Differential Relationships Among C-Reactive Protein, Attention Functioning, and Brain Structure in Bipolar Offspring With and Without Subthreshold Mood Symptoms

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ABSTRACT

Background: Bipolar disorder (BD) is a highly heritable mood disorder. Activated low-grade inflammation may not only play an adverse role in the pathophysiology of BD, but also contribute to a resilience process. The neuroinflammatory processes may underlie the attention deficit and alteration of gray matter volume (GMV) in the early stage and premorbid period of BD. Also, the differential inflammation-brain relationship may be identified as biological markers for BD pathology or resilience.

Methods: The present data were collected between March 2013 and June 2016. Sixty-four offspring of BD patients were recruited and subdivided into asymptomatic (n = 33, mean age = 17.8 years) and symptomatic (n = 31, mean age = 16.2 years) groups according to whether they manifested subthreshold mood symptoms. The diagnosis of BD was confirmed according to *DSM-IV* criteria. C-reactive protein (CRP) level, attention functioning, and GMV data were measured by ELISA, the Continuous Performance Test-Identical Pair test (CPT-IP), and 3.0 T magnetic resonance imaging, respectively. Their relationships were examined with mediation and moderation analyses.

Results: We observed a higher level of CRP and poorer attention in the symptomatic group than the asymptomatic group and found a significant group × CRP interactive effect on GMV in regions spanning right precentral and postcentral gyri ($P = .043$). CRP levels negatively mediated the relationship between the group and CPT-IP scores, and the group marginally moderated the relationship between pre/postcentral gyri volumes and CPT-IP scores ($P = .05$).

Conclusions: Symptomatic and asymptomatic bipolar offspring manifested differential inflammation-GMV-attention relationships, which may represent, respectively, an endophenotype or a resilience process for BD.

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Bipolar disorder (BD) is a highly heritable mood disorder characterized by recurrent episodes of depression alternating with periods of hypomania/mania. It affects approximately 1%–1.5% of the world's general population and is one of the main causes of disability, with high rates of morbidity and mortality.[1] The relatives of patients with BD have a mean prevalence of 11.9%, which is an approximately 10-fold higher risk than that of the general population.[2] Specially, the relatives of BD patients manifesting mild or identifiable prodromal symptoms (such as subthreshold mood symptoms) are more often found to escalate to the first episode of mania or depression, particularly when symptom load increases. These individuals may potentially possess endophenotype-related characteristics of a mood disorder.[3] On the other hand, there is still a significant proportion of relatives of patients with BD who are free of psychiatric symptoms across the peak age at onset and thus are considered to have protective features that prevent or delay the occurrence of the disorder.[4,5] Identifying the endophenotype-related and resilience-related markers may benefit early diagnosis, intervention, and prevention for BD.

Cognitive deficit is a core characteristic of BD patients across the different stages and mood states,[6] and even prior to the onset.[7] Attention deficit, among other cognitive domains, often occurs early and increases after a few major depressive and hypomanic/manic episodes.[8,9] Increased attention deficit has been reported during the occurrence of common prodromal symptoms prior to clinical mood episodes and may be predictive for increased risk of developing BD.[10] For instance, attention deficit was found in genetically at-risk individuals who manifest subthreshold mood symptoms[6,11] but was not observed in those without symptoms.[11,12]

Persistently activated low-grade inflammatory/immune system plays a crucial role in the pathophysiology of BD.[13,14] Elevated inflammatory biomarkers, such as high sensitivity C-reactive protein (hs-CRP), have been observed in patients with mania[15] and unipolar and bipolar depression,[16] as well as patients with BD at the stable state.[17] Although ample evidence has demonstrated the deleterious effect of inflammation on brain,[18,19] a recent study suggested that an adaptive inflammatory phenotype may also contribute to a resilience process.[20]

Clinical Points

- Inflammatory cytokines such as C-reactive protein (CRP) affect the brain structure differently in genetically at-risk offspring of parents with bipolar disorder (BD) depending on whether they manifest subthreshold syndromes.
- CRP mediates the differences in attention functioning of genetically at-risk offspring with and without symptoms, suggestive of differing risks for BD between these groups.
- Distinct correlations among CRP level, gray matter volume, and attention functioning may help explain whether individuals who are genetically at risk for BD manifest subthreshold syndromes.

