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Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8

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ABSTRACT

Background: Maternal infection during pregnancy has been repeatedly associated with increased risk for schizophrenia. Nevertheless, most viruses do not cross the placenta; therefore, the damaging effects to the fetus appear to be related to maternal antiviral responses to infection (e.g. proinflammatory cytokines). Fetal exposure to the proinflammatory cytokine interleukin-8 (IL-8) has been significantly associated with risk of schizophrenia in offspring. This study sought to determine the association between fetal exposure to IL-8 and structural brain changes among schizophrenia cases and controls.
Methods: Subjects were 17 cases diagnosed with schizophrenia from the Developmental Insult and Brain Anomaly in Schizophrenia (DIBS) study. Psychiatric diagnoses were determined among offspring with semi-structured interviews and medical records review. IL-8 was determined from assays in archived prenatal sera and volumetric analyses of neuroanatomical regions were obtained from T1-weighted magnetic resonance imaging in adulthood. Eight controls were included for exploratory purposes.
Results: Among cases, fetal exposure to increases in IL-8 was associated with significant increases in ventricular cerebrospinal fluid, significant decreases in left entorhinal cortex volumes and significant decreases in right posterior cingulate volumes. Decreases that approached significance also were found in volumes of the right caudate, the putamen (bilaterally), and the right superior temporal gyrus. No significant associations were observed among controls.
Conclusion: Fetal exposure to elevations in maternal IL-8 led to structural neuroanatomic alterations among cases in regions of the brain consistently implicated in schizophrenia research. In utero exposure to elevations in IL-8 may partially account for brain disturbances commonly found in schizophrenia.

1. Introduction

Previous studies have found structural changes throughout the brain in schizophrenia patients compared to non-

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psychiatric controls. Among these studies, increased ventricular size is the most well-replicated structural anomaly found in schizophrenia research (Gur et al., 2007; Wright et al., 2000). Decreases in whole brain volumes and temporolimbic regions also have been repeatedly found among schizophrenia patients (Gur et al., 2007; Wright et al., 2000). Further, studies of individuals who are in the prodrome of schizophrenia also have identified multiple structural brain changes, suggesting that brain disturbances associated with the disorder may have neurodevelopmental origins (Job et al., 2005; Pantelis et al., 2003). Despite these findings, few investigations have sought to determine the contributions of environmental risk factors to neuroanatomical changes found in schizophrenia.

Although genetic factors are believed to substantially contribute to the etiology of schizophrenia, concordance rates approximating 50% between monozygotic twins indicates the presence of substantial environmental influences (Cannon et al., 1998). Among the possible environmental contributors, maternal infections during pregnancy have been repeatedly linked to an increased risk for schizophrenia (Brown and Derkits, 2010). Nevertheless, many infections do not appear to cross the placenta; therefore the damaging influences to the fetal brain seem related to maternal antiviral responses to infection, such as increases in proinflammatory cytokines (Patterson, 2009). In a previous study using the birth cohort of the current investigation, increases in maternal levels of interleukin-8 (IL-8) during the second/third trimesters of pregnancy were associated with increased risk for schizophrenia among offspring (Brown et al., 2004).

Hence, we sought to examine whether fetal exposure to increases in maternal IL-8 during the second/third trimesters results in more pronounced structural brain alterations among individuals diagnosed with schizophrenia and other spectrum disorders (herein referred to as schizophrenia). Our primary hypothesis was that fetal exposure to IL-8 would result in increases in ventricular cerebrospinal fluid (CSF) volume among cases. In addition to the well-replicated association between increases in ventricular CSF and schizophrenia, this hypothesis was derived from animal studies indicating increased ventricular volumes following fetal exposure to immune activation (Patterson, 2009; Wright et al., 2000). Based on previous findings from studies of schizophrenia patients, prodromal research and animal studies, we also predicted that fetal exposure to increased maternal IL-8 would be associated with reduced volumes of temporal lobe regions, particularly in the hippocampus, parahippocampus, and the superior temporal gyrus, and reductions in basal ganglia volumes (Pantelis et al., 2003; Patterson, 2009; Wright et al., 2000). Exploratory analyses were conducted with control participants and on additional regions of interest (ROIs). Analyses of controls were conducted to obtain preliminary findings on whether there are differential vulnerabilities to fetal exposure to IL-8 among cases versus controls, as has been found in other studies (Cannon et al., 2008; Ellman et al., 2009).

2. Materials and methods

All subjects provided written informed consent and the study was approved by the Institutional Review Boards of the New York State Psychiatric Institute, the Kaiser Foundation Research Institute, and the University of California San Francisco VA Medical Center.

