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Maternal C-reactive protein concentration in early pregnancy and child autistic traits in the general population

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Abstract

Background—Exposure to elevated levels of inflammatory markers during pregnancy has been suggested as possible etiologic factor in the occurrence of autism spectrum disorder (ASD). In this study, we investigated the prospective relation between maternal C-reactive Protein (CRP) during early pregnancy and children's autistic traits in the general population.

Methods—In a large population-based cohort in the Netherlands, we measured maternal CRP levels before 18 weeks of gestation (N=4165). Parents reported on their children's autistic traits at age six years using the Social Responsiveness Scale, and the Pervasive Developmental Problem scale. Regression models were used to examine the relation between maternal CRP levels and autistic traits in children.

Results—Compared to the reference group (CRP <2.3 mg/L), elevated levels of CRP (>7.8 mg/L) in pregnant women were associated with higher Social Responsiveness Scale scores in children (B=0.055, 95% CI 0.033, 0.078); however, the effect was strongly attenuated after adjustment for several socioeconomic factors and in particular by maternal health-related factors including body mass index (fully adjusted model B=0.018, 95% CI -0.005, 0.042). We found no relation between maternal CRP levels and Pervasive Developmental Problem.

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Conclusions—Our results suggest that the association between elevated levels of maternal CRP in pregnancy and autistic traits in children is confounded by maternal health-related and socioeconomic factors. Further studies are needed to explore whether other maternal inflammatory markers during pregnancy, as a response to maternal inflammation, are associated with the development of autistic traits in the offspring.

Keywords

C-reactive protein; pregnancy; autistic traits; longitudinal; children

Autism spectrum disorder (ASD) is characterized by impairments in social interactions and communication and repetitive behavior.¹ Researchers have found high inheritance estimates for ASD, suggesting that ASD is largely caused by a genetic predisposition.² However, the increase in the incidence of ASD in the general population – although partly due to the combination of better case definition and/or broadening of the diagnostic concept³– and heritability estimates of maximal 80%,² highlight the importance of environmental risk factors in the etiology of ASD.

C-reactive protein (CRP) is an inflammatory marker that rises in response to infection or inflammation. Elevated levels of CRP are reported in patients with psychiatric disorders, for example schizophrenia.⁴⁻⁷ Therefore, it is suggested that raised CRP levels, as a result of an underlying inflammatory reaction, may have an etiological role in the development of psychiatric disorders. In addition, several epidemiological studies and animal models suggested an effect of maternal immune system activation during pregnancy on the risk of developing psychiatric diseases in offspring – for example, studies in humans showed a positive association of interferon gamma (IFN- γ), interleukin (IL)-4, IL-5, and IL-6 levels with the risk of developing ASD.^{8,9} In normal pregnancy, a mild systemic inflammatory reaction occurs in pregnant women which results in activation of the inflammatory network and therefore a raise of inflammatory markers. CRP is an inflammatory marker that is slightly elevated, up to 5.0 mg/L, during almost every normal pregnancy.^{10,11} Maternal CRP levels in pregnancy are increased as early as at four weeks of gestation, indicating that a maternal inflammatory response occurs when the implantation and development of the placenta takes place.¹¹ However, maternal CRP values higher than 5.0 mg/L may reflect a maternal inflammatory reaction to an underlying infection or a low-grade systemic inflammation. A recent population-based study in a Finnish National Birth cohort by Brown et al. demonstrated an association between maternal CRP levels during early pregnancy and the diagnosis ASD in the offspring.¹² They compared the data of 677 mother-child pairs with the diagnosis ASD to a matched control group and they showed that elevated maternal CRP levels (>58.4 mg/L) during the first trimester of pregnancy are related to the risk of developing ASD in the offspring. The authors concluded that a maternal immune reaction during pregnancy, which induces a release of inflammatory cytokines, leads to abnormal fetal brain maturation and, therefore, to the development of ASD in children.

