search for effective family interventions.

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SUPPLEMENTARY ONLINE CONTENT

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EMETHODS. DESCRIPTION OF THE MEASURES

Family Assessment Device

The General Functioning scale is a validated self-report measure of family health and pathology consisting of 12 Items. The items were selected to correlate highly with six scale scores (one from Problem Solving, four from Communication, two from Roles, one from Affective Responses, three from Affective Involvement and one from Behavior Control).^{1,2} Half of the items describe healthy functioning, e.g., ‘In times of crisis, we can turn to each other for support’. The other half describe unhealthy functioning, e.g., ‘There are a lot of unpleasant and painful feelings in our family’. Parents were asked to rate how well each item described their family by selecting from four different responses ranging from 1 to 4. We reverse-coded the six positively-worded healthy-functioning items so that a higher total FAD score indicated less well-functioning families. All 12 items were summed and divided by 12, yielding a score range from 1 to 4. The instruction read: “think about your (nuclear) family now”. In Dutch, the nuclear family (*gezin*) and extended family or family of origin are different words (*familie*) and concepts. Because questions do not reference specific family members or roles, mothers and fathers can respond regardless of their family’s structure. The FAD score will be referred to henceforth as the *poor family functioning score*. In the current study, internal consistencies (Cronbach’s alpha) ranged from 0.82 to 0.87 across time periods and reporters.

Child Problem Behaviors

The Child Behavior Checklist (CBCL) covers a broad range of emotional and behavioral problems of the child. The CBCL/1½–5 contains 99 problems items, which are scored on seven empirically based syndromes and three broadband scales (Internalizing, Externalizing, and Total Problems). Each item used a 3-point rating scale of 0 (not true), 1 (somewhat or sometimes true), and 2 (very true or often true), based on the preceding 2 months. The CBCL/6–18 has 118 problem items, also yielding syndrome scales and the same three broadband scales, with ratings based on the preceding 6 months. Good reliability and validity have been reported,³ and the scales have been found to be generalizable across 23 societies, including The Netherlands.⁴ We used the continuous Total Problems score (the sum of ratings on all problem items) as our mediator measure because it reflects all the behavioral and emotional problems tapped by the CBCL and is thus the best overall index of maladjustment.

Image Acquisition

Following a three-plane localizer scan, a high-resolution T1-weighted inversion recovery fast spoiled gradient recalled sequence was acquired with the parameters: $T_R = 8.77$ ms, $T_E = 3.4$ ms, $T_I = 600$ ms, flip angle = 10° , field of view (FOV) = 220 mm×220 mm, Acquisition Matrix = 220×220, slice thickness=1mm, number of slices=230.

Morphological Image Processing

Structural MRI data were processed through the FreeSurfer analysis suite, version 6.0.⁵ Briefly, nonbrain tissue was removed, voxel intensities were normalized for B1 inhomogeneity, whole-brain tissue segmentation was performed, and a surface-based model of the cortex was reconstructed. In our group, we have developed a metric of image quality which automatically characterizes the amount of motion/artifact based on signal intensities outside of the brain.⁶ In the image processing we additionally controlling for a metric for that described motion artifact and quality. Global metrics of volume were extracted (e.g., total brain volume and subcortical volume), and a number of subcortical and cortical structures (amygdala, orbitofrontal cortex, etc.) were automatically labeled. The averaged left and right hemispheres for all measures were used in primary analyses. In sensitivity analyses, all microstructural left and right hemispheres were used for their respective volumes. Surface reconstructions were visually inspected for accuracy and data not suitable for statistical analysis were excluded⁷ (eFigure 1 in the Supplement).

Covariates

Parental ethnicity was categorized into three groups: Dutch, non-Western, and other Western national origin.⁸ Parental education was classified in three levels: ‘low’ (maximum of three years general secondary school); ‘medium’ (>3 years general secondary school; intermediate vocational training); and ‘high’ (bachelor’s degree or higher academic education). Information about smoking (three categories: no smoking during pregnancy; smoked until pregnancy recognized; and continued smoking during pregnancy), alcohol intake during pregnancy (four categories: no alcohol consumption during pregnancy; alcohol consumption until pregnancy recognized; continued occasionally during pregnancy (<1 glass/week); and continued frequently during pregnancy (>=1 glass/week)) was assessed prenatally using self-report questionnaires. During pregnancy, marital status was scored dichotomously: “married/living together” and “separated/divorced.” Parity was scored dichotomously: previous pregnancies: 0 vs. ≥1. Parental psychopathological symptoms were assessed at 20 weeks of pregnancy using the Brief Symptom Inventory (BSI), a validated self-report questionnaire with 53 items pertaining the presence and severity of specific symptom dimensions. Each item is answered on a five-point scale, ranging from ‘0 = not at all’ to ‘4 = extremely’.^{9,10} High validity and reliability have been reported for the Dutch translation.¹¹ Harsh parenting was assessed when the child was 3 years old using the Parent-Child Conflict Tactics Scale,¹² a validated self-report questionnaire completed by mother and father. In our research group,¹³ a harsh discipline scale was confirmed using factor analysis. This resulted in a scale consisting of six items, representing constructs of psychological aggression and (mild) physical assault. Each item is answered on a thirteen-point scale, ranging from ‘0 = not at all’ to ‘12 = higher severity of harsh discipline’.