2.1. Description of the cohort

The subjects were derived from the Developmental Insult and Brain Anomaly in Schizophrenia (DIBS) study that was based on participants from the Prenatal Determinants of Schizophrenia (PDS) study, which ascertained cases of schizophrenia from a large birth cohort described in detail previously (Susser et al., 2000). Briefly, the PDS study included pregnant women ($n = 12{,}094$ live births) receiving obstetric care from the Kaiser Permanente Medical Care Plan (KPMCP) in Alameda County, California, as part of the Child Health and Development Study (CHDS). Maternal serum samples were collected during the pregnancies and were frozen and stored at $-20\,^{\circ}$ C.

2.2. Ascertainment and diagnosis

Case ascertainment and screening were accomplished following computerized record linkage between the CHDS and KPMCP identifiers from inpatient, outpatient, and pharmacy registries of CHDS participants who belonged to KPMCP from 1981 to 1997 (corresponding to the initiation of KPMCP computerized psychiatric registries and to the end of follow-up). Potential cases were diagnosed using DSM-IV criteria following assessment with the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994), chart review, and consensus of 3 experienced research psychiatrists.

All cases and controls were targeted for neuroimaging assessments in adulthood. DIBS study participants consisted of 23 cases of schizophrenia and 25 controls for MRI acquisition and analysis. Two cases were excluded due to unusable images from movement artifacts. The final sample was comprised of 17 cases and 8 controls with available MRI and available second/third trimester cytokine data. Among the 17 cases, 7 were diagnosed with schizophrenia, 4 with schizoaffective disorder, and 6 with other schizophrenia spectrum disorders. Demographic characteristics of the cases and controls are provided in Table 1. There were no significant differences in any demographic characteristics between cases and controls. There also were no significant differences in any demographic variables between DIBS cases and cases from the PDS study with available cytokine data that were not ascertained as part of the DIBS study (Table 2).

2.3. Interleukin-8 assay

Analyses were restricted to second/third trimester IL-8 values, because 1) maternal IL-8 during the second/third trimester was previously associated with schizophrenia in this birth cohort, whereas other proinflammatory cytokines (IL-6, TNF-α, IL-1β) were not associated with schizophrenia (Brown et al., 2004) and 2) we sought to reduce the probability of Type I errors by limiting the number of analyses.

Assays were conducted blind to diagnosis of the offspring and were carried out under Level II biohazard containment conditions. Each serum specimen was thawed and made 2 mM with respect to phenylmethylsulfonyl fluoride, a protease inhibitor, to prevent degradation of cytokines by serum

Table 1
Demographic characteristics of sample.

Characteristic	SSD ($n=17$)	No diagnosis ($n=8$)	p-value
% Female	5 (29.41%)	2 (25%)	0.819
Age at MRI scan	39.96 (1.78)	41.17 (1.69)	0.140
Birth weight	3508.73 (522.13)	3378.38 (572.20)	0.323
Gestational length (weeks)	40.39 (1.58)	40.14 (0.71)	0.722
Maternal age	28.12 (6.54)	26.88 (6.58)	0.662
Maternal earlier deliveries	1.88 (2.18)	0.75 (0.89)	0.173
Maternal education <=HS graduate*	12 (70.59)	4 (66.67)	0.858
Maternal race*			
White	12 (70.59%)	7(87.50%)	0.096
Black	5 (29.41%)	0	
Asian	0	1 (12.50%)	
IL-8 (pg/ml)	952.04 (1083.45)	315.24 (248.60)	0.118
Medication use			
Atypical antipsychotics	4 (23.53)	0	
Typical antipsychotics	3 (17.65)	0	
On any antipsychotic	7 (18.92)	0	
Anti-pyramidal drugs	3 (17.65)	0	
Mood stabilizers	2 (11.76)	0	
SSRIs	2 (11.76)	0	
Other anti-depressant (trazadone)	1 (5.88)	0	
Ritalin	1 (5.88)	0	
Methadone	1 (5.88)	0	

*Information was incomplete for 2 DIBS controls on maternal education and for 3 DIBS gravidas maternal race was incomplete; therefore this variable was extrapolated from child's race for these gravidas.

proteases. Measurement of IL-8 was conducted using the sandwich enzyme-linked immunosorbent assay described in detail in a previous study from this cohort (Brown et al., 2004).

2.4. Image acquisition and analysis

MR images were acquired using a 1.5-Tesla Siemens system. Coronal T1-weighted images were obtained from 3D MP-RAGE sequences (TR/TI/TE = 10/250/4 ms, resolution 1×1 mm^2, 1.4 mm slice thickness). MRI tissue segmentation and regional voluming in the Talairach coordinate system were used based on the cited methods (Collins et al., 1998; Kwan et al., 1999). These methods have been shown to be reliable (Manji et al., 2000) and valid (Fein et al., 2000) in previous studies. In-house software was used to: 1) remove the skull and meninges from the images; 2) coregister each of the interleaves of the spin-echo images to T1 images reformatted to the axial plane; 3) perform RF inhomogeneity correction in 3D; 4) define seeds (based on peaks in the 3D histogram of T1 pixel intensities) for the K-means cluster analysis; and 5) transfer the data to statistical software which performs the actual cluster analysis. The initial process was followed by computer-assisted editing of the data to separate cortical from subcortical gray matter, ventricular CSF from sulcal CSF, and to reclassify white matter incorrectly classified in the first pass into a category of white matter with an abnormal MRI signal or white matter signal hyperintensity (WMSH). This was followed by manual delineation of the boundaries of cortical regions, subcortical structures, the cerebellum, and the hippocampi. The transformation to the Talairach coordinate system involved piecewise linear transformations of 12 compartments for each subject's brain. Each subject's tissue contribution to the commonly