The definitive effect of inflammation on central nervous systems remains elusive. CRP is a well-established acute-phase protein acting as a sensitive marker for inflammatory reactions. Studies have demonstrated that individuals with elevated levels of CRP due to infection or autoimmunity have an increased risk of developing psychiatric disorders including BD,[13,21] and it is associated with severity of mood symptoms and cognitive dysfunction including attention deficit.[18]

Inverse association between CRP level and gray matter volume (GMV) or cortical thinness has been repeatedly observed in the general population[22,23] and patients with psychiatric disorder.[23,24] Inflammatory factors can influence the GMV by stimulating synaptic pruning and neurodevelopment,[25] impacting the brain microcirculation,[26] and increasing the susceptibility to internal and external stimulations.[27] The effect of inflammation on gray matter (GM) of specific areas is a speculative pathway accounting for the decline in cognitive functions, such as attention deficit, which may be partly caused by the effect of inflammation on specific attention networks.[28] We previously showed that interleukin (IL)-6 could affect attention via altering the volumes of the anterior cingulate cortex.[29] Moreover, inflammatory processes may serve as a pathway for the altered brain structure to induce cognitive deficit or mood symptoms.[30,31] Investigating the relationship between CRP with brain structure and cognitive function could help elucidate the mechanisms by which the interactions contribute to the different risks of developing BD.[32]

Given the above considerations, this study investigated the relationships among brain structure, plasma CRP level, and attention in 2 groups of bipolar offspring with and without symptoms, suggestive of different levels of risk for BD. We hypothesized that (1) CRP level would be higher in the symptomatic offspring group; (2) CRP level would mediate the group difference in attention functioning; and (3) the relationships among brain structure, CRP, and attention would be different between the two groups.

METHODS

Participants

The present study was part of the Recognition and Early Intervention on Prodromal Bipolar Disorder (REI-PBD) project, an ongoing longitudinal study of a cohort of bipolar offspring launched in 2013.[33] The data from the present study were collected from March 2013 to June 2016.

The inclusion criteria of participants were as follows: (1) having at least 1 biological parent diagnosed with BD according to *DSM-IV* criteria, (2) age from 8 to 28 years, and (3) ethnic Han Chinese. Based on whether they had specified subthreshold syndromes, the offspring were divided into symptomatic offspring and asymptomatic offspring. The subthreshold syndromes included (1) presence of 2 or 3 hypomanic symptoms for at least 4 days (not meeting *DSM-IV* hypomania episode criteria), (2) 2 or more symptoms of a major depressive episode present for at least 1 week but not meeting the *DSM-IV* major depressive episode criteria (falling short of the required number of symptoms or duration of 2 weeks), and (3) 1 or more attenuated psychotic symptoms present for at least 10 minutes for each manifestation and 2–7 manifestations per week for at least 3 months. The asymptomatic offspring had none of the subthreshold mood symptoms described above and were free of any *DSM-IV*–defined psychiatric disorder. Exclusion criteria were as follows: any current or lifetime *DSM-IV-TR* Axis I disorders, including drug or alcohol abuse; pregnancy; hypo/hyperthyroidism; traumatic brain injury; or inability to complete the MR scanning or having MR images that showed artifacts.

We applied the Structured Clinical Interview for *DSM-IV-TR* Axis I Disorders Patient Edition[34] or the Schedule for Affective Disorders and Schizophrenia for School-Aged Children: Present and Lifetime Version[35] (if age under 18 years) for screening psychiatric disorders. Only individuals who did not meet the criteria for a psychiatric disorder were included. We used a self-administered, 74-item symptom checklist[33] and a modified version of the Bipolar Prodrome Scale-Retrospective: Patient Version[36] adapted by the research team to the social and cultural context to assess current and past symptoms. The data of this study were censored in June 2016. This study was approved by the Institutional Research Board of The Affiliated Brain Hospital of Guangzhou Medical University. Written informed consent was obtained from all participants or their guardians (if age under 18 years) prior to enrollment in the study.

In total, 37 symptomatic and 32 asymptomatic bipolar offspring were recruited. One asymptomatic and 4 symptomatic individuals were excluded due to neurologic conditions or/and noncompliance with magnetic resonance imaging (MRI) scanning, leaving 31 asymptomatic and 33 symptomatic individuals in data analysis. The offspring had been followed up for up to 6 years. None of the bipolar offspring who were free of the subthreshold syndromes at baseline developed full-blown BD, and 4 bipolar offspring with subthreshold syndromes converted to BD onset during the follow-up years. The analysis of this study was cross-sectional, and the MRI data were compared at baseline.

