

## ORIGINAL ARTICLE

# Elevated maternal C-reactive protein and autism in a national birth cohort

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Autism is a complex neuropsychiatric syndrome with a largely unknown etiology. Inflammation during pregnancy may represent a common pathway by which infections and other insults increase risk for the disorder. Hence, we investigated the association between early gestational C-reactive protein (CRP), an established inflammatory biomarker, prospectively assayed in maternal sera, and childhood autism in a large national birth cohort with an extensive serum biobank. Other strengths of the cohort included nearly complete ascertainment of pregnancies in Finland ( $N = 1.2$  million) over the study period and national psychiatric registries consisting of virtually all treated autism cases in the population. Increasing maternal CRP levels, classified as a continuous variable, were significantly associated with autism in offspring. For maternal CRP levels in the highest quintile, compared with the lowest quintile, there was a significant, 43% elevated risk. This finding suggests that maternal inflammation may have a significant role in autism, with possible implications for identifying preventive strategies and pathogenic mechanisms in autism and other neurodevelopmental disorders.

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## INTRODUCTION

Autism is a complex neurodevelopmental syndrome of the central nervous system, which is characterized by severe disruptions in language and reciprocal social interactions, and a restricted repertoire of behaviors and interests.<sup>1</sup> The etiology of autism is largely unknown, although an important role for genetic factors has been suggested by family and twin studies.<sup>2</sup> New evidence, however, indicates that environmental etiologies may also contribute to risk of this disorder. In particular, a recent twin study suggests that the role of genetic factors may have been overestimated in previous studies, and the role of environmental etiologies may have been underestimated,<sup>3</sup> though previous studies indicate greater genetic and lesser environmental liability.<sup>4–6</sup>

Prenatal infection and immune dysfunction are biologically plausible potential causes of autism.<sup>7,8</sup> There is substantial evidence that the immune response alters the development of the central nervous system during fetal life,<sup>8,9</sup> and it has been proposed that maternal inflammation may represent a pathway by which infectious and other insults may give rise to neurodevelopmental disorders.<sup>8,10</sup> Preclinical studies demonstrated that maternal immune activation with synthetic double-stranded RNA (poly I:C) caused a delay in migration of cerebellar granule cells in lobule VII;<sup>11</sup> cerebellar abnormalities, including altered volume of the vermis, and decreased numbers of Purkinje cells have been demonstrated in the brains of autistic subjects, though other brain regions have been implicated.<sup>12,13</sup>

Epidemiological studies have provided intriguing clues for infectious or immune risk factors for autism. In a birth cohort with

known prenatal exposure to rubella, 8% were demonstrated to have autism.<sup>14</sup> In an intriguing investigation of 17 cytokines assessed in archived maternal serum samples from mid-pregnancy in the Early Markers of Autism Study, based in California, significantly increased levels of interferon- $\gamma$ , interleukin-4 and interleukin-5 were associated with autism spectrum disorders (ASD).<sup>15</sup> In an interesting Danish birth cohort study that used psychiatric registry linkages, elevated levels of tumor necrosis factor- $\alpha$  and tumor necrosis factor- $\beta$  in amniotic fluid were associated with increased ASD risk among offspring.<sup>16</sup> Further analyses revealed increased interleukin-4 and interleukin-5 in amniotic fluid of male ASD cases, and increased interleukin-10. Moreover, in this same cohort, amniotic fluid levels of Monocyte Chemoattractant Protein-1 were elevated in a subgroup of ASD cases diagnosed by ICD-10 criteria and in infantile autism cases.<sup>17</sup> These findings were interpreted as indicating an intrauterine inflammatory state compensated by an anti-inflammatory response. Prior studies, however, were limited to some degree by the lack of adjustment for multiple comparisons, and other methodologic issues (see 'Discussion'). In another Danish study, maternal hospitalization for viral infection in the first trimester and bacterial infection in the second trimester were associated with increased risks of ASD.<sup>18</sup> Moreover, maternal cytokine elevations, including interleukin-8 and tumor necrosis factor- $\alpha$ , have been associated previously with schizophrenia.<sup>19,20</sup>