In our study, we included unmarried partnered couples, but not unpartnered women. That is, not all couples were married but all women included in the study had a partner at baseline. In the Netherlands, many unmarried couples have a registered partnership.

Statistical analyses

Separate models estimated each brain measure as a depended variable. Maternal and paternal family functioning repeatedly measured each separately were included in the models as independent variables. We ran all models adjusting for all baseline previously mentioned confounders. The co-occurrence of childhood adversities could mediate or confound the associations of poor family functioning on childhood brain outcomes. Therefore, models additionally adjusted for harsh parenting, which was assessed at child age 3 to examine effect estimate change. The interaction between child sex and poor family functioning were entered into the model in a separate step. Adjustment for multiple comparisons was made using the Benjamini-Hochberg method¹⁴ to obtain a False Discovery Rate (FDR) of 0.05. In the primary analyses we examined global brain outcomes, i.e. total brain volume, total gray matter volume, total cerebral white matter volume, as well as the hippocampal and the amygdala volumes. This represents the first step of a hierarchical approach which is followed by models representing secondary analyses; the latter examined lobar volumes to further explore any finding in the first step. Against, the background of more than 100+ brain measures available in Freesurfer, a hierarchical approach is important as variable selection is mandatory even with a sample size of $n = 2583$.

The hippocampus and amygdala volumes are tested as the structural brain measure of interest in virtually any research on child stress and abuse. Hence, we account for all five brain structures and the two relevant exposure periods (prenatal and early childhood) in the multiple testing correction.

In secondary analyses we thus tested the association of family adjustment with brain lobar volumes given the finding with total brain volume. We tested other subcortical structures to test the specificity of the finding with the hippocampus. Additionally, we further explored the anatomical findings and present the results of poor family functioning and occipital lobe stratified by regions such as lateral, lingual, cuneus, and pericalcarine in Supplementary eTable 3 because of the association of prenatal family functioning and occipital lobe volumes.

We have included the total intracranial volume as a covariate in our models for subcortical brain measures to compensate for head size variability.

As a second step, we examined potential periods of heightened susceptibility to poor family functioning using maternal or paternal functioning measures at different time points (e.g., prenatal, mid-childhood and late-childhood) to simultaneously estimate the associations between maternal or paternal functioning with brain outcomes (i.e., whether parental functioning measured during different time points was associated in the same manner to child outcomes).^{15,16}

In sensitivity analyses, we calculated inverse probability weights to correct for lost to follow-up, i.e., to account for potential selection bias when including only participants with available data as compared with the full cohort recruited during pregnancy.¹⁷

Latent factors analysis

Maternal- and paternal-reported family functioning were modeled as latent variable via common confirmatory factor analytic (CFA) methods (eFigure 2). The models were allowed to correlate, and were estimated with the robust maximum likelihood estimator using standardized latent variables. The association between the latent construct of family functioning and preadolescent brain morphology captures covariation across raters, or the extent to which a given dimension is reflected both across parents (i.e., a “between-rater” dimension factor). Similarly, a latent construct for maternal- and paternal-reported child problem behavior was estimated (Figure 1). The latent constructs showed good model fit as judged with the comparative fit index (CFI, acceptable fit $\geq .90$ ¹⁸). The association of parental family functioning factor and preadolescent brain morphology were performed using structural equation modeling. The goodness of fit of these models was compared with the Bayesian information criterion (BIC) and Akaike’s information criterion (AIC). A lower value for AIC and BIC indicates a better fit.¹⁹ The latent child problem behavior factor was used in mediation model to test whether the associations between prenatal maternal-reported family functioning and child problem behavior factor was mediated by hippocampal volumes (Figure 1).