Table 2
Demographic characteristics of DIBS and non-DIBS cases.

Characteristic	DIBS cases ($n=17$)	Non-DIBS cases ** ($n=42$)	p-value
% Female	5 (29.41%)	15 (35.71%)	0.643
Birth weight (g)	3508.73 (522.13)	3332.48(562.67)	0.245
Gestational length (weeks)*	40.39 (1.58)	40.59 (2.26)	0.738
Maternal age	28.12 (6.54)	27.91 (5.59)	0.90
Maternal earlier deliveries	1.88 (2.18)	2.05 (1.90)	0.77
Maternal education <=HS graduate*	12 (70.59)	24 (66.67)	0.775
Maternal race*			
White	12 (70.59%)	17 (41.46%)	0.775
Black	5 (29.41%)	23 (56.10%)	
Asian	0	1 (2.443%)	
IL-8 (pg/ml)	952.04 (1083.45)	1020.81(1644.09)	0.875

*Information was incomplete for 8 non-DIBS cases on maternal education, for 1 non-DIBS case on gestational length, and for 1 non-DIBS case on maternal and child race. For 3 DIBS gravidas maternal race was incomplete; therefore this variable was extrapolated from child's race for these gravidas.
**Non-DIBS cases were defined as any cases of schizophrenia with available IL-8 data who were part of the larger PDS cohort, but not ascertained in the present t2:16 study.

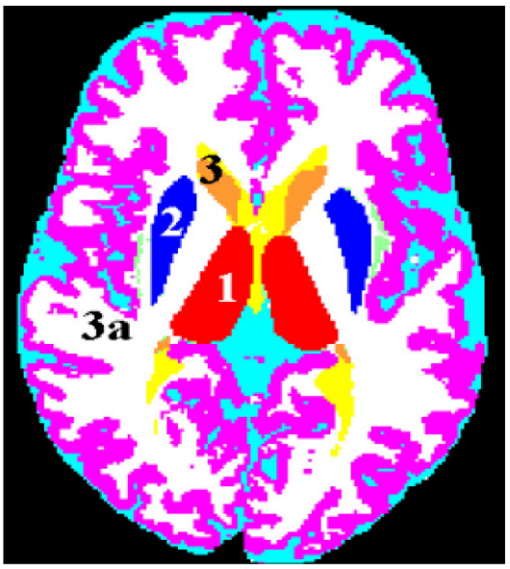

Fig. 1. Axial MRI tissue segmented image. This figure displays manually segmented areas of the thalamus (1); putamen (2); head of caudate (3); tail of caudate (3a).

defined ROI was then computed by superimposing the subject-specific ROI on the subject's segmented image, and counting the (segmented) pixels contained in the ROI. Using this approach, reliability studies for 20 normal subjects on two separate occasions revealed inter- and intraoperator correlations of 0.91 to 0.99 for ventricular CSF, sulcal CSF, cortical gray matter, white matter, and % white matter signal hyperintensities. All cortical volumes, including superior temporal gyrus (Brodmann area 22), entorhinal cortex (Brodmann area 28), inferior frontal cortex (Brodmann areas 44 and 45), dorsolateral prefrontal cortex (DLPFC, Brodmann areas 9 and 46), anterior cingulate (Brodmann areas 24, 25, and 32), and posterior cingulate (Brodmann area 31) were obtained based on the methods above.

The thalamus (see Fig. 1; Deicken et al., 2002), and hippocampus (see Fig. 2; Deicken et al., 1999; Talairach and Tournoux, 1988) were manually traced using the cited methods. Reliability studies of hippocampal and thalamic volumes ($n=10$) of normal controls revealed interoperator correlations of 0.92 to 0.96.

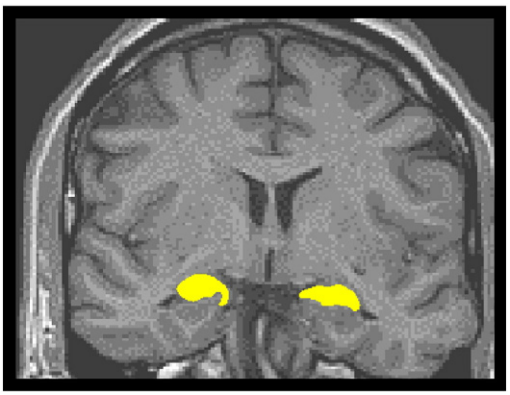

Fig. 2. ROI tracing of hippocampus. This figure shows a representative coronal MR image with the hippocampal areas shown in yellow.