C-reactive protein (CRP) is an acute-phase reactant, which is a well-established marker of low-grade inflammation from both infectious and noninfectious exposures but which may also

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become substantially elevated during acute infection, chiefly by the secretion of interleukin-6 (IL-6).<sup>21</sup> Hence, in the present study, we tested the hypothesis that elevated prospectively measured maternal CRP during early gestation is related to an increased risk of childhood autism in offspring. The study was conducted in the Finnish Prenatal Study of Autism, which capitalizes on a large and representative sample of pregnancies from a national birth cohort with prospectively collected and archived maternal serum specimens from an extensive biobank and well-validated offspring diagnoses of virtually all childhood autism cases in Finland from national registries of both hospital admissions and outpatient treatment.

## MATERIALS AND METHODS

The methods are described in detail in Lampi *et al.*<sup>22</sup>, and will be summarized here. The Finnish Prenatal Study of Autism is based on a nested case-control design. The sampling frame was defined so that all members of the cohort were within the age of risk of autism. For this purpose, the sampling frame consisted of all offspring born in Finland from 1987 to 2005, and subjects were followed up until 2007 (see 'Case and control identification').

### Description of the cohort and biobank

All offspring in the Finnish Prenatal Study of Autism were derived from the Finnish Maternity Cohort, which consists of over 1 million pregnancies with archived prenatal serum specimens that were drawn beginning in 1983. Sera are drawn during the first and early second trimesters from over 98% of pregnant women in Finland, following informed consent, for screening of HIV, syphilis and hepatitis. One maternal serum sample is obtained for each pregnancy. After the screening, serum samples are stored as one aliquot at  $-25^{\circ}\text{C}$  in a single, centralized biorepository at the National Institute of Health and Welfare in Oulu, Finland. All of the serum samples in the Finnish Maternity Cohort can be linked with offspring by a unique personal identification number, which has been assigned to all residents of Finland since 1971.

### Finnish Medical Birth Registry

The Finnish Medical Birth Registry includes comprehensive and standardized data on demographics, pregnancy, the prenatal period and the neonatal period up to age 7 days on all births in Finland. The registry includes the personal identification number codes of mothers and live born children that can be used to link the subjects with the other registries.

### Case and control identification

The Finnish Hospital and Outpatient Discharge Registry was used to identify all recorded diagnoses for psychiatric hospital admissions and outpatient visits. Computerized data are available from 1987 to the present. The registry contains the personal and hospital identification code and primary/secondary psychiatric diagnoses.

In order to identify the autism cases for the present study, we conducted a record linkage between the Finnish Medical Birth Registry and the Finnish Hospital Discharge and Outpatient Registry, using the personal identification numbers. Cases with childhood autism (ICD-10 F84.0) in the sampling frame were followed up from 1987 to 2007. Over this time period, there were 1.2 million births. The total number of childhood autism cases in the study sample was 1132.

In order to validate the registry diagnoses, 80 cases of infantile autism from the Finnish Hospital and Outpatient Discharge Registry were assessed using the Autism Diagnostic Interview-Revised. Among these cases, 77 (96%) met the criteria for childhood autism by the Autism Diagnostic Interview-Revised.<sup>23</sup>

The childhood autism cases were matched 1:1 to controls drawn from the birth cohort who were without ASD or severe/profound mental retardation (MR) on date of birth, sex, birthplace and residence in Finland. A total of 677 cases and 677 matched controls had maternal sera available for CRP testing and were included in all analyses.

The study was approved by the ethical committees of the hospital district of Southwest Finland, National Institute of Health and Welfare, and the Institutional Review Board of the New York State Psychiatric Institute. Informed consent was obtained before acquisition of all maternal serum

specimens after the nature and possible consequences of the procedure and data derived from serum analyses were explained.