Table 1. Demographic and Clinical Characteristics and Level of CRP in Asymptomatic and Symptomatic Bipolar Offspring[a]

	Symptomatic offspring (n = 33)	Asymptomatic offspring (n = 31)	Statistics	*P*
Demographic				
Sex, female/male, n	16/17	12/19	$\chi^2 = 0.621$	.46
Age, y	16.21 ± 5.94	17.77 ± 5.3	$t_{62} = 1.11$	.27
Handedness, right/left, n	32/1	29/2	$\chi^2 = 0.425$	.61
Education, y	8.85 ± 4.31	10.23 ± 3.67	$t_{62} = 1.37$	.18
BMI	19.71 ± 2.73	19.52 ± 2.32	$t_{62} = 0.292$	.77
TONI-III IQ	24.39 ± 9.78	25.77 ± 8.08	$F_{1,58} = 0.25$	.62
Clinical				
HDRS	8.41 ± 10.35	0.52 ± 1.0	$t_{62} = 4.31$	< .001
HARS	6.5 ± 8.93	0.52 ± 0.93	$t_{62} = 3.54$	.001
YMRS	2.91 ± 3.32	0.61 ± 1.61	$t_{62} = 3.69$	.001
BPRS	24.03 ± 7.1	18.26 ± 0.63	$t_{62} = 5.63$	< .001
CPT-IP	3.09 ± 0.87	3.54 ± 0.68	$F_{1,58} = 5.17$	.027
Cytokine				
hs-CRP-OD	0.24 ± 0.11	0.20 ± 0.06	$t_{62} = 2.63$	.011

[a]Values expressed as mean ± SD unless otherwise noted. CPT-IP score and hs-CRP-OD were corrected for age, sex, and IQ scores. All were $P_{corrected} < .05$ after Holm-Bonferroni correction.

Abbreviations: BMI = body mass index, BPRS = Brief Psychiatric Rating Scale, CPT-IP = Continuous Performance Test-Identical Pairs test, CRP = C-reactive protein, HARS = Hamilton Anxiety Rating Scale, HDRS = Hamilton Depression Rating Scale, hs-CRP-OD = high sensitive C-reactive protein-optical density, TONI-III IQ = Test of Nonverbal Intelligence-III intelligence quotient, YMRS = Young Mania Rating Scale.

Demographic and Clinical Variables

We used the Hamilton Depression Rating Scale (HDRS)[37] and Hamilton Anxiety Rating Scale (HARS),[38] Young Mania Rating Scale (YMRS),[39] and Brief Psychiatric Rating Scale (BPRS)[40] to measure the severity of depression and anxiety, mania, and psychotic symptoms, respectively. Test of Nonverbal Intelligence-III (TONI-III)[41] and the Continuous Performance Test-Identical Pairs test (CPT-IP)[42] were applied to measure general intelligence and attention function, respectively.

Participants' demographic information was analyzed using independent-samples *t* test (for age and years of education), χ^2 test (for sex and handedness), and univariate ANOVA controlling for age and sex. Performance of the CPT-IP attention task was assessed using univariate ANOVA controlling for age, sex, and TONI score. The analysis was repeated after controlling for all clinical variables. Data normality was checked using the Kolmogorov-Smirnov test, and equality of variance was assessed using the Levene test. All data except for the two binary variables of age and sex satisfied the normality and equal variance assumptions. A statistical threshold was set as $P < .05$, 2-tailed.

Participants' clinical variables were determined to be non-normally distributed according to the Kolmogorov-Smirnov test ($P < .05$), and thus the non-parametric independent samples Mann-Whitney *U* test was performed. Holm-Bonferroni correction was carried out to correct for multiple testing where appropriate.

GM Image Acquisition and Processing

MRI scanning was conducted within 3 days after clinical assessment. Image data were acquired on a Philips Achieva X-series 3.0 Tesla scanner with an 8-channel SENSE head coil. A T2WI sequence was performed to identify any brain organic disease in each participant, such as focal cortical dysplasia, cerebral infarction, encephalitis, and brain tumor. Any participant showing signs of organic brain disorder was excluded. The high-resolution sagittal T1-weighted images were acquired with the following parameters: repetition time (TR) = 8.2 ms; echo time (TE) = 3.7 ms; FOV = 256 × 256 mm^2; voxel sizes = 1 × 1 × 1 mm^3; matrix size = 256 × 256; slice number = 188; slices thickness = 1 mm.

The 3D T1 data were segmented and extracted using Computational Anatomy Toolbox (CAT12, http://www.neuro.uni-jena.de/cat/) in Statistical Parametric Mapping 12 (SPM12, http://www.fil.ion.ucl.ac.uk/spm/) and then smoothed with an 8 mm Gaussian kernel. The smoothed, normalized, and modulated GM images were analyzed using independent samples *t* test including 2 groups (symptomatic vs asymptomatic offspring). The model additionally incorporated 2 variables representing the CRP levels in each group, so as to assess the possible interaction of group and CRP on GMV. Total intracranial volume (TIV), age, and sex were entered as covariates of no interest.