The head of the caudate (see Fig. 1) was defined on the transaxial plane as the mass of gray matter comprising the lateral walls of the lateral ventricles bounded inferiorly and laterally by the anterior limb of the internal capsule and superiorly and laterally by the external capsule. Region placement for the caudate and the putamen began inferiorly when the operator could see clearly at least on one side the anterior limb of the internal capsule dividing the caudate head and the putamen. The boundary for the head of the caudate continued superiorly until the thalamus could no longer be visualized and the anterior horn of the lateral ventricles became confluent with the posterior horn. The body of the caudate was defined as the portion above the thalamus after the confluence of the anterior and posterior horns of the lateral ventricles. The caudate tail was defined as a structure of gray matter closely adjacent anterolaterally to the posterior horns of the lateral ventricles and posterolaterally to the thalamus until it became confluent with the body of the caudate. Reliability studies of caudate volumes ($n=10$) of normal controls revealed interoperator correlations of 0.92 to 0.93.

The putamen (see Fig. 1) is bounded laterally by the external capsule, and postero- and antero-medially by the internal capsule. The boundary between the putamen and the globus pallidus is noted by the difference between the two structures and when possible a strip of white matter was identified between the gray matter masses of the two structures. Regional tracing for the putamen extended superiorly until no more gray matter pixels could be detected medially to the claustrum band. Reliability studies of putamen volumes ($n=10$) of normal controls revealed interoperator correlations of 0.93 to 0.94.

All ROIs were divided by intracranial volume to correct for head size. Ratios were chosen instead of controlling for intracranial volumes, in order to minimize degrees of freedom given the modest sample size.

2.5. Statistical analyses

As is often the case with cytokine distributions, there was a wide range of IL-8 values (3.63 pg/ml to 3717.7 pg/ml) and this distribution was skewed. IL-8 values were log-transformed due to this non-normal distribution, as well as ease of interpretation. Multiple regressions were conducted in SAS version 9.1 (SAS, Inc., Cary, N.C.). Standardized parameter estimates were calculated in addition to traditional unstandardized estimates to allow for interpretation of the magnitude of the relationship between IL-8 and volumetric measures, as represented in standardized units. Standardized estimates represent the standard deviation changes in volumetric ratios that result from a change of one standard deviation in log IL-8 values. Maternal education at birth (HS graduate or below versus higher education) was added to the models to control for the potential influences of postnatal adversity. Maternal education has been highly correlated with other measures of socioeconomic status in the PDS study and is often used as a proxy variable for postnatal adversity in studies of prenatal risk factors to control for the potential contributions of postnatal disadvantages to brain alterations (Schlotz and Phillips, 2009).

Age of participants, participants' sex, and medication status were explored as potential control variables and/or confounders by examining the relationship between these variables and IL-8 levels and volumetric measures. Statistical significance was based on $p<0.05$; all tests were two-tailed. Primary analyses included all analyses with a priori, directional hypotheses; whereas secondary analyses were used for exploratory purposes.

3. Results

There were no significant differences in age or sex between cases and controls (see Table 1). In addition, among several demographic variables examined, age ($r=0.0575$, $p=0.785$), ($F=0.35$, $df=1, 24$, $p=0.559$), and medication status of cases (on antipsychotics or not; $F=0.01$, $df=1, 24$, $p=0.924$) were not significantly related to IL-8 levels. Further, there were no differences in brain volumes between cases that were and were not taking antipsychotics at the time of the assessment (results available upon request).

In primary analyses among cases (see Table 3 and Fig. 3), fetal exposure to increases in maternal IL-8 levels was significantly associated with increases in ventricular CSF volume among cases ($p=0.045$). Fetal exposure to IL-8 also was related to significant decreases in left entorhinal cortex volumes ($p=0.037$) and decreases that approached signifi-

Table 3
Results of unadjusted and adjusted regression analyses of maternal IL-8 and brain volumes in cases and controls: primary analyses.