### CRP assay

CRP measurements were carried out blind to case/control status. CRP was measured on the clinical chemistry analyzer Architect c8200 (Abbott Laboratories, Abbott Park, IL, USA) using a latex immunoassay (Sentinel, Milan, Italy). During the course of the study, the precision between series expressed as the coefficient of variation (mean  $\pm$  s.d.) was  $5.1 \pm 2.3\%$  and the systematic error (bias) (mean  $\pm$  s.d.) was  $-2.7 \pm 7.4\%$ . Assay sensitivity is  $0.10 \text{ mg dl}^{-1}$ .

### Covariates

The covariates included maternal age, paternal age, number of previous births, socioeconomic status, preterm birth, low birth weight, maternal/parental history of psychiatric disorders and gestational week of the maternal blood draw. In accord with the extant epidemiologic literature, covariates were considered for inclusion in the statistical models based on their meeting both of the following criteria: (1) association with maternal CRP ( $P < 0.1$ ); (2) association with childhood autism ( $P < 0.1$ ).<sup>24-26</sup> Given that we are presently preparing several manuscripts on the covariates in relation to autism risk in this cohort, we elected to include only the  $P$  values for the bivariate results of covariates in relation to autism (Table 1).

### Statistical analysis

The analysis was based on a nested case-control design, in which the controls for each case were matched from the population at risk (the Finnish Prenatal Study of Autism birth cohort) on selected factors, elaborated in 'Case and control identification.' We first examined maternal CRP as a continuous measure. Given the skewed distribution of CRP, the variable was log transformed before analysis.

In order to facilitate interpretation of the data, we then examined maternal CRP as a categorical variable. In this analysis, maternal CRP levels were categorized in quintiles. The quintiles for the case and control groups in the analyses were derived from the cut-points of CRP levels that defined the quintiles for this biomarker in the control group. Separate analyses were conducted for CRP in each quintile and autism in relation to the reference group. We hypothesized that a significant association would be observed for CRP classified in the highest quintile compared with the reference group, which was defined as the lowest quintile. This analytic scheme has been commonly used in many previous epidemiologic studies including those on CRP<sup>27</sup> and cholesterol.<sup>28</sup> In order to assess whether the risk of autism was greater at even higher CRP levels, we classified CRP in deciles in a further analysis. We hypothesized that a significant association would be observed for CRP classified in the highest decile compared with the reference group, which was defined as the lowest decile. Appropriate to the nested case-control study design, point and interval estimates of odds ratios were obtained by fitting conditional logistic regression models for matched sets. Statistical significance was judged at  $P < 0.05$ . After examining the main effects, we then investigated whether the effect of maternal CRP on autism risk was modified by sex. For this purpose, sex and sex by CRP interaction terms were added to the statistical model. The interaction term was deemed to be statistically significant based on  $P < 0.05$ . In a supplementary analysis, we examined the relationships between maternal CRP and childhood autism in subgroups stratified by the presence or absence of concurrent MR. For this purpose, the following ICD-10/9 codes, respectively, for MR, were used: F70/317, F71/318.0, F72/318.1, F73/318.2, F78 (no ICD-9 code) and F79/319. Statistical analyses were performed with SAS software (SAS 9.2, SAS Institute, Cary, NC, USA).

## RESULTS

The total sample of childhood autism cases in the study was 1132. Of these, 677 cases were included in this study. These cases did not differ from the 455 cases that were not included on maternal age ( $P = 0.78$ ), paternal age ( $P = 0.31$ ), sex ( $P = 0.11$ ), previous numbers of births ( $P = 0.41$ ), maternal socioeconomic status ( $P = 0.91$ ), gestational age ( $P = 0.38$ ) or birth weight ( $P = 0.37$ ).

## Covariates

Relationships between the covariates and maternal CRP levels, and childhood autism, are provided in Table 1. These covariates included maternal age, paternal age, number of previous births, maternal socioeconomic status, preterm birth, low birthweight, maternal/parental history of psychiatric disorders and gestational week of the blood draw. Greater maternal age, greater paternal age, parental schizophrenia spectrum disorders, parental affective disorders and 'any maternal psychiatric disorder' were associated with an increased risk of autism. None of the covariates met the *a priori* criteria for confounding, which are based on standard epidemiologic texts (see 'Materials and methods').<sup>24–26</sup> Specifically, no covariate was associated with both maternal CRP level and childhood autism. For further reassurance, however, we adjusted for gestational age of the blood draw and number of previous births, which were associated with maternal CRP at a high level of statistical significance. Moreover, given previous associations between CRP and depression,<sup>29</sup> we adjusted for maternal lifetime history of depression (ICD-8 300.40, 300.41, 296.00, 296.20, 298.00, ICD-9 196.1 A-F, 196.3 A-F, 296.8 A, 300.4 A, ICD-10 F32, F33, F34.1, F41.2, F43.20, F43.21, F43.22) in a separate analysis.