Generalized estimating equation analysis

Using multivariable linear regression with generalized estimating equations (GEE),^{15,16} we simultaneously estimated the associations between maternal or paternal functioning measured prenatally, in mid-childhood and in late-childhood with brain outcomes (i.e., whether parental functioning measured during different time points was associated in the same manner to child outcomes). In addition, to test the unique period of susceptibility, we tested the interaction with the child’s age in the associations between poor family functioning and brain measures (poor family functioning \times exposure period interaction p -value = .001). This analysis tested if the association of poor family functioning (as reported by both mothers and fathers) with child brain morphology depends on the age of the child by comparing the estimates of the repeatedly assessed

poor family functioning. Although this approach retains the interpretation of a set of separate multiple regressions (by providing a single estimate of effect for exposure at each time point), it also takes the variance into account between family functioning over time, while assessing the differences in associations between poor family functioning and brain morphology. All models were adjusted for potential effects of confounders, including socioeconomic factors and maternal or paternal psychopathology at baseline. The GEE retains the MCAR assumption for the missing data.

Table 1. Concurrent associations of poor family functioning and brain morphology.

| <i>Mother reported poor family functioning</i> | Brain morphology (N = 2,583) | | | | | | | | | | | | | | |
|--|---------------------------------------|------|---|---------------------------|---------------------------------------|------------------|---|------|--|-------------------------|------|------------------|-------------------------|------|------|
| | <i>Global brain measures</i> | | | | | | <i>Specific brain volumetric measures</i> | | | | | | | | |
| | Total brain volume (cm ³) | | Cerebral white matter, (cm ³) | | Total gray volume, (cm ³) | | Amygdala volume, (cm ³) | | Hippocampus volume, (cm ³) | | | | | | |
| | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | | | |
| Age 9 poor family functioning (FAD), per score | | | | | | | | | | | | | | | |
| Model 1 | -9.13 (-17.7 to -0.59) | .036 | .081 | -4.10 (-7.98 to -0.22) | .038 | .081 | -5.05 (-10.1 to -0.03) | .049 | .081 | 0.02 (-0.01 to 0.04) | .234 | .234 | 0.03 (-0.02 to 0.09) | .194 | .234 |
| Model 2 | -1.13 (-9.61 to 7.34) | .793 | .886 | -1.60 (-5.51 to 2.31) | .423 | .823 | 0.36 (-4.57 to 5.29) | .886 | .886 | 0.01 (-0.02 to 0.04) | .834 | .834 | 0.02 (-0.03 to 0.07) | .463 | .834 |
| Model 3 | -0.50 (-3.17 to 4.07) | .789 | .790 | -1.27 (-5.20 to 2.66) | .527 | .658 | 0.90 (-4.04 to 5.85) | .720 | .720 | 0.01 (-0.01 to 0.04) | .359 | .658 | 0.02 (-0.03 to 0.08) | .425 | .658 |

Note: Linear regression analysis of EAD and brain morphology outcome. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption. Model 3 is additionally adjusted for harsh parenting assessed when child was 3 years old. Global brain measures are not adjusted for total ICV. *p_{FDR} = false discovery rate correction for multiple testing. Number of tests = 5 outcomes and exposure period (late childhood). Critical value for FDR = 0.05.

eTable 2. The associations of poor family functioning and lobar measures.

| Brain morphology (N = 2,583) | | | | | | | | | | | | |
|---|-----------------------------------|------|------------------|----------------------------------|------|------------------|-----------------------------------|------|------------------|------------------------------------|-------|------------------|
| Lobar measures | | | | | | | | | | | | |
| Mother reported poor family functioning | Temporal lobe, (cm ³) | | | Frontal lobe, (cm ³) | | | Parietal lobe, (cm ³) | | | Occipital lobe, (cm ³) | | |
| | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} |
| Poor prenatal family functioning (FAD), per score | | | | | | | | | | | | |
| Model 1 | -0.16 (-0.8 to 0.53) | .652 | .869 | 0.31 (-0.68 to 1.31) | .534 | .854 | -1.25 (-2.01 to -0.49) | .001 | .004 | -0.80 (-1.25 to -0.35) | <.001 | .004 |
| Model 2 | 0.05 (-0.71 to 0.80) | .903 | .903 | 0.70 (-0.38 to 1.78) | .206 | .408 | -0.58 (-1.40 to 0.24) | .167 | .408 | -0.70 (-1.19 to -0.21) | .005 | .040 |
| Age 5 poor family functioning (FAD), per score | | | | | | | | | | | | |
| Model 1 | -0.04 (-0.85 to 0.77) | .928 | .928 | -0.91 (-2.07, 0.26) | .127 | .254 | -0.09 (-0.98 to 0.81) | .851 | .928 | -0.48 (-1.01 to 0.48) | .074 | .197 |
| Model 2 | 0.05 (-0.78 to 0.89) | .898 | .903 | -0.80 (-2.00, 0.39) | .189 | .408 | 0.33 (-0.59 to 1.24) | .486 | .648 | -0.32 (-0.86 to 0.23) | .255 | .408 |
| p Homogeneity for age of FAD assessment* | | .093 | | | .148 | | | .091 | | | | .030 |