ASD is a neurodevelopmental disorder that represents the extreme end of the normal distribution of autistic traits in the general population.¹³ Autistic traits are defined as clinical deficits in communication, social interaction and repetitive behavior that do not meet the

criteria for the diagnosis of ASD.¹³ Previous studies have shown that autistic traits in the general population are common, heritable, and continuously distributed.^{13,14} Environmental and genetic risk factors are consistent across the normal distribution of autistic traits, indicating an etiologic overlap between ASD at the end of the spectrum and subthreshold autistic traits.¹⁵ Furthermore, several studies have emphasized on the importance of establishing the course and neurobiology of the continuum of the autistic traits in the general population.^{16,17} To date, it is uncertain whether maternal low-grade inflammation during pregnancy is related to the development of autistic traits across the range of severity in the general population. Consequently, it is not known whether high levels of maternal inflammation markers during pregnancy are a precipitating factor for ASD in susceptible offspring or more generally determine the risk of autistic symptoms.

Against this background, we investigated the prospective relation between elevated levels of maternal high sensitivity CRP during early pregnancy and autistic traits in children at the age of 6 years. We hypothesized that elevated levels of maternal CRP during early pregnancy, as a marker of low-grade systemic inflammation and unspecific immune reaction in the mother, increases the risk of autistic-like traits across the spectrum in the general population. As several studies of CRP point to the importance of confounding,^{4,7,18,19} we carefully tested whether any relation in our study was confounded by socioeconomic status, life style factors and other health related outcomes.

Methods

Participants

This study was embedded within the Generation R Study, a population-based birth cohort in the Netherlands which follows children from fetal life onwards.^{20,21} Briefly, pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. The study was approved by the Medical Ethical Committee of the Erasmus Medical Center; and written informed consent was obtained from adult participants.

Of 7069 women enrolled in early pregnancy, blood samples were available in 6398 women and CRP was successfully measured in 5321 women. We excluded pregnant women with twin pregnancies ($n = 114$) due to possible differences in neurodevelopment of children born to singleton or twin pregnancies. This left a total of 5207 women. Of those, 4165 women (80%) reported autistic symptoms in their children at the age of 6 years. These mother-child pairs were included in the analyses.

We compared the 4165 mother-child pairs included in the analyses with the 1042 pairs excluded because of missing data on autistic traits. The maternal CRP levels were not different between mother-child pairs included in the study and those who were excluded. However, we found that the mothers excluded from analyses were younger (mean difference = -3.00 years, $p < 0.001$), less educated (21.9% versus 52.5% higher education, $p < 0.001$), and were mostly of a non-Western ethnic background (59.4% versus 26.7%, $p < 0.001$) than the mothers included in the analyses. In addition, excluded mothers smoked more during pregnancy (26.3% versus 14.6%, $p < 0.001$) and had a higher prenatal

psychopathology score (mean difference=0.15, $p<0.001$) than those who were included. Excluded children were smaller at birth (mean difference=-111 grams in birthweight, $p<0.001$).

Measurements

Maternal venous blood samples were collected in early pregnancy [mean=13.4 (1.9), range=5.9-17.9 weeks] and were transported to the regional laboratory in Rotterdam, the Netherlands, for further processing and storage. Blood samples were stored at -80°C at one location. High sensitivity CRP concentrations were measured in EDTA plasma samples in one laboratory in 2009. CRP levels were analyzed using an immunoturbidimetric assay on the Architect System (Abbot Diagnostics B.V., Hoofddorp, the Netherlands).²² The total precision (interassay variation) for CRP was 0.9% at 12.9 mg/L and 1.3% at 39.9 mg/L. The lowest level of detection was 0.2 mg/L.

Parents scored autistic traits in their children at age 6 years with the Social Responsiveness Scale (SRS), and Pervasive Developmental Problem scale (PDP), subscale of the Child Behavior Checklist (CBCL).

The SRS is a 65-item questionnaire that represents parental observations of a child's social behavior in a naturalistic setting, and provides a quantitative measure of autistic traits in children at the age of 4 to 18 years.²³ Higher SRS scores indicate more autistic problems. Several studies investigated the relevance of the SRS as a valid and reliable instrument for assessment of autistic traits in the general population or in the clinical setting.^{13,23,24} They reported high sensitivity and specificity of the SRS for diagnosis of ASD and confirmed that the SRS is a valid quantitative measure of autistic traits in clinical settings and in large-scale research studies of autism spectrum conditions. To minimize subject burden, we used a short version of the SRS with 18 items. In a sample of 2719 children from the Interactive Autism Network²⁵, the correlation between total scores derived from the 18-item SRS short-form and the complete SRS (65 items) was $r=0.99$ ($p<0.001$).