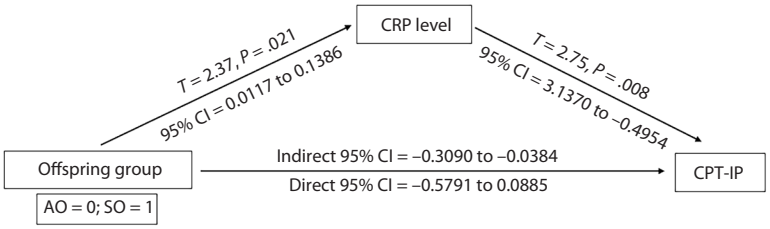
Peripheral hs-CRP Level Measurements

Serum samples were separated from whole blood samples using centrifugation at 3,000 g for 10 min at 4°C and were then stored in 1 mL aliquots at −80°C. Enzyme-linked immunosorbent assay (ELISA) was applied to measure the level of hs-CRP according to the instructions of the manufacturer (Cusabio). All samples were assayed in triplicate.

Data Analysis

In order to delineate the interrelations between offspring group, CRP level, GMV, and CPT-IP performance, we performed the following sequence of analyses. We first tested

Figure 1. CRP Level Mediated a Negative Relationship Between Group and CPT-IP Scores[a]



[a]Group (AO = 0, SO = 1) had a positive effect on CRP-optical density level, which in turn showed a negative association with attention score. The remaining direct effect became insignificant after controlling for the significantly negative indirect mediation effect. The CRP-optical density levels were log-transformed to improve data normality.
Abbreviations: AO = asymptomatic offspring, CPT-IP = Continuous Performance Test-Identical Pairs Test, CRP = C-reactive protein, SO = symptomatic offspring.

whether CRP level mediated any between-group difference in CPT-IP performance, while controlling for important confounding variables including age, sex, TIV, and TONI IQ. Then, we examined brain region(s) in which GMV correlated with CRP level in the offspring groups, using a general linear model including the variables of group and CRP, while controlling for age, sex, and TIV. We also tested whether there was any interactive effect of group and CRP on the GMV. A statistical threshold was set as whole-brain voxel-level family-wise error–corrected at $P < .05$, which is a stringent thresholding criterion. Subsequently, we explored whether the brain region(s) where the GMV was correlated significantly with CRP level also showed significant associations with the CPT-IP performance in the offspring groups. The mediation and moderation analyses were performed using the PROCESS macro implemented in SPSS, which utilized a bootstrapping approach.[43] Bootstrapping is a non-parametric approach to test hypotheses without making inherent assumptions on the data distribution. Bootstrapping was carried out using a bias-corrected procedure with 5,000 samples, which outputs 95% confidence intervals (95% CIs). Significance was determined based on whether the 95% CIs encompassed zero.[44] To rule out the possibility that certain unclassified data characteristics may bias the bootstrapped results, we additionally performed permutation testing (5,000 times) on the mediation and moderation results. The mediation permutation analysis was conducted using the Lavaan toolbox of RStudio (https://lavaan.ugent.be/), and the moderation permutation analysis was conducted using the lmPerm toolbox of RStudio.

RESULTS

Demographic, Clinical, and Cognitive Analysis

As shown in Table 1, the symptomatic and asymptomatic offspring groups were matched on age, sex, years of education, handedness, body mass index (BMI), and intelligence quotient (IQ) (all $P > .05$). The symptomatic group manifested higher scores on the HDRS ($t_{62} = 4.31$, $P < .001$), HARS ($t_{62} = 3.54$, $P = .001$), YMRS (standardized $t_{62} = 3.69$, $P = .001$), and BPRS (standardized $t_{62} = 5.63$, $P < .001$) than the asymptomatic group (all $P_{corrected} < .01$ after Holm-Bonferroni correction). After controlling for age, sex, and TONI IQ scores, the symptomatic group showed poorer performances on the CPT-IP attention task than the asymptomatic offspring ($F_{1,58} = 5.17$, $P = .027$).

hs-CRP Level

Symptomatic offspring showed higher hs-CRP level-optical density than the asymptomatic offspring (mean [SD]: 0.24 [0.11] vs 0.2 [0.06]) after controlling for age, sex, and IQ scores ($t_{62} = 2.63$, $P = .011$).

As between-group comparison was conducted both on CPT-IP score and on CRP level, we conducted Holm-Bonferroni correction on the two tests. Both group differences remained significant after the correction (all $P_{corrected} < .05$).