Brain region	Control participants ($n=8$) unadjusted models				Control participants ($n=8$) models adjusted for maternal education			
	Parameter estimate	95% CI	β	p-value	Parameter estimate	95% CI	β	p-value
Ventricular regions								
Ventricular cerebrospinal fluid	0.002	−0.006, 0.011	0.249	0.552	−0.003	−0.023, 0.016	−0.386	0.631
Cortical/limbic structures								
Entorhinal cortex (right)	0.000005	−0.0003, 0.0003	0.013	0.975	0.0002	−0.0004, 0.0008	0.731	0.393
Entorhinal cortex (left)	−0.00004	−0.0004, 0.0003	−0.097	0.820	0.0002	−0.0007, 0.001	0.481	0.562
Hippocampus (right)	0.0002	−0.00003, 0.0004	0.649	0.082†	0.0004	−0.00003, 0.0008	1.281	0.061†
Hippocampus (left)	−0.00001	−0.0002, 0.0001	−0.077	0.856	−0.00002	−0.0004, 0.0004	−0.166	0.858
Superior temporal gyrus (right)	0.000002	−0.001, 0.001	0.002	0.996	0.0005	−0.002, 0.002	0.544	0.518
Superior temporal gyrus (left)	−0.0001	−0.001, 0.001	−0.104	0.806	0.0004	−0.002, 0.003	0.377	0.661
Subcortical structures								
Caudate (right)	0.0001	−0.0005, 0.0007	0.184	0.662	0.0002	−0.0001, 0.0006	0.978	0.142
Caudate (left)	0.0002	−0.0002, 0.0005	0.449	0.264	0.0002	−0.0005, 0.0008	0.471	0.508
Putamen (right)	0.0001	−0.0004, 0.0006	0.238	0.571	0.0002	−0.0009, 0.001	0.508	0.573
Putamen (left)	0.0002	−0.0003, 0.0006	0.367	0.371	0.0003	−0.0007, 0.001	0.759	0.381
Brain region	Schizophrenia cases ($n=17$) unadjusted models				Schizophrenia cases ($n=17$) models adjusted for maternal education			
	Parameter estimate	95% CI	β	p-value	Parameter estimate	95% CI	β	p-value
Ventricular regions								
Ventricular cerebrospinal fluid	0.002	−0.00005, 0.004	0.473	0.055†	0.002	0.00005, 0.004	0.487	0.045*
Cortical/limbic structures								
Entorhinal cortex (right)	−0.00001	−0.00007, 0.00004	−0.138	0.597	−0.00001	−0.00007,0.00004	−0.124	0.630
Entorhinal cortex (left)	−0.00005	−0.00009, −0.000005	−0.525	0.030*	−0.00005	−0.00009, −0.000003	−0.519	0.037*
Hippocampus (right)	0.00002	−0.00005, 0.00009	0.172	0.509	0.00002	−0.00005, 0.0001	0.169	0.531
Hippocampus (left)	−0.00002	−0.0001, 0.00006	−0.142	0.588	−0.00003	−0.0001, 0.00006	−0.160	0.523
Superior temporal gyrus (right)	−0.0002	−0.0004, 0.00007	−0.353	0.165	−0.0002	−0.0004, 0.00002	−0.379	0.079†
Superior temporal gyrus (left)	−0.0001	−0.0003, 0.0001	−0.223	0.390	−0.0001	−0.0003, 0.0001	−0.242	0.320
Subcortical structures								
Caudate (right)	−0.00006	−0.0001, −0.000002	−0.497	0.042*	−0.00006	−0.0001, 0.0000002	−0.495	0.051†
Caudate (left)	−0.00003	−0.0001, 0.00007	−0.147	0.573	−0.00003	−0.0001, 0.00008	−0.144	0.594
Putamen (right)	−0.0001	−0.0002, 0.00003	−0.393	0.118	−0.0001	−0.0002, 0.00002	−0.409	0.094†
Putamen (left)	−0.0001	−0.0002, 0.000008	−0.456	0.066†	−0.0001	−0.0002, 0.00001	−0.467	0.061†

This table includes the results from the primary multiple regression analyses with and without controlling for maternal education at birth. Unstandardized parameter estimates, 95% confidence intervals of the unstandardized estimates, standardized parameter estimates (β), and p-values for regression analyses of the relationship between the log of IL-8 and brain volumes/icv are presented. *Denotes a result that was significant at $p<0.05$. †Denotes a result between $p=0.05$ and $p=0.10$.