## CRP as a continuous variable

We first examined maternal CRP modeled as a continuous variable in relation to risk of childhood autism in offspring. The analysis revealed a significant association between increasing maternal CRP and risk of autism (OR = 1.12, 95% CI = 1.02–1.24, *P* = 0.02). For further reassurance, we adjusted for number of previous births and gestational week of the blood draw. There was a slight increase in the magnitude of association (OR = 1.14, 95% CI = 1.02–1.27), and no change in the significance level (*P* = 0.02). Moreover, the finding was essentially unchanged following adjustment for maternal lifetime depression (OR = 1.12, 95% CI = 1.01–1.24, *P* = 0.028).

## CRP as a categorical variable

The results for maternal CRP level by quintile and childhood autism are presented in Table 2. There was a greater than 40% increase in risk of childhood autism following exposure to elevated maternal CRP, defined *a priori* as a CRP level in the highest quintile (> 5.84 mg dl<sup>-1</sup>), compared with maternal CRP in the lowest quintile (0.10–0.92 mg dl<sup>-1</sup>) (OR = 1.43, 95% CI = 1.02–2.01, *P* = 0.039). The findings for maternal CRP by decile and

**Table 1.** Covariates in relation to maternal early gestational CRP levels in controls and in relation to risk of childhood autism

Covariates <sup>a</sup>	CRP				Relationship between covariates and childhood autism
	≥ median	< median	χ <sup>b</sup> /t	P	P
Maternal age (mean, s.d.)	29.8 (5.3)	29.6 (5.4)	−0.63	0.53	0.002
Paternal age (mean, s.d.)	32.5 (6.4)	32.2 (6.3)	−0.81	0.42	0.005
Previous births (N (%) ≥ 2)	102 (58.9)	71 (41.0)	7.34	0.007	0.35
<i>Maternal socioeconomic status<sup>b</sup></i>					
Upper white collar (N (%))	40 (43.5)	52 (56.5)	9.91	0.02	0.74
Lower white collar (N (%))	135 (53.2)	119 (46.9)			
Blue collar (N (%))	72 (61.5)	45 (38.5)			
Other (N (%))	44 (43.5)	57 (56.4)			
Preterm birth ((N (%)) < 37 weeks)	13 (40.6)	19 (59.3)	1.19	0.27	0.19
Low birth weight ((N (%)) < 2500 g)	10 (50.0)	10 (50.1)	0		0.09
<i>Family history (N (%) for each diagnostic category)</i>					
Maternal SSD	3 (50)	3 (50)	0	0.99	0.15
Parental SSD	4 (44.4)	5 (55.6)	0.12	0.73	0.03
Maternal affective	8 (57.1)	6 (42.9)	0.29	0.59	0.13
Maternal depression	6 (54.5)	5 (45.5)	0.09	0.76	0.10
Parental affective	13 (46.4)	15 (53.6)	0.15	0.69	0.03
Maternal PDD	0	0	—	—	0.32
Parental PDD	0 (0)	1 (100)	1.00	0.32	1.00
Any maternal psychiatric disorder	9 (50)	9 (50)	0	0.99	0.04
Any parental psychiatric disorder	28 (43.7)	36 (56.2)	1.13	0.29	0.11
Gestational week of blood draw (mean, s.d.) <sup>c</sup>	11.4 (3.5)	10.3 (3.3)	−5.62	<0.001	0.53

Abbreviations: PDD, Pervasive developmental disorder; SSD, Schizophrenia spectrum disorder.