Between-Group GMV Comparison

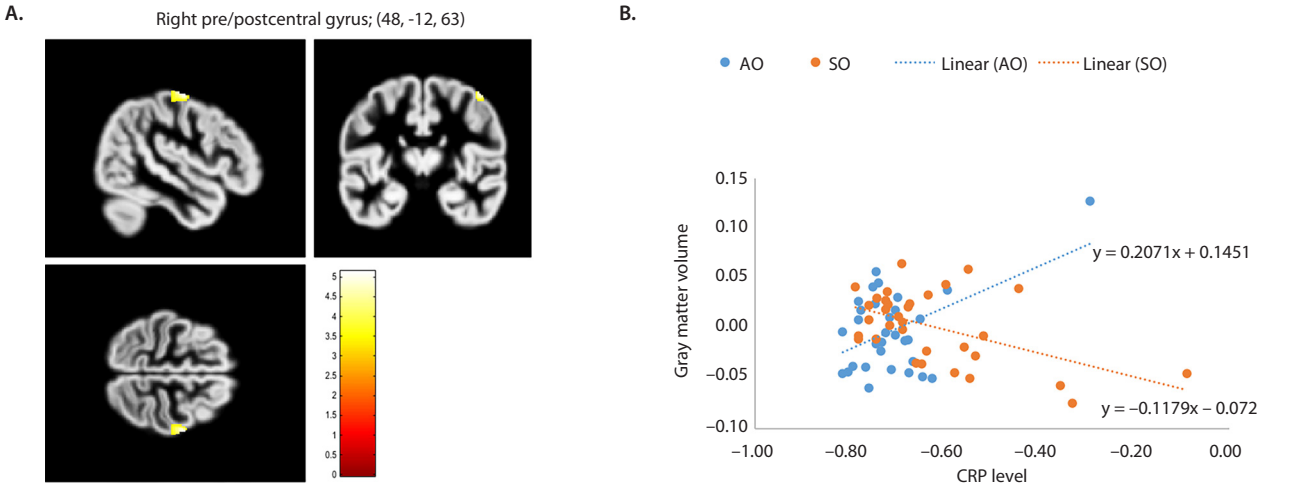
Based on a group-level independent-samples t test model that controlled for age, sex, TIV, and CRP level, we found that the asymptomatic offspring showed greater GMV than the symptomatic offspring in the left medial cerebellum (locus-of-maxima = −8, −72, −21, peak-level $t = 5.07$, $P = .049$, cluster size = 505). The reverse contrast generated no cluster that reached our predetermined voxel-wise statistical threshold.

If the CRP level was not controlled for, the same between-group difference was observed. Specifically, the asymptomatic offspring showed greater GMV than the symptomatic offspring in the left medial cerebellum (locus-of-maxima = −8, −72, −21, peak-level $t = 5.30$, $P = .023$, cluster size = 835). The reverse contrast generated no significant result.

Converted vs Non-Converted Offspring (Exploratory Analysis)

During the period of study and 6-year follow-up, 9 symptomatic offspring converted to full-blown BD. No asymptomatic offspring converted to BD. We conducted some preliminary exploratory analyses comparing the converted and non-converted offspring on CRP level and CPT-IP scores.

Figure 2. Partial Correlation Plot Showing Significant Group × CRP Interactive Effects on Gray Matter Volume in a Region Spanning the Right Precentral and Postcentral Gyri After Whole-Brain Voxel-wise FWE Correction of $P < .05$[a]



[a]CRP level was significantly and positively associated with the right pre/postcentral gyri volumes in the asymptomatic offspring, but significantly negatively associated in the symptomatic offspring. The CRP-optical density levels were log-transformed to improve data normality.
Abbreviations: AO = asymptomatic offspring, CPT-IP = Continuous Performance Test-Identical Pairs Test, CRP = C-reactive protein, FWE = family-wise error, SO = symptomatic offspring.

When the 9 converters were compared with all other offspring who had not converted (N = 55), the converters showed quantitatively greater CRP level, which was not significant (mean difference = 0.024, $t_{62} = 0.524$, $P = .602$, correcting for age, sex, and IQ). However, if the converters were compared with the asymptomatic offspring, they showed marginally greater CRP level (mean difference = 0.061, $t_{38} = 1.693$, $P = .099$ correcting for age, sex, and IQ).

In terms of the CPT-IP score, when the 9 converters were compared with all other offspring who had not converted, the converters showed quantitively reduced CPT-IP score (mean difference = 0.37, $F_{1,58} = 1.221$, $P = .274$, correcting for age, sex, and IQ). When the converters were compared with the asymptomatic offspring, they showed marginally reduced CPT-IP performance (mean difference = 0.555, $F_{1,34} = 2.971$, $P = .094$, correcting for age, sex, and IQ).