The CBCL is a 99-item questionnaire completed by parents based on their observations of a child's emotions and behavior in the preceding two months.²⁶ The PDP subscale is one of the five scales that can be derived from the CBCL preschool, consistent with the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) diagnostic categories. It has a good predictive validity to identify preschoolers at risk of ASD.²⁷ The PDP is a useful screening instrument for autistic problems when compared to the Autism Diagnostic Observation Schedule-Generic.^{28,29} The correlation between the CBCL PDP and the SRS scores in this study sample was $r=0.6$, $P\text{-value}<0.001$ ($N=4165$).

Information on parental age, parental education, maternal ethnic background, marital status, household income, maternal history of smoking and parity was collected during the first prenatal visit in pregnancy using questionnaires. Information on child's history of breast feeding was collected when the children were 6 months. Parental education was defined by the highest completed educational level. We created four categories of parental education level according to the classification of Statistics Netherlands: primary (no education or only primary school), secondary (lower or intermediate vocational education), higher education

phase 1 (higher vocational training), and higher education phase 2 (higher academic education). Maternal ethnic background was defined based on the country of birth according to Statistics Netherlands. Household income was divided in three categories: low income (1200 euro per month), middle income (1200-2000 euro per month), and high income (2000 euro per month). Maternal smoking was assessed at enrollment and in mid and late pregnancy. We used the Brief Symptom Inventory, a validated self-report questionnaire with 53 items, to measure maternal psychopathology in early pregnancy.³⁰ Brief Symptom Inventory encompasses eight subscales: depression, anxiety, hostility, psychoticism, paranoid ideation, interpersonal sensitivity and obsessive-compulsive. We used the Global Severity Index, an overall measure of maternal psychopathology. Higher scores indicated more problems. Maternal weight and length were measured at enrollment (first prenatal visit) and were used to calculate body mass index (BMI). Information on a child's gender and birthweight was obtained from midwives and hospital registries (at birth). Information on gestational age at birth was established using ultrasound examination during the first prenatal visit of pregnancy.

Statistical analysis

All children with data on maternal CRP in pregnancy and with SRS or PDP assessments were included in the analyses. In our study sample, missing values of the covariates ranged between 0 and 8.9%, except for maternal psychopathology (13.4%) and SRS scores (18.7%). Missing values were imputed using multiple imputations. Thirty copies of the original dataset were generated. In these copies, the missing values were replaced by values randomly generated from the predictive distribution on the basis of the association between the variable with missing values and other variables.

Maternal CRP during early pregnancy was the main determinant in all analyses. We performed the analyses with continuous and categorical CRP. In the continuous analyses, the values for CRP concentrations were divided by standard deviation in the sample, so that the association between CRP levels and autistic traits can be interpreted as standard deviation (SD) change in the determinant. We categorized maternal CRP levels in quartiles (corresponding CRP levels: 2.3, 4.3 and 7.8 mg/L) and CRP levels <2.3 mg/L were considered as the reference group in the analyses.

SRS and PDP scores were the outcomes in the analyses. The SRS scores were transformed using square root to satisfy the assumption of normality. After the transformation of the SRS scores, the residuals of the linear regression models had a normal distribution. To facilitate clinical interpretation, we used the 98th percentile of a Dutch norm group for the CBCL PDP scale as cut-off score to classify children with PDP within the clinical range versus those with scores in the non-clinical range (n=119).³¹ To examine the relation between maternal CRP and children's autistic traits, we used linear regression for SRS scores and logistic regression for CBCL PDP dichotomized scores.

Identification of the confounders was grounded on understanding of the causal network associated with maternal inflammation and child's autistic traits using a causal diagram.^{4,5,32} For example existing literature suggests that maternal body mass index is related to both maternal CRP levels³² and children's neurodevelopment³³ and could potentially act as a

confounder in the analyses. This method is widely used for selection of variables that could interfere with the causal effect of the investigated association in order to control for confounding. For the univariate association of the covariates with SRS and CRP, please see Supplementary Table 1 and 2. The association between maternal CRP and children's autistic traits was examined in five steps: *Model 1* adjusted for children's gender and age at the time of assessment. *Model 2* additionally adjusted for maternal age, maternal educational levels, maternal ethnic background, marital status, maternal history of smoking and maternal psychopathology in pregnancy. *Model 3* additionally adjusted for paternal age, paternal educational levels and household income. *Model 4* additionally adjusted for parity, time of blood sampling during pregnancy and maternal body mass index. *Model 5* additionally adjusted for children's gestational age, birthweight and breast feeding at 6 months that could potentially mediate the relation between maternal CRP in pregnancy and children's autistic traits.