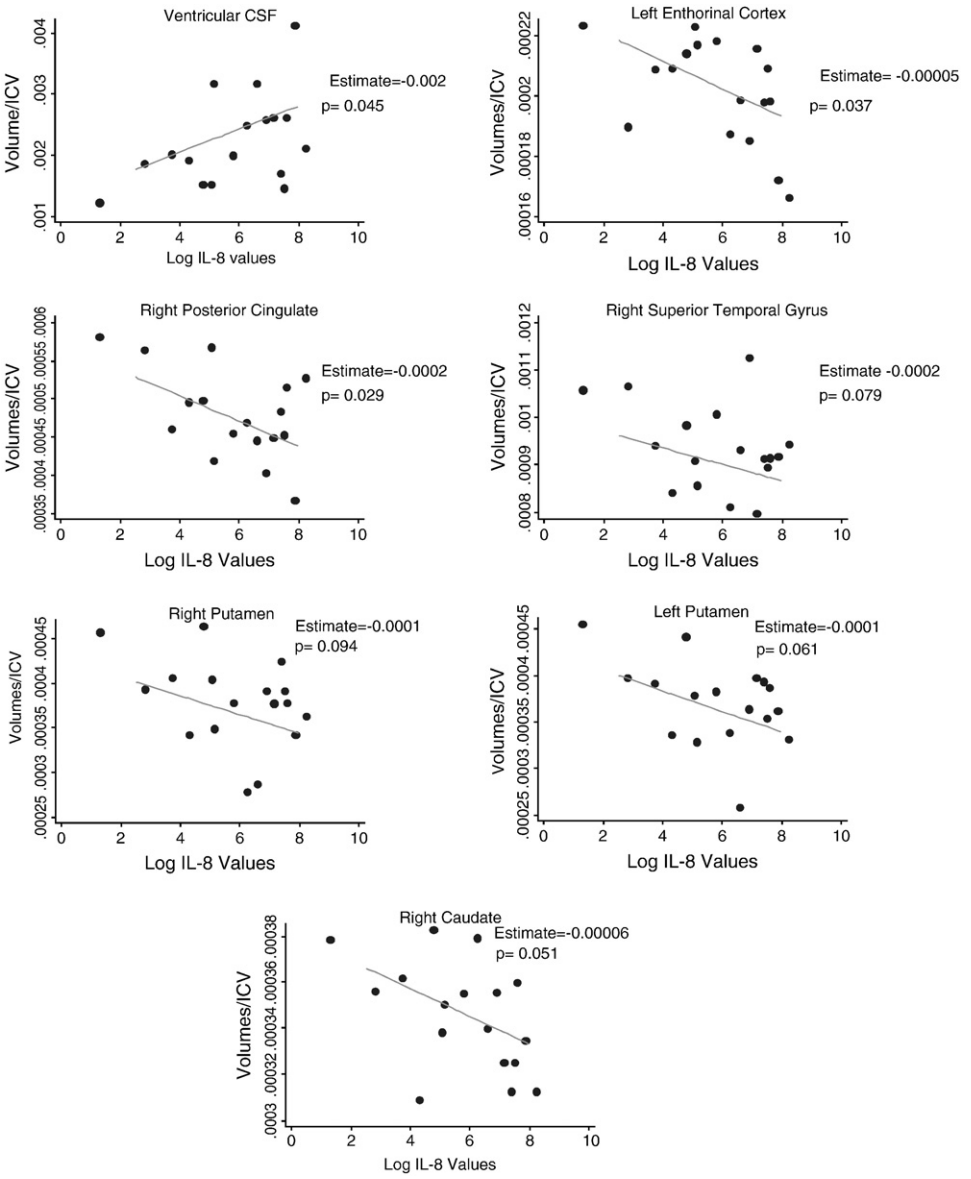

Fig. 3. Relationship between IL-8 and volumetric changes among schizophrenia cases controlling for maternal education. This figure displays raw ROI/icv values by log-transformed IL-8 values, as well as regression lines controlling for maternal education (maternal education was held constant at HS graduate or below for each regression line).

cance in the right superior temporal gyrus ($p = 0.079$), the right caudate ($p = 0.051$), and the putamen bilaterally ($p = 0.061$ and $p = 0.094$, right and left, respectively). There were no associations between fetal exposure to IL-8 and hippocampal volumes.

In secondary analyses among cases (Table 4 and Fig. 3), fetal exposure to increases in maternal IL-8 levels was related to volumetric decreases in the right posterior cingulate ($p = 0.029$). No significant relationships were observed between maternal IL-8 levels and any of the additional brain volumes examined among cases or in exploratory analyses among controls, although non-significant increases were observed for the right hippocampus ($p = 0.061$), right anterior cingulate ($p = 0.097$), and the right posterior cingulate ($p = 0.088$) among controls.

4. Discussion

These results provide the first evidence that fetal exposure to increases in a maternal cytokine is associated with structural neuroanatomic alterations that have been consistently linked to schizophrenia (Wright et al., 2000). Among schizophrenia cases, increases in maternal IL-8 during the second/third trimesters of pregnancy were related to increases in ventricular CSF. In addition, we observed significant associations between maternal IL-8 and decreases in left entorhinal cortex and right posterior cingulate volumes, and volumetric decreases that approached significance in the right caudate, the putamen bilaterally, and the right superior temporal gyrus among cases.

Table 4
Results of unadjusted and adjusted regression analyses of maternal IL-8 and brain volumes in cases and controls: secondary analyses.