Note: Linear regression analysis of FAD and lobar measures. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption. Global brain measures are not adjusted for total ICV. *p_{FDR} = false discovery rate correction for multiple testing. Number of tests = 4 outcomes and the 2 relevant exposure periods (prenatal and early childhood). Critical value for FDR = 0.05. *p Homogeneity for age of FAD assessment = Multiple-partial test used to test whether exposure from different time points of poor family functioning relates in the same manner to brain morphology outcome.

Table 3. The associations of poor family functioning and occipital lobe stratified by regions.

| | | Brain morphology (N = 2,583) | | | | | | | |
|---|---------|---------------------------------------|---------------------------------------|--------------------------------------|---|-----------------------|------|-----------------------|------|
| | | Occipital lobe anatomical region | | | | | | | |
| Mother reported poor family functioning | | Lateral occipital, (cm ³) | Lingual occipital, (cm ³) | Cuneus occipital, (cm ³) | Pericalcarine occipital, (cm ³) | | | | |
| | | B (95% CI) | p | B (95% CI) | p | B (95% CI) | p | | |
| Poor prenatal family functioning (FAD), per score | | | | | | | | | |
| | Model 1 | -0.54 (-0.79 to -0.29) | <.001 | -0.12 (-0.28 to 0.04) | .138 | -0.09 (-0.19 to 0.02) | .056 | -0.05 (-0.12 to 0.02) | .176 |
| | Model 2 | -0.47 (-0.61 to -0.09) | .007 | -0.11 (-0.28 to 0.06) | .223 | -0.07 (-0.16 to 0.04) | .247 | -0.04 (-0.11 to 0.03) | .242 |
| Age 5 poor family functioning (FAD), per score | | | | | | | | | |
| | Model 1 | -0.27 (-0.56 to 0.02) | .066 | -0.09 (-0.29, 0.09) | .328 | -0.06 (-0.18 to 0.05) | .246 | -0.05 (-0.13 to 0.03) | .240 |
| | Model 2 | -0.15 (-0.45 to 0.14) | .311 | -0.07 (-0.27, 0.13) | .483 | -0.05 (-0.16 to 0.07) | .402 | -0.04 (-0.13 to 0.04) | .296 |

Note: Linear regression analysis of FAD and occipital anatomical region. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption.

eTable 4. The associations of poor family functioning and subcortical brain morphology.

| <i>Mother reported poor family functioning</i> | Brain morphology (N = 2,583) | | | | | | | |
|---|-------------------------------------|--------------------------------------|------------------------------------|---|------------------------|------|-----------------------|------|
| | <i>Global brain measures</i> | | | <i>Specific brain volumetric measures</i> | | | | |
| | Thalamus volume, (cm ³) | Accumbens volume, (cm ³) | Caudate volume, (cm ³) | Putamen volume, (cm ³) | | | | |
| | B (95% CI) | p | B (95% CI) | p | B (95% CI) | p | | |
| Poor prenatal family functioning (FAD), per score | | | | | | | | |
| Model 1 | 0.02 (-13.3 to -6.20) | .623 | -0.01 (-0.02 to -0.01) | .243 | -0.07 (-0.14 to -0.01) | .038 | 0.06 (-0.02 to 0.14) | .140 |
| Model 2 | 0.03 (-0.05 to 0.11) | .433 | -0.01 (-0.02 to 0.01) | .323 | -0.04 (-0.12 to 0.03) | .236 | 0.01 (-0.08 to 0.09) | .870 |
| Age 5 poor family functioning (FAD), per score | | | | | | | | |
| Model 1 | -0.04 (-0.13 to 0.05) | .356 | -0.01 (-0.02 to 0.01) | .298 | -0.05 (-0.13 to 0.03) | .241 | 0.06 (-0.03 to 0.15) | .210 |
| Model 2 | -0.04 (-0.13 to 0.05) | .528 | -0.01 (-0.02 to 0.01) | .392 | -0.02 (-0.10 to 0.06) | .605 | -0.04 (-0.13 to 0.05) | .528 |

Note: Linear regression analysis of FAD and brain morphology outcome. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption.

eTable 5. The associations of paternal-reported poor family functioning and brain morphology.