Gender differences are well established in ASD symptoms; in addition, evidence suggests gender-specific vulnerability to maternal inflammation in pregnancy.³⁴ Therefore, we explored an interaction between children's gender and maternal CRP levels in relation to autistic traits.

Results

Characteristics of the study participants are presented in Table 1. In our sample, the mean age of women at enrollment was 30.7 years. Most of the women completed higher education (52.4%) and had a Dutch ethnic background (61.0%). In total, 14.5% of the mothers continued smoking during pregnancy and 6.1% of the parents were from low income families. Mean gestational age at birth was 40.0 weeks and mean birthweight of the children was 3454 grams. Mean CRP level during early pregnancy was 6.49 mg/L.

We found an association between maternal CRP levels and SRS scores in the basic model adjusted for child's gender and age at the time of assessment (CRP >7.8 mg/L; Table 2). However, this association was strongly attenuated after adjustment for confounding factors. Maternal socioeconomic factors including age, educational level, ethnic background, marital status, history of smoking and psychopathology changed the effect size to $B = 0.029$ (95% CI 0.008, 0.051) (Model 2). Model 3 shows that the paternal socioeconomic factors including age, educational level and household income, did not materially change the effect size in the association of continuous maternal CRP levels with SRS scores ($B = 0.028$, 95% CI -0.006 , 0.050). The maternal health related factors such as parity, time of blood sampling during pregnancy and body mass index, additionally attenuated the relation: $B = 0.019$ (95% CI -0.005 , 0.042) (Model 4). Model 5 shows that the child related factors including gestational age, birthweight and breast feeding at 6 months did not influence the observed association. Similar results and confounding patterns emerged from the analyses of maternal CRP levels in quintiles with the SRS scores.

We found no relation between maternal CRP levels and CBCL PDP in the clinical range (fully adjusted model OR 1.4, 95% CI 0.8, 2.4) (Table 3). There was no interaction between

maternal CRP and the children's gender in the association with children's autistic traits at age 6 years.

Comment

In this large, population-based study, we found no relation between elevated levels of maternal CRP during early pregnancy and parent-reported autistic traits in children at the age of 6 years. Our findings suggest that the relation between maternal CRP levels and child autistic traits is strongly confounded by maternal socioeconomic and health-related factors such as educational level and body mass index.

Several studies examined the effect of elevated maternal inflammatory markers during pregnancy, as a response to a maternal infection or inflammation, on the risk of autism in children. For example, Goines et al., and Parker-Athill and colleagues examined the effect of high maternal cytokine profiles on the development of offspring psychiatric diseases and found an association between elevated maternal IL-4, IL-5, IL-6 and IFN- γ during pregnancy and the development of ASD in children.^{8,9} In addition, a population-based study in a national birth cohort by Brown et al. reported an association between elevated levels of maternal CRP in early pregnancy and the diagnosis of ASD in children.¹² These results suggest an association between raised maternal inflammatory markers during pregnancy and the development of ASD in children. Therefore, we investigated the prospective relation between elevated levels of maternal high sensitivity CRP during early pregnancy, as a marker of low-grade systemic inflammation and unspecific immune reaction in the mother, and autistic traits across the whole spectrum in the children at the age of 6 years. However, after adjustment for confounding factors we did not find an association between maternal CRP levels during pregnancy and the risk of autistic traits in children.