	Control participants ($n=8$) unadjusted models				Control participants ($n=8$) models adjusted for maternal education			
	Parameter estimate	95% CI	β	p-value	Parameter estimate	95% CI	β	p-value
Ventricular measures								
Sulcal cerebrospinal fluid	0.008	−0.012, 0.028	0.386	0.345	0.016	−0.015, 0.046	1.023	0.202
Cortical/limbic regions								
DLPFC (right)	0.0002	−0.0005, 0.0008	0.214	0.612	−0.0002	−0.002, 0.001	−0.324	0.674
DLPFC (left)	−0.0002	−0.0008, 0.0005	−0.232	0.580	−0.00007	−0.002, 0.002	−0.111	0.905
Inferior frontal cortex (right)	0.0003	−0.0005, 0.001	0.361	0.379	0.0002	−0.002, 0.002	0.221	0.806
Inferior frontal cortex (left)	0.0001	−0.0005, 0.0007	0.211	0.616	0.0003	−0.001, 0.002	0.582	0.512
Anterior cingulate (right)	0.0008	−0.0006, 0.002	0.500	0.207	0.0008	−0.0003, 0.002	0.779	0.097†
Anterior cingulate (left)	−0.002	−0.004, 0.0009	−0.527	0.180	−0.001	−0.007, 0.004	−0.423	0.547
Posterior cingulate (right)	0.0003	−0.0003, 0.001	0.460	0.252	0.0005	−0.0001, 0.001	0.930	0.088†
Posterior cingulate (left)	−0.0005	−0.001, 0.0004	−0.473	0.237	0.00005	−0.002, 0.002	0.047	0.947
Subcortical regions								
Thalamus (right)	0.0001	−0.0005, 0.0008	0.182	0.665	0.0006	−0.0008, 0.002	0.855	0.279
Thalamus (left)	0.0001	−0.0004, 0.0006	0.219	0.602	0.0003	−0.0006, 0.001	0.730	0.376
White/gray matter								
White matter	0.006	−0.018, 0.030	0.233	0.578	−0.012	−0.042, 0.017	−0.625	0.265
Cortical gray matter	−0.017	−0.041, 0.006	−0.594	0.120	−0.002	−0.046, 0.042	−0.077	0.901
Subcortical gray matter	0.0002	−0.001, 0.001	0.163	0.700	0.0002	−0.002, 0.003	0.187	0.842
Brain region	Schizophrenia cases ($n=17$) unadjusted models				Schizophrenia cases ($n=17$) models adjusted for maternal education			
	Parameter estimate	95% CI	β	p-value	Parameter estimate	95% CI	β	p-value
Ventricular measures								
Sulcal cerebrospinal fluid	0.0004	−0.006, 0.007	0.039	0.882	0.0006	−0.005, 0.007	0.056	0.825
Cortical/limbic regions								
DLPFC (right)	−0.000004	−0.0004, 0.0004	−0.006	0.983	−0.00002	−0.0004, 0.0003	−0.025	0.919
DLPFC (left)	0.000004	−0.0004, 0.0004	0.005	0.985	−0.00001	−0.0004, 0.0004	−0.015	0.952
Inferior frontal cortex (right)	0.0000004	−0.0003, 0.0003	0.001	0.998	−0.00001	−0.0003, 0.0003	−0.014	0.956
Inferior frontal cortex (left)	−0.00001	−0.0003, 0.0003	−0.026	0.922	−0.00002	−0.0003, 0.0002	−0.043	0.866
Anterior cingulate (right)	−0.0002	−0.0006, 0.00008	−0.381	0.131	−0.0002	−0.0006, 0.00007	−0.392	0.123
Anterior cingulate (left)	−0.0001	−0.0006, 0.0004	−0.148	0.571	−0.0001	−0.0007, 0.0004	−0.157	0.556
Posterior cingulate (right)	−0.0002	−0.0003, −0.00002	−0.537	0.026*	−0.0002	−0.0003, −0.00002	−0.543	0.029*
Posterior cingulate (left)	−0.0001	−0.0003, 0.0001	−0.271	0.292	−0.0001	−0.0003, 0.0001	−0.285	0.266
Subcortical regions								
Thalamus (right)	0.00001	−0.0001, 0.0001	0.048	0.854	0.00001	−0.0001, 0.0001	0.035	0.892
Thalamus (left)	0.00005	−0.00008, 0.0002	0.193	0.459	0.00005	−0.00009, 0.0002	0.189	0.482
White/gray matter								
White matter	−0.0005	−0.006, 0.005	−0.049	0.853	−0.0004	−0.006, 0.005	−0.046	0.866
Cortical gray matter	−0.002	−0.008, 0.005	−0.138	0.598	−0.002	−0.007, 0.004	−0.161	0.491
Subcortical gray matter	−0.00005	−0.0003, 0.0002	−0.090	0.732	−0.00004	−0.0003, 0.0003	−0.082	0.759

This table includes the results from the secondary multiple regression analyses with and without controlling for maternal education at birth. Unstandardized parameter estimates, 95% confidence intervals of the unstandardized estimates, standardized parameter estimates (β), and p-values for regression analyses of the relationship between the log of IL-8 and brain volumes/icv are presented. *Denotes a result that was significant at $p<0.05$. †Denotes a result between $p=0.05$ and $p=0.10$.