| | | Brain morphology (N = 2,583) | | | | | | | | | | | | | | | |
|---|--|---|------|---------------------------------------|------------|-------------------------------------|------------------------------------|--|---------------------------|------------------|------------|--------------------------|------|------|--------------------------|------|------|
| | | Global brain measures | | | | | Specific brain volumetric measures | | | | | | | | | | |
| Father reported poor family functioning | Total brain volume, (cm ³) | Cerebral white matter, (cm ³) | | Total gray volume, (cm ³) | | Amygdala volume, (cm ³) | | Hippocampus volume, (cm ³) | | | | | | | | | |
| | | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | | | | | |
| Poor prenatal family functioning (FAD), per score | | | | | | | | | | | | | | | | | |
| Model 1 | -23.1 (-34.3 to -12.1) | <.001 | .005 | .002 | .005 | -7.98 (-13.1 to -2.92) | <.001 | .005 | -15.2 (-21.7 to -8.69) | .881 | .881 | -0.03 (-0.04 to 0.03) | .881 | .881 | -0.01 (-0.08 to 0.05) | .664 | .772 |
| Model 2 | -7.34 (-18.9 to 4.16) | .211 | .422 | .375 | .564 | -2.40 (-7.72 to 2.91) | .137 | .387 | -5.08 (-11.8 to 1.61) | .842 | .842 | -0.04 (-0.04 to 0.03) | .842 | .842 | -0.02 (-0.09 to 0.06) | .668 | .808 |
| Age 9 poor family functioning (FAD), per score | | | | | | | | | | | | | | | | | |
| Model 1 | -13.2 (-23.7 to -2.73) | .013 | .026 | .002 | .005 | -5.82 (-10.6 to -1.03) | .021 | .035 | -7.19 (-13.3 to -1.06) | .695 | .772 | 0.007 (-0.02 to 0.04) | .695 | .772 | 0.03 (-0.03 to 0.09) | .359 | .513 |
| Model 2 | -8.95 (-19.3 to 1.45) | .092 | .387 | .074 | .387 | -4.39 (-9.20 to 0.42) | .155 | .387 | -4.37 (-10.4 to 1.65) | .728 | .808 | 0.06 (-0.03 to 0.04) | .728 | .808 | 0.03 (-0.04 to 0.09) | .395 | .564 |
| p Homogeneity for age of FAD assessment* | | .069 | | .055 | | .132 | | .600 | | .162 | | | | | | | |

Note: Linear regression analysis of FAD and brain morphology outcome. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for paternal age, ethnicity, education, parity, marital status, paternal psychopathology, smoking and alcohol consumption. Global brain measures are not adjusted for total ICV. *p_{FDR} = false discovery rate correction for multiple testing. Number of tests = 5 outcomes and the 2 relevant exposure periods (prenatal and early childhood). Critical value for FDR = 0.05. *p Homogeneity for age of FAD assessment = Multiple-partial test used to test whether exposure from different time points of poor family functioning relates in the same manner to brain morphology outcome.

eTable 6. The associations of paternal-reported poor family functioning and lobar measures.

| <i>Father reported poor family functioning</i> | Brain morphology (N = 2,583) | | | | | | | | | | | |
|---|-----------------------------------|------|----------------------------------|-----------------------|-----------------------------------|------------------|------------------------------------|------|------------------|------------------------|------|------------------|
| | Lobar measures | | | | | | | | | | | |
| | Temporal lobe, (cm ³) | | Frontal lobe, (cm ³) | | Parietal lobe, (cm ³) | | Occipital lobe, (cm ³) | | | | | |
| | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} | B (95% CI) | p | p _{FDR} |
| Poor prenatal family functioning (FAD), per score | | | | | | | | | | | | |
| Model 1 | -0.87 (-1.85 to 0.11) | .083 | .226 | -1.10 (-2.52 to 0.32) | .128 | .260 | -1.09 (-2.17 to -0.01) | .166 | .266 | -0.25 (-1.25 to -0.35) | .442 | .593 |
| Model 2 | 0.51 (-1.56 to 0.53) | .334 | .605 | -0.75 (-2.26 to 0.75) | .327 | .605 | -0.61 (-1.76 to 0.52) | .288 | .845 | 0.09 (-0.58 to -0.77) | .783 | .845 |
| Age 9 poor family functioning (FAD), per score | | | | | | | | | | | | |
| Model 1 | 0.28 (-0.64 to 1.20) | .552 | .791 | 0.17 (-1.12 to 1.47) | .791 | .791 | 0.38 (-0.62 to 1.39) | .453 | .791 | -0.14 (-0.74 to -0.47) | .658 | .791 |
| Model 2 | 0.38 (-0.55 to 1.31) | .426 | .686 | 0.33 (-0.98 to 1.65) | .620 | .827 | 0.54 (-0.48 to 1.56) | .300 | .686 | -0.03 (-0.63 to -0.58) | .930 | .930 |
| <i>p Homogeneity for age of FAD assessment*</i> | | .122 | | | .376 | | | .110 | | | | .930 |