Our results differ from the findings of the study by Brown and colleagues¹² on the prospective association of maternal CRP levels during pregnancy and a diagnosis of ASD in children. There are two possible explanations for these different results. First, in this study, the outcome measure was autistic traits in children, while in the study by Brown et al. the outcome measure was the diagnosis ASD. Despite the fact that socio-economic and genetic risk factors are suggested to be associated with the continuum of autistic symptoms –and ASD at the extreme end of this continuum–, our findings on autistic traits in a non-clinical population-based sample might differ from the results found in a national cohort including children diagnosed with ASD. Second, Brown et al. considered several confounding factors in their study including child related factors (preterm birth and low birthweight), parental socioeconomic factors (maternal and paternal age, number of previous births, socioeconomic status and gestational time of maternal blood sampling) and parental history of psychiatric disorders. However, maternal educational level as socioeconomic factor and maternal body mass index as health related factor, were not included in the analyses. As the present study suggests that these factors are important confounders in studies investigating maternal CRP and children's autistic traits, residual confounding remains an alternative explanation for the inconsistencies between the studies.

Observational studies as the study by Brown et al. or the present study attempt to control confounding by conditioning the associations on a large number of factors associated with maternal CRP levels or children's neurodevelopment. Nevertheless, the association of interest may be further influenced by other unmeasured paternal factor or an unmeasured maternal health conditions that precede both the exposure and outcome. Furthermore, socioeconomic status or general health are factors with many levels and a model that uses a surrogate measure of socioeconomic status or health could possibly fail to completely block the confounding paths. Therefore, observational studies, even those considering a large number of socioeconomic and health-related confounding factors, cannot fully address the causality of the association between elevated levels of CRP and various health outcomes. To evaluate the possible causality of intrauterine influences, it has been proposed to compare the associations of maternal exposures (e.g. CRP levels) to those of the respective paternal exposures on behavioral outcomes in the offspring. Any similarity between the results would point to the confounding effect of familial or shared environmental factors, such as socioeconomic status. However, Mendelian randomization, in which natural assortment of genetic material at conception is used for randomization of exposed and non-exposed groups is probably the only approach to rule out that results are explained by residual confounding in studies on CRP.³⁵

To our knowledge, the present study is one of the largest prospective population-based, non-clinical studies that examine the effect of elevated CRP levels in mothers during early pregnancy on autistic traits in children. However, the study has several limitations. First, we measured maternal CRP levels only at one time point in pregnancy. As a result of the relatively short half-life time of CRP (<12 hours), it was not possible to determine whether the elevated CRP levels were a result of an acute or chronic maternal inflammatory reaction. Only if data on repeated measurements of CRP are available during pregnancy, one is able to distinguish consequences of acute inflammation and chronic conditions in pregnant women. Second, there was no measure of maternal autistic traits available. Third, as in many observational studies we observed two types of non-response effect: non-response at baseline and loss to follow-up that could introduce bias in the association, and influence the generalizability of the findings. However, loss to follow-up was not associated with the CRP levels.

In conclusion, the findings of the present study suggest that the relation between maternal CRP levels and child autistic traits is strongly confounded by maternal socioeconomic and health-related factors. Residual confounding remains a major problem on associations with CRP levels. Further studies with more specific inflammatory markers are needed to find out whether a raised maternal inflammatory marker during pregnancy, as a response to maternal inflammation, predicts for the development of autistic traits in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of participants, n=4165

	N for valid observation	Mean (SD)
Maternal characteristics		
Age at enrollment, years	4165	30.7 (4.7)
Educational levels, %	4027	
No education or primary school		17.6
Secondary school		30.0
Higher education phase 1		23.7
Higher education phase 2		28.7
Ethnicity, %	4159	
Dutch		61.0
Other Western		12.4
Non Western		26.6
Marital Status, married/with partner, %	3989	90.5
History of smoking	4122	
Never		77.1
Quit when pregnancy known		8.4
Continued in pregnancy		14.5
Psychopathology in pregnancy, score	3606	0.25 (0.33)
Paternal characteristics		
Age at enrollment, years	3786	33.4 (5.4)
Educational level, %	3158	
No education or primary school		19.0
Secondary school		25.2
Higher education phase 1		20.3
Higher education phase 2		35.6
Household income, %	3797	
<€1200		6.1
>€1200 and <€2000		15.0
>€2000		78.9
Maternal health characteristics		
Parity, primipara, %	4147	59.7
Time of blood sampling in pregnancy, weeks	4165	13.4 (1.9)
Body mass index at enrollment	4142	24.3 (4.2)
Children's characteristics		
Age at time of assessment, months	3440	74 (6)
Gender, boy, %	4165	49.8
Gestational age at birth, weeks	4165	40.0 (1.7)
Birthweight, grams	4163	3454 (548)
Breast feeding at 6 months, yes, %	3331	32.9
Social Responsiveness Scale, score	3388	0.22 (0.24)

	N for valid observation	Mean (SD)
Pervasive Developmental Problem Scale, score	4025	2.26 (2.54)
Maternal C reactive protein, mg/L	4165	6.49 (9.38)

Numbers are mean (SD) unless otherwise indicated.