<sup>a</sup>There were no missing data on any covariates other than those noted in footnotes 2 and 3. <sup>b</sup>Frequency missing = 91 cases, 113 controls. <sup>c</sup>Frequency missing = 31 cases, 26 controls.

**Table 2.** Maternal early gestational CRP levels by quintile in childhood autism cases and matched controls

CRP by quintile (%), (Range (mg dl <sup>-1</sup> ))	Cases (N, %)	Controls (N, %)	OR (95% CI)	P
≤ 20 (0.10–0.92)	119 (17.6%)	137 (20.2%)	1	NA
21–40 (0.93–1.77)	112 (16.5%)	134 (19.8%)	0.97 (0.68–1.37)	0.85
41–60 (1.78–3.18)	142 (20.1%)	137 (20.2%)	1.21 (0.86–1.69)	0.27
61–80 (3.19–5.83)	140 (20.7%)	135 (19.9%)	1.21 (0.86–1.69)	0.27
> 80 (5.84–88.9)	164 (24.2%)	134 (19.7%)	1.43 (1.02–2.01)	0.039

Abbreviations: CRP, C-reactive protein; CI, confidence interval; OR, odds ratio.

**Table 3.** Maternal early gestational C-reactive protein (CRP) levels by decile in childhood autism cases and matched controls

CRP by decile (%) [Range (mg dl <sup>-1</sup> )]	Cases, N (%)	Controls, N (%)	OR (95% CI)	P
≤10 (0.10–0.57)	45 (6.5)	71 (10.5)	1	NA
11–20 (0.58–0.92)	74 (10.9)	66 (9.7)	1.76 (1.06–2.92)	0.03
21–30 (0.93–1.31)	51 (7.5)	68 (10.0)	1.15 (0.70–1.89)	0.58
31–40 (1.32–1.77)	61 (9.0)	66 (9.7)	1.51 (0.89–2.57)	0.13
41–50 (1.78–2.42)	69 (10.2)	69 (10.2)	1.62 (0.98–2.66)	0.06
51–60 (2.43–3.18)	73 (10.8)	68 (10.0)	1.68 (1.02–2.78)	0.04
61–70 (3.19–4.33)	80 (11.8)	66 (9.7)	1.92 (1.17–3.14)	0.01
71–80 (4.34–5.83)	60 (8.9)	69 (10.2)	1.37 (0.83–2.26)	0.22
81–90 (5.84–9.54)	89 (13.1)	67 (9.9)	2.08 (1.28–3.40)	0.003
91–100 (9.55–88.90)	75 (11.1)	67 (9.9)	1.80 (1.09–2.97)	0.02

Abbreviations: CRP, C-reactive protein; CI, confidence interval; OR, odds ratio.

childhood autism are presented in Table 3. We observed an 80% increase in risk of childhood autism following exposure to elevated maternal CRP, defined *a priori* as a CRP level in the highest decile ( $>9.55$  mg dl<sup>-1</sup>), compared with the lowest decile (0.10–0.57 mg dl<sup>-1</sup>) (OR = 1.80, 95% CI = 1.09–2.97,  $P=0.02$ ). The findings were not altered appreciably following adjustment for number of previous births and gestational week of the blood draw (highest versus lowest quintile: OR = 1.46, 95% CI = 1.01–2.11,  $P=0.045$ ; highest versus lowest decile: OR = 1.83, 95% CI = 1.06–3.17,  $P=0.03$ ). The findings were also essentially unchanged adjusting for maternal lifetime depression in both the quintile and decile analysis (quintile: OR = 1.41, 95% CI = 1.00–1.98,  $P=0.049$ ; decile: OR = 1.79, 95% CI = 1.08–2.97,  $P=0.023$ ).

#### CRP and childhood autism by sex of offspring

Given the established differences in risk of autism by sex,<sup>30</sup> we conducted a supplementary analysis to assess effect modification by sex on the relationship between maternal CRP and risk of childhood autism. There were associations between maternal CRP and risk of autism in both sexes, with a numerically greater association for females, but the findings fell short of statistical significance (males: OR = 1.10, 95% CI = 0.98–1.24,  $P=0.09$ ; females: OR = 1.20, 95% CI = 0.97–1.49,  $P=0.10$ ). There was no statistical evidence of interaction between maternal CRP and sex on the relationship with autism ( $P=0.50$ ).