Note: Linear regression analysis of FAD and lobar measures. Betas are averaged from 10 imputed datasets. Model 1 is adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for paternal age, ethnicity, education, parity, marital status, paternal psychopathology, smoking and alcohol consumption. Global brain measures are not adjusted for total ICV. **p*_{FDR} = false discovery rate correction for multiple testing. Number of tests = 4 outcomes and the 2 relevant exposure periods (prenatal and early childhood). Critical value for FDR = 0.05. **p Homogeneity for age of FAD assessment* = Multiple-partial test used to test whether exposure from different time points of poor family functioning relates in the same manner to brain morphology outcome.

Table 7. Inverse probability weighting approach for the associations of poor prenatal family functioning and brain morphology.

| <i>Mother reported poor family functioning</i> | Brain morphology (N = 2,583) | | | | | | | | | |
|---|--|---|---------------------------------------|---|--|----------|------------------------|------|------------------------|------|
| | <i>Global brain measure</i> | | | <i>Specific brain volumetric measures</i> | | | | | | |
| | Total brain volume, (cm ³) | Cerebral white matter, (cm ³) | Total gray volume, (cm ³) | Amygdala volume, (cm ³) | Hippocampus volume, (cm ³) | <i>p</i> | | | | |
| | B (95% CI) | <i>p</i> | B (95% CI) | <i>p</i> | B (95% CI) | <i>p</i> | | | | |
| Poor prenatal family functioning FAD, per score | -9.50 (-17.9 to -1.13) | .026 | -4.19 (-8.07 to -0.32) | .034 | -5.16 (-10.1 to -0.31) | .037 | -0.02 (-0.05 to 0.005) | .107 | -0.08 (-0.13 to -0.02) | .004 |
| Age 5 poor family functioning (FAD), per score | -5.47 (-14.7 to 3.81) | .248 | -1.86 (-6.19 to 2.40) | .388 | -3.59 (-8.98 to 1.78) | .190 | -0.004 (-0.03 to 0.03) | .798 | -0.02 (-0.07 to 0.04) | .518 |

Note: Inverse probability weighting under missing data of poor prenatal family functioning and brain morphology outcome. Betas are averaged from 10 imputed datasets. Models are adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume), maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption. Global brain measures are not adjusted for total ICV.

Table 8. The associations of poor family functioning and microstructural measures of brain morphology stratified by hemispheres.

| <i>Mother reported poor family functioning</i> | Brain morphology (N = 2,583) | | | | | | | |
|---|---|----------------|-------------------------------------|---|-------------------------|--|--------------------------|------|
| | <i>Specific brain volumetric measures</i> | | | <i>Specific brain volumetric measures</i> | | | | |
| | Left amygdala | Right amygdala | Amygdala volume, (cm ³) | Left hippocampus | Right hippocampus | Hippocampus volume, (cm ³) | | |
| | B (95% CI) | <i>p</i> | B (95% CI) | <i>p</i> | B (95% CI) | <i>p</i> | | |
| Poor prenatal family functioning (FAD), per score | -0.12 (-0.27 to 0.03) | .110 | -0.009 (-0.025 to .006) | .243 | .039 (-0.066 to -.012) | .005 | -0.037 (-0.066 to -.009) | .011 |
| Age 5 poor family functioning (FAD), per score | -0.006 (-0.023 to .010) | .444 | .003 (-0.015 to .020) | .748 | -0.005 (-0.035 to .025) | .828 | -0.014 (-0.046 to .018) | .391 |

Note: Linear regression analysis of FAD and brain morphology outcome stratified by hemispheres. Betas are averaged from 10 imputed datasets. Models are adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume). Model 2 is additionally adjusted for maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption.