On the other hand, comparison of converters versus non-converters within the symptomatic group revealed no significant difference ($t_{31} = 0.424$, $P = .675$) in CRP level or in CPT-IP score ($F_{1,27} = 0.045$, $P = .833$).

These preliminary results suggest that elevated CRP level and reduced CPT-IP score could be inherent characteristics of offspring with subclinical BD symptoms, which are absent in asymptomatic offspring. It is also important to note that the marginally significant results could be due to the small sample size of converters, resulting in low statistical power.

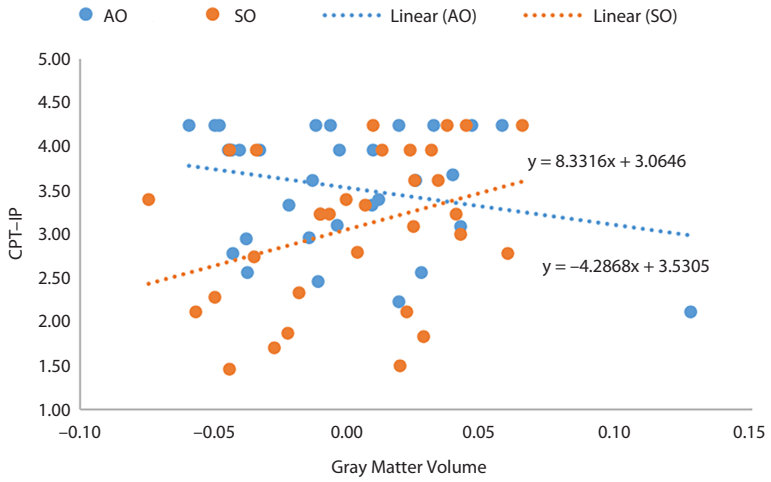
Further exploratory analysis revealed that 2 asymptomatic offspring became symptomatic during follow-up. The log-transformed baseline CRP levels of these 2 asymptomatic offspring were −0.804 and −0.699, respectively (while the average CRP level of the remaining 29 asymptomatic offspring was −0.709). The baseline CPT-IP score of the 2 asymptomatic offspring were 2.78 and 4.24, respectively (while the average CPT-IP score of the remaining 29 asymptomatic offspring was 3.542).

Relationship Among the Group, CRP, GMV, and CPT-IP Performance

We first examined whether CRP level mediated the significant association between offspring group and CPT-IP performance. As seen in Figure 1, the mediation analysis indeed showed that CRP level mediated a negative relationship between group and CPT-IP scores (95% CI = −0.3090 to −0.0384) after controlling for age, sex, and IQ score. The total effect of group on CPT-IP score was significantly negative (95% CI = −0.7180 to −0.0456). After accounting for the significantly negative indirect mediation effect, the remaining direct effect became insignificant (95% CI = −0.5791 to 0.0885). Decomposition of this result revealed that group (asymptomatic = 0, symptomatic = 1) exerted positive effect on CRP levels ($t = 2.37$, $P = .021$, 95% CI = 0.0117 to 0.01386), and CRP levels exerted negative effect on the CPT-IP (t = −2.75, $P = .008$, 95% CI = −0.3137 to −0.4954). This mediation effect was also significant in the permutation test ($P = .05$).

We then tested the association of CRP and brain GM structure. We obtained a significant group × CRP interactive effect on GMV in a region spanning of right precentral and postcentral gyri, which survived whole-brain voxel-wise FWE correction of $P < .05$ (locus-of-maxima = 48, −12, 63, peak-level $t = 5.12$, $P = .043$, cluster size = 226), after controlling for age, sex, and TIV (Figure 2). Specifically, in the asymptomatic group, the relationship between CRP and GM was positive ($t = 3.21$, $P = .0022$), while the same relationship was negative in the symptomatic group ($t = 2.93$, $P = .0049$). The same results were obtained after controlling for the clinical variables. This moderation effect of group on

Figure 3. Association Between CPT-IP Score and Right Pre/Postcentral Gyri Volumes[a]



[a]The asymptomatic group showed no association between precentral/postcentral gray matter and CPT-IP performances, whereas the symptomatic group showed a positive relationship between the right precentral/postcentral gray matter and CPT-IP performances. The CRP-optical density levels were log-transformed to improve data normality.
Abbreviations: AO = asymptomatic offspring, CPT-IP = Continuous Performance Test-Identical Pairs Test, CRP = C-reactive protein, SO = symptomatic offspring.

the relationship between CRP and GMV was also significant in the permutation test ($P < .001$).