#### CRP and childhood autism by MR status

For maternal CRP measured as a continuous variable in relation to childhood autism, the relationships were similar for cases with MR (OR = 1.17, 95% CI = 0.94–1.45,  $P=0.17$ ) and without MR (OR = 1.11, 95% CI = 0.99–1.25,  $P=0.06$ ). For maternal CRP measured as a categorical variable (highest quintile versus lowest quintile), the findings were also similar between cases with (OR = 1.43, 95% CI = 1.02–2.01,  $P=0.16$ ) and without (OR = 1.41, 95% CI = 0.96–2.08,  $P=0.08$ ) MR.

## DISCUSSION

In summary, elevated maternal CRP, prospectively documented during pregnancy, was related to a significant increase in risk of childhood autism in offspring from a large national birth cohort. This acute-phase reactant protein is synthesized by hepatocytes in response to IL-6, though other cytokines, including interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$ , also have roles in CRP induction.<sup>21,31</sup> Infection of pregnant mice or rats, as well as administration of poly I:C or lipopolysaccharide, induce histopathologically observed cerebellar anomalies, social interaction deficits<sup>32</sup> and diminished prepulse inhibition,<sup>33</sup> all of which have been demonstrated in autism.<sup>7</sup> Maternal immune activation induces release of proinflammatory cytokines, including IL-6, within the fetal brain

and in the placenta leading to downregulation of placental growth hormone, with potentially detrimental effects on the fetal brain development.<sup>34</sup> IL-6 was elevated in the cerebellum of autistic subjects,<sup>35</sup> consistent with maternal immune activation-exposed offspring.<sup>11</sup> IL-6 is also essential for maternal immune activation effects on abnormal behaviors and changes in brain gene expression in offspring.<sup>32</sup> In primary cortical neuronal cultures from E18 rats, IL-6 significantly reduced the number of primary dendrites, nodes and total dendrite length.<sup>36</sup> CRP, as a marker of low-grade inflammation, may also elicit endothelial dysfunction, resulting in vascular damage and impaired placental development.<sup>37,38</sup>

As noted above, three previous epidemiologic studies have related elevations in select maternal cytokines and chemokines to ASD.<sup>15–17</sup> Although the findings are intriguing and novel, the conclusions from these studies are tempered somewhat by certain limitations. In the first study,<sup>15</sup> the three positive findings (elevations in interferon- $\gamma$ , interleukin-4 and interleukin-5 in mid-pregnancy maternal sera) were derived from 17 comparisons, and there was no control for multiple comparisons; hence, it is possible that one of more of the findings were due to chance. Second, the participants represented a subsample of pregnancies from women residing in a single county, but information was not available on nonparticipating mothers and offspring.

The second study, in which elevations in certain cytokines were observed in amniotic fluid from pregnancies of ASD cases, was also based on multiple comparisons ( $N=16$ ), and was noted by the authors as an 'exploratory study'.<sup>16</sup> Second, two separate analyses were conducted: the first was based on the entire sample, while the second was derived from subjects born after 1994, and the findings differed somewhat between the analyses. Although the authors clearly justified this strategy by the fact that ICD-10 was implemented in 1994, this appeared to have been a supplementary analysis. In the third study, amniotic fluid Monocyte Chemoattractant Protein-1 was elevated in ASD cases also diagnosed by ICD-10 criteria, and this chemokine was also increased when the phenotype was restricted to 'infantile autism,' which more closely represents the cases in the present study.<sup>17</sup> However, there were no associations between ASD and two other chemokines in that study.