Table 9. The associations of poor family functioning and lobar measures stratified by hemispheres.

| | | Brain morphology (N = 2,583) | | | | | |
|---|--|--|---------------------------------------|--|---|----------------------|---|
| | | Lobar measures | | | | | |
| <i>Mother reported poor family functioning</i> | | Temporal lobe, (cm³) | Frontal lobe, (cm³) | Parietal lobe, (cm³) | Occipital lobe, (cm³) | | |
| | | B (95% CI) | p | B (95% CI) | p | B (95% CI) | p |
| Poor prenatal family functioning (FAD), per score | | | | Left lobar measures | | | |
| | | | | | | Right lobar measures | |
| | | | | | | Left lobar measures | |
| | | | | | | Right lobar measures | |
| Age 5 poor family functioning (FAD), per score | | | | Left lobar measures | | | |
| | | | | | | Right lobar measures | |
| | | | | | | Left lobar measures | |
| | | | | | | Right lobar measures | |

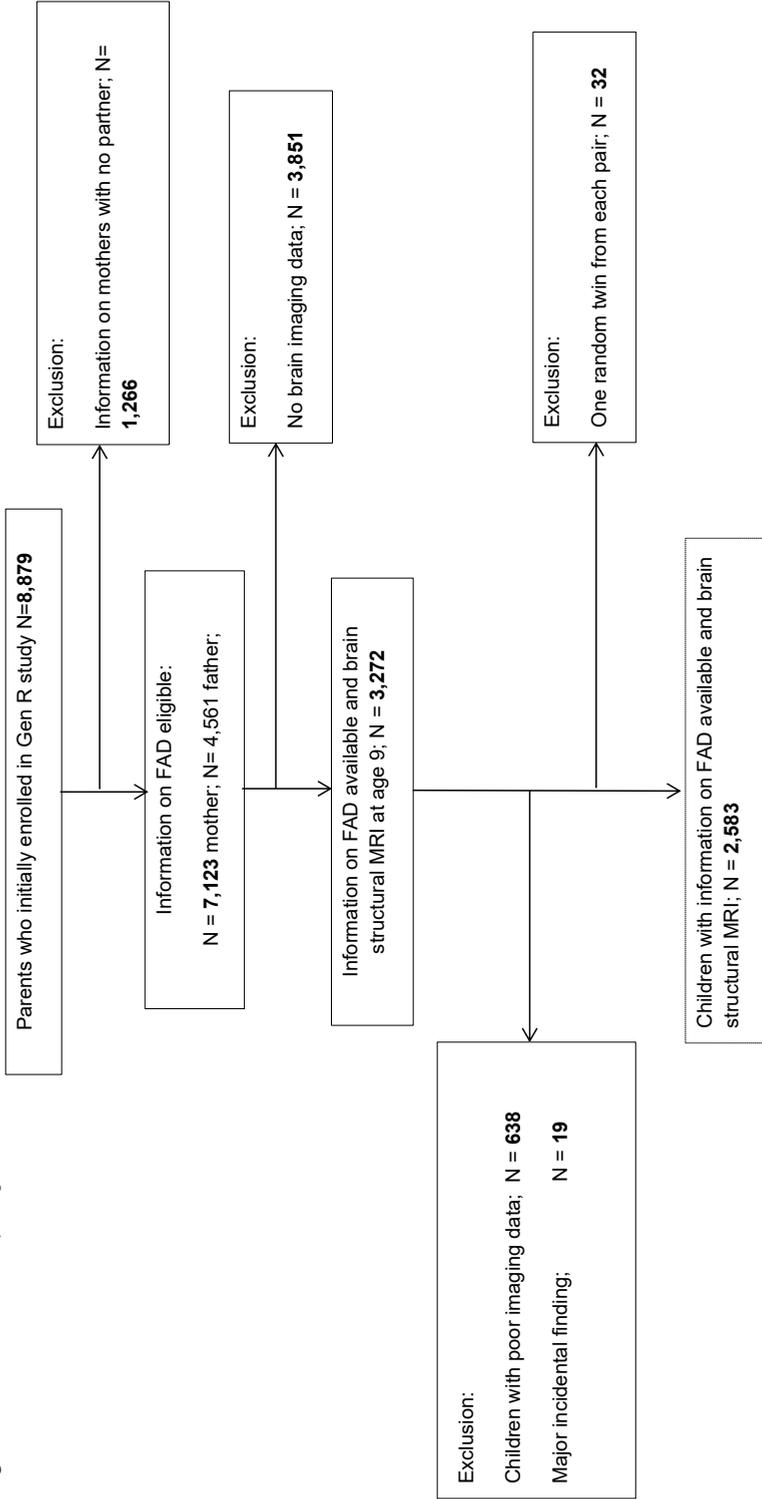
Note: Linear regression analysis of FAD and lobar measures stratified by hemispheres. Betas are averaged from 10 imputed datasets. Models are adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume), maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption.

eTable 10. Correlation coefficients between maternal and paternal report of poor family functioning.