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Maternal C-reactive protein (CRP) levels in early pregnancy and Social Responsiveness Scale scores in children at the age of 6 years. The Generation R Study.

Table 2

Social Responsiveness Scale					
	Model 1 ¹	Model 2 ²	Model 3 ³	Model 4 ⁴	Model 5 ⁵
CRP mg/L	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
Continuous ⁶	0.009 (0.003, 0.015)	0.005 (0.000, 0.011)	0.005 (0.000, 0.011)	0.004 (−0.002, 0.009)	0.004 (−0.002, 0.009)
2.3	Reference	Reference	Reference	Reference	Reference
2.4-4.3	0.008 (−0.013, 0.030)	0.003 (−0.017, 0.024)	0.001 (−0.020, 0.022)	−0.001 (−0.022, 0.021)	0.000 (−0.021, 0.021)
4.4-7.8	0.030 (0.008, 0.052)	0.014 (−0.007, 0.035)	0.013 (−0.008, 0.035)	0.008 (−0.013, 0.030)	0.008 (−0.014, 0.030)
>7.8	0.055 (0.033, 0.078)	0.029 (0.008, 0.051)	0.028 (−0.006, 0.050)	0.019 (−0.005, 0.042)	0.018 (−0.005, 0.042)

The B's are not interpretable since the mathematically transformed scores were used in the analyses.

¹ Basic model adjusted for children's age at the time of assessment and gender.

² Additionally adjusted for maternal age, maternal educational levels, maternal ethnic background, marital status, maternal history of smoking and maternal psychopathology in pregnancy.

³ Additionally adjusted for paternal age, paternal educational levels and household income.

⁴ Additionally adjusted for parity, time of blood sampling during pregnancy and maternal body mass index.

⁵ Additionally adjusted for children's gestational age, birthweight and breast feeding at 6 months.

⁶ The values for continuous CRP concentrations were divided by standard deviation in the sample.

Maternal C-reactive protein (CRP) levels in early pregnancy and Pervasive Developmental Problems in children at the age of 6 years. The Generation R Study.

Table 3

Pervasive Developmental Problems In the Clinical Range					
	Model 1 ¹	Model 2 ²	Model 3 ³	Model 4 ⁴	Model 5 ⁵
CRP mg/L	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Continuous ⁶	1.0 (0.9, 1.2)	1.4 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)
2.3	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
2.4-4.3	0.9 (0.6, 1.1)	0.8 (0.4, 1.4)	0.8 (0.4, 1.4)	0.7 (0.4, 1.4)	0.8 (0.4, 1.4)
4.4-7.8	1.3 (0.8, 2.3)	1.2 (0.7, 2.1)	1.2 (0.7, 2.1)	1.1 (0.7, 2.0)	1.1 (0.6, 2.0)
>7.8	1.7 (1.1, 2.8)	1.5 (0.9, 2.6)	1.5 (0.9, 2.6)	1.4 (0.8, 2.4)	1.4 (0.8, 2.4)

Pervasive Developmental Problems in the Clinical Range were defined as scores above the 98th percentile of a Dutch norm population

Pervasive developmental problems were defined as the scores in the highest 2% of the Child Behavior Checklist pervasive developmental problems scale (n=119)

¹ Basic model adjusted for children's age at the time of assessment and gender.

² Additionally adjusted for maternal age, maternal educational levels, maternal ethnic background, marital status, maternal history of smoking and maternal psychopathology in pregnancy.

³ Additionally adjusted for paternal age, paternal educational levels and household income.

⁴ Additionally adjusted for parity, time of blood sampling during pregnancy and maternal body mass index.

⁵ Additionally adjusted for children's gestational age, birthweight and breast feeding at 6 months.

⁶ The values for continuous CRP concentrations were divided by standard deviation in the sample.