Inflammation, brain structure and cognition interrelations among individuals with differential risks for bipolar disorder

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Abstract

Neuro-inflammation might impact on clinical manifestations and cognition function via changing the volumes of key brain structures such as the anterior cingulate cortex (ACC) in bipolar disorder (BD). In this study, we investigated the interrelations among interleukin (IL)-6 cytokine level, grey matter (GM) volume of the anterior cingulated cortex (ACC), and attention function among offspring of parents diagnosed with BD. The offspring were categorized as being either asymptomatic or symptomatic based on whether they manifested pre-defined sub-threshold mood symptoms. We found that the symptomatic offspring showed significantly higher serum levels of IL-6 than the asymptomatic offspring ($F_{(1, 59)} = 67.65$, $p<0.001$). On the brain level, we obtained significant interactive effect of group and IL6 level on the ACC GM ($P_{FWE}=0.017$). Specifically, the GM volume of the rostral ACC was negatively associated with the levels of IL-6 in the asymptomatic offspring ($P_{FWE}=0.021$), but not the symptomatic offspring ($P_{FWE}>0.05$). Mediation analyses revealed that the GM volume of the rostral ACC significantly mediated the negative association between the IL-6 levels and attention performance in the asymptomatic offspring (bootstrapping Confidence Interval (CI) = -6.0432 to -0.0731) but not the symptomatic offspring (bootstrapping CI = -0.3197 to 1.3423). Our data suggest that the asymptomatic and symptomatic bipolar offspring may exhibit different neurocognitive-inflammatory profiles, which could be further validated as viable biosignatures for BD risk and resilience.

Key words: Bipolar disorder; Offspring; Interleukin-6; Cognition; Neuroimaging

Introduction

Bipolar disorder (BD) is a highly heritable mental illness, characterized by episodes of depression and hypo/mania, typically starting with depression during adolescence then progressing to hypo/mania in the early adulthood. Neuro-inflammation may play an important role in the pathogenesis of BD (Goldstein et al., 2009; Maes and Carvalho, 2018). Genetic research showed that inflammation-related genes were over-expressed in genetically at-risk offspring of parent with BD in a dose-dependent relationship: An "inflammatory signature" (defined as the overexpression of a set of inflammatory genes) was found in 85% of BD offspring as a whole, 45% of a subgroup who were unaffected, and in 100% of those who had already developed a mood disorder, compared to 19% in the healthy controls (Padmos et al., 2008).

At the protein level, serum inflammatory cytokines such as interleukin (IL)-1, IL-6 and corticotrophin releasing hormone (CRF) were elevated in BD offspring and patients (Duffy et al., 2012; Sayana et al., 2017). Cerebrospinal fluid IL-6 level was correlated with mood symptoms severity in depressive patients, while peripheral administration of Etanercept that was used to antagonist pro-inflammatory cytokine tumor necrosis factor (TNF)-alpha, eliminated depressive-like behavior in animal chronic stress models (Krügel et al., 2013). Moreover, neuroimaging studies demonstrated that microglia, the level of which is an indicator of immune response in the brain, was activated in the brain regions implicated in mood disorders such as the anterior cingulate cortex (ACC) (Capuron et al., 2005). Furthermore, post-mortem studies of BD patients showed that the number and size of glial cells were reduced

(Rajkowska et al., 2001), perhaps due in part to relative overexpression of IL-1 beta, IL-I receptor, and c-Fos leading to excessive inflammatory reactions and excitatory toxic effects (Rao et al., 2010), and finally to brain regional atrophy and cognitive impairments (Naaldijk et al., 2016).

Cognitive impairment is a core feature of bipolar disorder. Cognitive deficits of some domains emerge early and even prior to the onset of the illness, while deficits in other domains such as attention are moderately detectable at the early phases of the illness and become severe after a number of episodes (i.e. depression and mania), possibly partly affected by the side effects of medications such as valproate (Elias et al., 2017; Lin et al., 2017; Porter et al., 2016). A few studies showed that levels of peripheral serum inflammatory cytokines were negatively associated with cognitive functioning in BD patients (Dickerson et al., 2013; Doganavsargil-Baysal et al., 2013), but little is known about the mechanisms by which inflammatory cytokines affect the brain structure associated with cognitive impairment (Bauer et al., 2014).