Given the differential levels of CRP in the two offspring groups, it could also be that the group × CRP effect on the GM was due to a generally nonlinear relationship between CRP and GM, rather than a modulating effect of group. We conducted additional analyses to rule out this possibility. First, we tested whether there was a quadratic relationship between CRP and GMV among the total participant sample, but found no significant effect ($F_{2,61} = 0.289$, $P = .75$). Second, we divided all participants into a high-CRP and a low-CRP group based on median split and tested the CRP-GMV relationship in each group (controlling for age, sex, and TIV). However, the CRP-GMV relationship was not significant in either the high-CRP ($r = -0.061$, $P = .759$) or the low-CRP ($r = 0.297$, $P = .11$) group. In contrast, the same relationship was significantly positive in the asymptomatic group ($r = 0.445$, $P = .018$) and negative in the symptomatic group ($r = -0.496$, $P = .005$). Thus, the results suggested that our findings were not due to nonlinear relationship between CRP level and GMV.

Finally, we examined whether the GMV of the significant precentral/postcentral cluster was associated with CPT-IP performance in the offspring groups. We found a marginally significant moderating effect of the offspring group on the association between the GMV and the CPT-IP performance (bootstrapping 95% CI = −0.0359 to 13.1126, $t = 1.99$, $P = .05$). Specifically, the asymptomatic group showed no association between precentral/postcentral GM and CPT-IP performance (bootstrapping 95% CI = −6.0033 to 4.7691), whereas the symptomatic group showed a positive association between the right precentral/postcentral GM and CPT-IP performances (bootstrapping 95% CI = 0.4206 to 11.422) (Figure 3). This moderation effect of group on the association between precentral GMV and CPT-IP score was also significant in the permutation test ($P = .0185$).

Given that 1 mediation and 2 moderation analyses were performed, Holm-Bonferroni correction was conducted on the permutation-based P values to correct for multiple testing. All of the mediation and moderation effects remained significant after correction ($P_{corrected} < .05$).

DISCUSSION

This study revealed distinct relationships among peripheral inflammation (CRP), brain structure (GMV), and cognition (attention) in a cohort of bipolar offspring with and without subthreshold mood symptoms. During the 6-year follow-up, none of the asymptomatic offspring developed BD, who may possess resilience-related characteristics. On the contrary, the symptomatic offspring showed a high risk (25%, 9/33) of transitioning to BD onset during the 6-year follow-up, who may thus possess endophenotype markers prior to disease onset.

In line with our first hypothesis, we observed significant higher CRP levels in the symptomatic offspring relative to the asymptomatic offspring. CRP is a sensitive marker involved in chronic inflammatory-related disorders such as BD, and a moderate increase in its plasma level reflects an underlying cellular stress phenomenon.[45] Previous prospective studies and meta-analysis have demonstrated that elevated level of CRP could independently predict the onset of BD in the general and high-risk populations.[46,47] Some studies suggested that inflammation was a consequence of stressors from generic and environmental factors and that it further exerted a deleterious effect on specific brain areas, which

contributed to developing stress-induced psychiatric disorders such as BD.[13,48] Emerging evidence suggested that the initial elevated inflammation may be a protective reaction to reduce the adverse consequences and maintain normal mental function,[20,49] but prolonged dysregulated inflammation might contribute to the severity of symptoms and mood episode.[50] The CRP level of our symptomatic offspring probably reflected a dysregulated inflammation pattern and contributed to the development of BD. The mean CRP level of our asymptomatic offspring was slightly over the normal range, suggesting that a mild inflammatory condition might be protective for the asymptomatic offspring.

Consistent with our second hypothesis, we observed significantly poorer CPT-IP scores in the symptomatic group than the asymptomatic group and found that this group difference in attention functioning was mediated by the group difference in CRP levels. Specifically, we found that CRP levels were significantly and negatively associated with attention performances. Between the groups, the different CRP levels reflect different magnitudes of inflammatory conditions, which may in turn induce distinct inflammatory effects on the brain, which contributed to the different attentional performance.[19] Our results showed that across the symptomatic and asymptomatic offspring, higher inflammation was linked with compromised attention function.

One main finding of this study is that we observed a significant group × CRP interactive effect in the region spanning the right precentral and postcentral gyri, of which the GMV showed positive correlation with attention cognition in the symptomatic offspring. Such correlation was absent in the asymptomatic offspring. Precentral and postcentral gyri are traditionally known as the primary motor and sensory areas, respectively, while their roles in cognitive function are relatively less understood. A study by Hwang et al[51] showed increased activity in the precentral and postcentral gyri during an attentional task in healthy subjects, suggesting that the regions were involved in top-down attentional responding. Moreover, Pagliaccio et al[7] observed blunted activity in precentral and postcentral gyri during a similar attentional task in both individuals with BD and those at familial risk of BD, suggesting that the regions may be involved in the development of BD.