Ventricular enlargement is arguably the most well-replicated neuromorphologic anomaly in schizophrenia (Wright et al., 2000). Further, ventricular enlargement has been previously associated with hypoxia-associated OCs, suggesting that this structural change may have risk factors that are neurodevelopmental in origin (Cannon et al., 2002). Lastly, fetal exposure to activation of proinflammatory cytokines in rodents has been linked to increased ventricular CSF, which is the only known neuromorphologic volumetric finding among animal studies to date (Patterson, 2009). Cumulatively, these findings suggest that fetal exposure to IL-8 may contribute to the known increases in ventricular volumes in schizophrenia.

Many of the other volumetric changes found in the present study also have been observed in schizophrenia and prodromal populations (Borgwardt et al., 2007; Pantelis et al., 2003; Wright et al., 2000). Specifically, parahippocampal and superior temporal gyrus (STG) volume reductions have been repeatedly

found among patients with schizophrenia and these regions have been shown to be decreased in the available prodromal studies, as well (Pantelis et al., 2003; Wright et al., 2000). Similar to the present study, in first-episode and prodromal patients basal ganglia volumes were decreased (Corson et al., 1999; Pantelis et al., 2003).

Interestingly, no significant associations were observed among controls. This pattern of null findings may support the assertion that a genetic or environmental factor associated with schizophrenia is necessary for IL-8 to exert damaging influences on the fetal brain, although studies with larger samples are necessary to confirm this possibility.

IL-8 is a proinflammatory chemokine produced by multiple cells involved in mobilizing, activating and degranulating neutrophils (Janeway et al., 2005). Although IL-8 is integral in the initial immune response to infection, elevations in IL-8 also are sometimes associated with other maternal conditions during pregnancy that increase risk for schizophrenia, such as preeclampsia, obesity and anemia (Basso et al., 2005; Dalman et al., 1999; Fain, 2006; Insel et al., 2008; Laskowska et al., 2007; Schaefer et al., 2000). It is possible that additional obstetric insults may add to, interact with, or precede the effects of IL-8 to cause disruptions in fetal neuronal development among cases, which should be teased apart in future studies.

There were several limitations in the present study that should be noted. Given the multiple tests conducted in this study, it is possible that spurious findings arose from type I error. It appears unlikely, however, that our results are entirely due to chance, as they are consistent with many previous studies in clinical and preclinical studies of schizophrenia. Further, there is the possibility of type 2 error, given the modest sample sizes. It should considered, however, that the present study is the first of its kind with access to serological evidence of prenatal events and follow-up brain imaging data. Nevertheless, it will be essential to attempt to replicate the results of the present study with larger samples.

In addition, the sample was limited to slightly more than 25% of cases from the overall cohort, raising the potential for selection bias. However, cases who participated in the DIBS study and cases from the original cohort who were not DIBS subjects did not differ with regard to several demographic variables and IL-8 levels; therefore, it is unlikely that case ascertainment bias accounts for our findings. Bias also would be mitigated by the fact that the sample was derived from a population-based study, in contrast to many clinical imaging studies, which draw upon hospital or clinic-based samples.

Lastly, the use of antipsychotics may have influenced our results. Although this is a possibility, IL-8 values were not significantly related to current antipsychotic use, which significantly decreases the likelihood that medication use confounded our findings. Nonetheless, long-term medication use, which is difficult to assess retrospectively, could have influenced the observed brain volumes.

In conclusion, the present study is the first to suggest that brain anomalies previously associated with schizophrenia may be partially attributable to in utero insults, such as fetal exposure to elevated IL-8. These findings support our previous association between fetal exposure to IL-8 and risk of schizophrenia in this birth cohort (Brown et al., 2004). Future research is necessary to replicate this finding and to delineate whether fetal exposure to IL-8 interacts with risk genes for schizophrenia. Our findings underscore the potential importance of prenatal contributions to schizophrenia, with implications for prevention, early intervention, and treatment strategies.

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Contributors

Dr. Ellman conducted all of the statistical analyses, contributed substantially to the conceptualization of the paper, study design, data interpretation, and was the main writer of the manuscript. Dr. Deicken supervised all of the imaging acquisition and imaging analyses for the study and contributed to writing of the manuscript. Drs Vinogradov, Kremen, and Poole contributed to the conceptual design, to data collection, data interpretation, and manuscript writing. Mr. Kern and Dr. Tsai aided in data management and consultation pertaining to statistical analyses, interpretation of the data, and contributed to manuscript writing. Dr. Schaefer contributed to case/control ascertainment and manuscript writing. Dr. Brown contributed to all areas pertaining to study conceptualization, study design, participant ascertainment, interpretation of the findings, and manuscript writing.

Conflict of interest

The authors have no financial disclosures and/or conflicts to report.

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