The ACC plays a vital role in mood regulations. Its structural volume was found to be decreased during the early course of BD patients (Drevets et al., 2008; Yu et al., 2019). Phillips et al. proposed that deficient control of the ACC over the amygdala was a principle abnormality of BD (Phillips and Swartz, 2014). Increased ACC activity and decreased amygdala-ACC functional connectivity during emotion regulation tasks were found in BD offspring and suggested to be a specific marker of BD risk (Acuff et al., 2018; Manelis et al., 2015). Moreover, postmortem studies showed that ACC volume was reduced due to loss of glia, the key cell of the immune

system in the brain (Ongur et al., 1998). A follow-up study showed that metabolic changes in the ACC existed prior to the onset of depression (Kumano et al., 2007), and this manifestation was seen for a few occasions prior to discrete hypomanic episodes (Akiskal et al., 1995; Duffy, 2010). Furthermore, inflammatory cytokines had impacts on the ACC, whose activity in turn was correlated to the number of errors on an attention task (Capuron et al., 2005; Critchley et al., 2005). In cognitive tasks demanding conflict monitoring, the ACC activation level reflected the degree of intentional effort (Duncan and Owen, 2000) and was found to be abnormal in BD (Manelis et al., 2015). Under the negative effect of cytokines, the ACC may need to be more involved in order to exert greater cognitive controls to maintain normal cognitive performance, as was previously observed (Capuron et al., 2005). The above evidence might suggest that inflammatory reactions affect the ACC in the early course of BD, whose interactions may be closely linked to attention function, which needs to be closely researched.

Offspring of parent with BD have a high risk of developing mood disorders, as evidenced by two well-conducted longitudinal studies showing approximately 25% bipolar offspring subsequently developed BD. Besides, an even larger proportion would suffer disturbances in mood regulations that fall short of a DSM-defined psychiatric diagnosis, including minor depression, subthreshold hypomanic, and mood liability (Duffy et al., 2014; Mesman et al., 2013). This subclinical group has frequently been labelled as ‘unaffected’ in bipolar research. However, growing studies showed that compensatory reactions and/or etiological processes observed in the

symptomatic offspring of parents—who fall short of a psychiatry diagnosis—distinguish them from those who were free of symptoms (Duffy et al., 2009; Hajek et al., 2008; Hanford et al., 2016; Lin et al., 2015). As well stated by McGorry (McGorry et al., 2003), though symptomatic individuals are not "patients", clinical attention should be paid to them so as to ameliorate symptoms and facilitate early identification and intervention when a disorder occurs. Identifying biomarkers that prevent symptom manifestations (i.e. resilience or protective factors) and those predictive of disease development—which may respectively be found in asymptomatic and symptomatic offspring—is critical, given the huge burden of BD on the individual's life and the society, the great difficulty of diagnosing and treating the disease, and the immense need for early disease identification and prevention.

Given these considerations, this study explicitly investigated the interrelations between cytokine levels (i.e. IL6), the ACC structural volume, and attention function in the genetically at-risk offspring of bipolar patients, in order to elucidate whether the symptomatic and asymptomatic high-risk individuals may exhibit different inflammation-brain-cognition profiles that may be biomarkers for resilience and risk for BD. We tested the following hypotheses: i) IL6 levels will be correlated with the structural volume of the ACC; ii) the ACC structural volume will predict participants' performance on an attention task and iii) the ACC structural volume will mediate the correlation between IL-6 levels and attention performance. In view of the distinct etiological characteristics of asymptomatic and symptomatic bipolar offspring, we explicitly examined whether the effect of IL-6 on ACC structure, as well as the

mediating effect of ACC on the association between IL-6 and attention, may be different across asymptomatic and symptomatic bipolar offspring.

Materials and methods

Participants

The present study was part of the Recognition and Early intervention on Prodromal Bipolar Disorder (REI-PBD) project, launched in 2013 by the Global Mood and Brain Science Initiative as previously described (Lin et al., 2018b; Lin et al., 2015; Mansur et al., 2017). Briefly, the project followed up a cohort of bipolar off spring whose parents were diagnosed with BD. The data of the current study was derived from the above ongoing project (March 2013-June 2016). This study was approved by the Institutional Review Board of the Guangzhou Brain Hospital. All participants (if aged 18 years or above) or their guardian (if aged under 18 years) gave written informed consents.