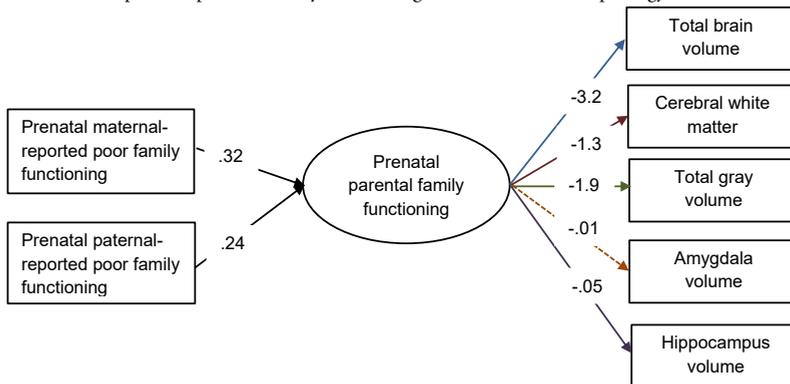
| | 1 | 2 | 3 | 4 | 5 |
|---------------------------|-------|-------|-------|-------|---|
| Poor family functioning | | | | | |
| 1 Prenatal, mother report | - | | | | |
| 2 Prenatal, father report | .45** | - | | | |
| 3 Age 5, mother report | .38** | .26** | - | | |
| 4 Age 9, mother report | .37** | .22** | .52** | - | |
| 5 Age 9, father report | .25** | .39** | .35** | .45** | - |

**Correlation is significant at the 0.01 level (2-tailed).

Figure 1. Inclusion of the study sample

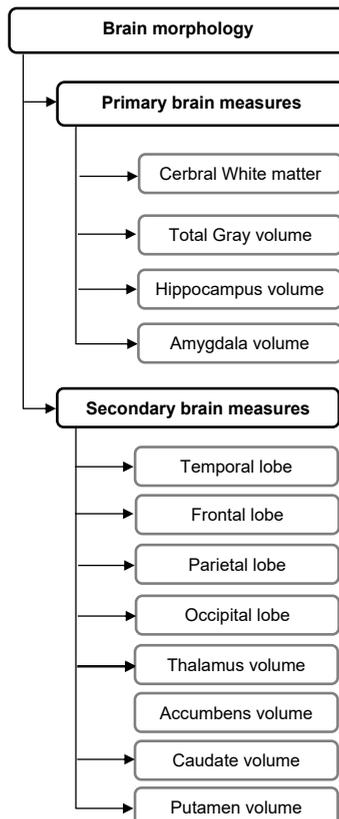


eFigure 2. Path model of prenatal parental family functioning factor and brain morphology.



Note: Structural equation modeling of parental family functioning factor and preadolescent brain morphology. Numeric values are standardized path regression coefficients of latent factor. Models are adjusted for child age at brain MRI scan, child sex and total ICV (total intracranial volume), maternal age, ethnicity, education, parity, marital status, maternal psychopathology, smoking and alcohol consumption. The dotted line represents the non-significant associations.

eFigure 3. The list of primary and secondary brain morphology



RESULTS.

Complementary sensitivity analyses

Latent factor model

The latent variable models suggest that prenatal parental poor family functioning factor is associated with smaller offspring total brain volume, cortical gray, white matter, and hippocampal volumes in late childhood, but not with amygdala volume (eFigure 2). The association between the latent construct of prenatal maternal- and paternal-reported family functioning and preadolescent brain morphology captures covariation across raters, or the extent to which a given dimension is reflected across parents (i.e., a “between-rater” dimension factor). Maternal and paternal family functioning were positively correlated. This model indicated good fit to the data. Overall, results were very similar to those using prenatal maternal-reported family functioning.

Generalized estimating equation

Tests for homogeneity of the associations of poor family functioning with hippocampal volumes at different child ages showed evidence for an interaction indicating that results differed by child age. The GEE estimates of poor family functioning and brain outcomes were very similar to the results in Table 5, only the CIs varied slightly because this method takes into account within-individual correlation across the time points.

Inverse probability weighting approach

We calculated inverse probability weights to reduce a possible selection bias in this cohort study, thereby adding to the representatives of the study population with respect to the full cohort recruited during pregnancy. That is, we corrected for potential selection bias that can arise when only parent and children with available predictor and outcome data were included.¹⁷ Overall, we used information available for all participants at recruitment to predict probability of participation in the study and used the inverse of those probabilities as weights in the analyses so that result would be representative for the initial population of this cohort study (eSupplementary Table 7).

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