These limitations were addressed in the present study by several methodologic advantages. First, we sought to test a single hypothesis, related to a single exposure, so multiple comparison issues do not arise. Although other alterations in immune mediators may be relevant, the study is consequently more parsimoniously interpretable as indicative of the hypothesized elevated maternal proinflammatory state. Second, the cases and controls were derived from a large, population-based national birth cohort including all childhood autism cases diagnosed in Finland through psychiatric registries, which cover the entire population. This indicates that the potential for selection bias was



minimal. Third, the diagnoses were restricted to the childhood autism subtype, and were validated by directly administered Autism Diagnostic Interview-Revised interviews. Finally, the sample size of the present study was substantially larger than the previously cited studies on prenatal cytokines and autism.

Our results are consistent with findings from a previous national registry-based study, which demonstrated associations between hospitalizations for infections during pregnancy and ASD.<sup>18</sup> Although that study did not include direct assessment of maternal biomarkers during pregnancy, it is well-established that CRP levels are markedly increased following infection, and these increases are induced by IL-6 and other cytokines.<sup>21</sup>

As reviewed by Patterson<sup>8</sup> and Onore *et al.*,<sup>39</sup> a variety of immunologic anomalies have also been observed in children with autism. Elevated cytokines and chemokines, activated microglia, and increased mRNA transcript levels of immune-related genes were observed in postmortem brain specimens of individuals who had ASD.<sup>39–41</sup> Levels of several immune proteins differ in ASD, and elevated proinflammatory cytokines in plasma were related to impaired communication and social interaction.<sup>39</sup> Other peripheral immune abnormalities have been observed in ASD cases.<sup>39,42–45</sup> In addition, autoantibodies to the fetal brain have been demonstrated in plasma from mothers of children with ASD and in the children.<sup>46–49</sup>

We wish to note some caveats of this study. First, although there was no evidence of confounding following extensive testing of many covariates, there is the possibility of residual confounding by unmeasured factors. Second, elevated maternal CRP may not be specific to childhood autism among developmental disorders. Notably, maternal IL-6 was associated in another study with risk of developmental delay without autism,<sup>15</sup> and significantly elevated amniotic fluid levels of interleukin-5 and IL-6 were observed in other cases of childhood psychiatric disorder.<sup>16</sup> Although we do not believe that this detracts from the significance of the findings reported here, it would be worthwhile in future studies to assess whether maternal CRP is also related to other types of ASD and other developmental disorders. The similar magnitudes of association between maternal CRP and childhood autism in the supplemental analysis stratified by concurrent MR suggests that this relationship is not accounted for by this developmental disorder. Third, given that the median age of onset in the sample was 4 years, a small proportion of cases who later developed autism may have been misclassified as controls, as the most recently born subjects would not have been followed up through a portion of the period of risk. However, this is unlikely to have had an appreciable effect on the results. Finally, the possibility that maternal depression may have confounded the findings should be considered. However, the associations were essentially unchanged after adjustment for maternal lifetime depression and cumulative, rather than current, depressive episodes were previously related to peripheral CRP.<sup>50</sup> In addition, according to a meta-analysis, the relationship between CRP and depression in women was weak ( $d = 0.14$ ) and not statistically significant ( $P < 0.08$ ).<sup>29</sup> Furthermore, use of serotonin reuptake inhibitors, which have been related to autism, when taken during pregnancy<sup>51</sup> were not associated with CRP levels.<sup>52</sup>

In conclusion, we demonstrated that elevated maternal CRP is related to an increased risk of autism in offspring. These findings, if replicated, may have important implications for elaborating the role of immune system dysfunction in autism. Elevated maternal CRP may represent a common pathway by which infections and other inflammatory insults elevate risk for autism. Studies of biomarker-based prenatal infections in relation to autism, and their association with maternal CRP may substantiate this hypothesis. These findings may also have important implications for prevention, given that many standard approaches already exist to reduce the incidence of infections.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Dr Brown contributed to hypothesis generation, study design, data analysis and manuscript writing. Dr Sourander contributed to hypothesis generation, study design, data collection, and manuscript writing. Dr Hinkka-Yli Salomäki contributed to the study design, data analysis, and manuscript writing. Dr McKeague contributed to the study design, data analysis, and manuscript writing. Dr Sundvall contributed to study design, supervision of the CRP assay and manuscript writing. Dr Surcel contributed to hypothesis generation, study design, data collection and manuscript writing.

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