Participants were offspring of parent who was diagnosed with bipolar type I or type II. Parents with BD were inpatients or out-patients who received psychiatric services in the Guangzhou Brain Hospital. The offspring were referred by their parent or self-referred through advertisement. All the offspring were free of psychotropic medication at the time of study. The diagnosis of BD was established according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. As described previously, the inclusion criteria for asymptomatic offspring were as

follows: i) having at least one biological parent diagnosed with BD; ii) aged 8-28 years old, and iii) none of the sub-threshold syndrome as described below and free of any DSM-IV defined psychiatric disorders. In total 32 asymptomatic individuals were recruited, among which one was excluded due to noncompliance with the Magnetic Resonance Imaging (MRI) scan protocol, leaving 31 participants in the data set.

The symptomatic offspring included in this study met at least one of the following sub-threshold syndromes: i) two or three hypomanic symptoms present for at least 4 days (not meeting DSM-IV hypomania episode criteria); ii) two or more DSM-IV defined major depressive symptoms 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); iii) one 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 attenuated psychotic symptoms included: ideas of reference, odd ideas, odd beliefs, unusual perceptual experiences, bizarre thoughts or speech, grandiosity, suspicious ideas, paranoid ideas, odd mannerisms, hallucinations, disorganized/catatonic behaviors; iv) two or more hyperactivity/impulsivity symptoms defined for attention deficit hyperactivity disorder (ADHD) were observable by teachers, peers, and/or parents. Notably, the symptomatic offspring included in this study who manifested ADHD or attenuated psychotic symptoms also displayed either sub-threshold depressive or hypomanic syndromes. Kiddie-Schedule for Affective Disorders(K-SAD), Structured Clinical Interview for DSM-IV Axis II Disorders(SCID-II), and the following clinical scales were applied to assess the

participants' symptomology, including the Hamilton Depression/Anxiety Rating Scale (HAM-D/A), the Young Mania Rating Scale (YMRS), and the Brief Psychiatric Rating Scale (BPRS). In total 37 symptomatic individuals were included, among which 4 were excluded due to neurological condition or noncompliance with the MRI protocol, leaving 33 participants in the data set.

Cognitive performance and peripheral IL-6 level measurements

Attention was measured by the Continuous Performance Test-Identical Pair test (CPT-IP), derived from the MATRICS Consensus Cognitive Battery (MCCB). Serum samples were separated from whole blood samples by centrifugation at 3000g for 10 min at 4°C, and were then stored in 1ml aliquots at -80°C. Levels of IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (CUSABIO) (Yu, et al., 2017) . All samples were assayed in triplicate.

Participants' IL6 levels were log-transformed and analyzed using univariate ANOVA, controlling for age, sex and Test of Nonverbal Intelligence (TONI-III) intelligence quotient (IQ) score. The analysis was repeated after additionally controlling for all clinical variables (HAMD, HAMA, YMRS, BPRS). The analysis was also repeated without incorporating the covariates (Supplementary Material). The logged IL6 levels were normally distributed as assessed with the

Kolmogorov-Smirnov test, and satisfied the equality of variance assumption as assessed with the Levene's test.

Demographic, behavioural and clinical analyses

Participants' demographic information was analyzed using independent-samples t-test (for age and education), Chi-square test (for sex and handedness), and univariate ANOVA controlling for age and sex (for TONI-III IQ score) (Table 1). Performance score on 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. The analysis was also repeated without including the covariates (Supplementary Material). Data normality was checked using the Kolmogorov-Smirnov test, and equality of variance was assessed using the Levene's test. All data except for the two binary variables of sex and handedness satisfied the normality and equal variance assumptions. Statistical threshold was set as $p<0.05$, two-tailed.

Participants' clinical variables were determined to be non-normally-distributed according to the Kolmogorov-Smirnov test ($p<0.05$), thus the non-parametric independent-samples Mann-Whitney U test was performed.

Structural imaging data acquisition and processing

Participants' T1-weighted high-resolution anatomical scans were acquired with a 3T Phillips scanner equipped with an 8-channel SENSE head coil, utilizing an interleaved sequence (188 sagittal slices, TR/TE/flip angle =8.2 ms/3.7 ms/7°, matrix = $256\times256mm^2$, FOV = $256\times256\times188mm^3$, voxel size = $1\times1\times1mm^3$). Data processing and analyses were conducted using the Computational Anatomy Toolbox (CAT12) (http://dbm.neuro.uni-jena.de/cat/) and embedded functions of Statistical Parametric Mapping (SPM12) (Wellcome Department of Cognitive Neurology, London, UK). The T1 images were reoriented, cropped, and bias-corrected before being affine-registered using customized age-specific tissue probability map generated using the Template-O-Matic (TOM8) toolbox (Wilke et al., 2008). Then, the images were segmented and normalized following the standard Diffeomorphic-Anatomical-Registration-Using-Exponentiated-Lie-Algebra (DARTEL) procedure (Ashburner, 2007), using customized normalized DARTEL template generated on the total participant sample (see Supplementary Materials for details). The resulted modulated normalized grey matter (GM) images were then smoothed using an 8mm full-width-at-half-maximum (FWHM) Gaussian Kernel. Image quality check was performed after segmentation to ensure high weighted average image quality for all participants.