Moreover, we observed a different direction of relationship between the CRP and the GMV of pre/postcentral gyri in our asymptomatic (positive) and symptomatic (negative) groups. This result was not due to a generally nonlinear relationship between CRP level and GMV, but rather due to inherent differences between the two offspring groups. We consider 2 possibilities for explaining this result. First, inflammatory cytokines such as CRP are known to affect the brain structure and function in mental conditions.[27,52] On the other hand, certain neurogenesis factors such as brain-derived neurotrophic factor (BDNF) have been shown to promote neural plasticity and cell growth and thus may modulate the inflammatory neurotoxic effect on the brain.[53–55] There may also be other anti-inflammatory agents such as the T lymphocyte pathway[56] that may reduce the inflammatory neurotoxic effect on the brain. Future research may continue to explore important agent factor(s) that modulate the brain-CRP relationship in BD. In this context, the higher levels of BDNF or other endogenous anti-inflammatory agents could represent a resilience factor that renders an individual less affected by CRP. Second, the differential CRP-brain relationship in the two offspring groups could be caused by external factors such as stress. In general, short-term stress might stimulate and enhance immuno-protective processes that help orchestrate the immune defense against the detrimental effects of stressful events, whereas a prolonged stress might exacerbate pathological immune response.[57] The resulted inflammation might influence the neurogenesis and synaptic plasticity resulting in GM structural alteration via neuroendocrine and neurohormonal pathways, which can be adjustable and reversible.[58] Thus, it could be that the asymptomatic and symptomatic offspring groups experienced different levels and durations of stress. The asymptomatic group may experience relatively short-lasting and mild stress, resulting in adaptive, protective, and compensatory neural growth (ie, a positive CRP-brain relationship). In contrast, the symptomatic group may experience relatively long-lasting and severe stress, resulting in brain atrophic processes due to excessive inflammatory responses (ie, a negative CRP-brain relationship). In this context, if the stress level for an asymptomatic individual becomes long-lasting and severe in the future, this individual could switch to show a detrimental effect of immune responses on brain volume (ie, a negative CRP-brain relationship). This speculation needs to be verified by further longitudinal follow-up research on the inflammatory and brain measures of BD offspring who switch from asymptomatic to symptomatic state.

The present study showed a different baseline CRP-attention-GM relationship in our asymptomatic and symptomatic offspring. However, we still need to consider the modest statistical strength of our small sample (n = 64). Few studies have directly investigated the inflammation-cognition-brain profile in high-risk individuals. We previously observed a mutual relationship among IL-6, anterior cingulate cortex volume, and attention, which were different between asymptomatic and symptomatic groups.[29] Notably, a recent study by Nielson et al[59] reported little difference in CRP level between BD patients, unaffected relatives of BD patients, and healthy controls, which differs from our findings. One possibility might be the different dietary customs between the Danish and Chinese Han populations. The Western-type diet tends to be more calorically rich compared to the Chinese Han diet. The different dietary cultures can result in different levels of organismal metabolism[60] and compositions of gut microbiota,[61] which in turn could have impacts on the inflammatory/immune systems.[62] The mean BMIs of the subjects of Nielson and colleagues' study were higher than

those of ours, which could serve as a proinflammatory factor and impact the gut-brain axis.[61] We speculate that those factors might be involved in the pathophysiology of BD and partly explained the different results. Under the premise of scarcity of relevant studies, our results still could provide a significant reference for future study.

Limitations

Several limitations need to be considered when interpreting the findings. First, the sample size was modest. Second, we did not include a healthy control group. Third, we could not rule out the possibility that our asymptomatic offspring would develop subclinical/clinical symptoms in the future because they were still in the peak age at onset, despite none of our asymptomatic individuals developing full-blown BD during the 6 years of follow-up. Finally, it is worth noting that this is a cross-sectional study that does not allow us to establish the direction of causality for the relationships, eg, between CRP and CPT-IP.

CONCLUSIONS

In conclusion, our data suggest that there are distinct correlations among CRP level, attention, and GM in symptomatic and asymptomatic offspring of patients with BD. The higher level of CRP, poorer attention, and positive correlation between the GMV of precentral/postcentral regions and attention functioning may represent an endophenotype-related characteristic or a risk biomarker for the development of BD, whereas the opposite correlation observed in asymptomatic offspring may represent a resilience or protective factor for BD.

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