The smoothed, normalized and modulated GM images were analyzed using independent-samples t-test including two groups (asymptomatic vs. symptomatic offspring). The model additionally incorporated two variables representing the IL6 levels in each group, so as to assess the possible interaction of group and IL6 on GM

volumes. Total intracranial volume (TIV), age and sex were entered as covariates of no interest. We focused specifically on the region of interest (ROI) of the ACC. The ACC ROI template was constructed using the Wake Forest University (WFU)-Pickatlas software, and included bilateral rostral, dorsal and subgenual anterior cingulate (BA 24, 25, 32). In order to more precisely locate any effects in the ACC, we further divided the ACC ROI mask into a relatively dorsal and caudal portion (above the genu, $z>10$; relatively caudal, $y<30$), a rostral portion (anterior and not inferior to the genu, $y>30$, $z>0$), and a subgenual portion (below the genu, $z<0$). We then examined the GM volume of each of the subdivision of the ACC on the key contrasts of interest (e.g. group × IL6 effect). Moreover, given the important involvement of the limbic structures such as the amygdala and the insula in emotion processing, we also included those regions in the complementary analyses (Supplementary Materials). Statistical thresholds for the resulted t-contrast maps were determined as family-wise-error (FWE)-corrected $p<0.05$ at the peak level within the ACC (and amygdala and insula) ROI or in the whole brain. Mean GM volumes were then extracted from the significant cluster(s) and analyzed further using SPSS v.21 (IBM Corp.) (see below).

Cytokine-brain-cognition analysis

After respectively assessing the effect of offspring group on attention and on the relationship between IL6 level and brain structural volume, we proceeded to test

whether the brain structural volume mediated any association between the IL6 level and attention, and whether such mediation effect was different in the symptomatic and asymptomatic offspring groups. The mediation analyses were performed using the PROCESS macro implemented in SPSS, which utilized a bootstrapping approach (Hayes, 2013). Bootstrapping is a non-parametric approach to test hypotheses without making inherent assumptions on the data distribution. First, we assessed, for the combined participant sample and for each of the symptomatic and asymptomatic offspring groups, the mediating effect of brain structural volume for the relationship between IL6 level (predictor) and attention performance (dependent variable), controlling for age, sex, TIV and TONI IQ. Second, we explicitly tested the moderating effect of offspring group on the mediating effect. Bootstrapping was carried out using a bias-corrected procedure with 5000 samples, which outputs confident-intervals (CIs). Significance was determined on whether the CIs encompassed zero (Preacher and Hayes, 2008).

Results

Demographic, clinical, behavioural AND cognitive:

Symptomatic and asymptomatic offspring groups were matched on age, sex, years of education, IQ and handedness (all $p>0.05$, Table 1). As expected, the symptomatic group showed higher clinical scores than the asymptomatic group on the HAMD (standardized $t = 4.31$, $p<0.001$), HAMA (standardized $t = 3.54$, $p<0.001$), YMRS

(standardized t = 3.69, p<0.001), and BPRS (standardized t = 5.63, p<0.001) measures (Table 1). After controlling for age, sex and TONI score, the symptomatic offspring group showed poorer performance on the CPT-IP attention task than the asymptomatic offspring group ($F_{(1, 59)}$ = 5.17, p = 0.027) (Table 1). Attention performance also showed significant positive association with the HAMD score after controlling for other clinical variables ($F_{(1, 53)}$ = 4.45, p = 0.04). The effect of group remained marginally significant if controlling for the HAMD score ($F_{(1, 56)}$ = 3.42, p = 0.07).

IL-6 cytokine levels:

The symptomatic group showed significantly higher serum IL-6 levels than the asymptomatic group ($F_{(1, 59)}$ = 67.65, p<0.001), even after controlling for all clinical variables ($F_{(1, 54)}$ = 26.37, p<0.001) (Table 1). No clinical variables had significant effect on the IL-6 levels ($F_{(1, 54)}$< 3.23, ps>0.075).

Cytokine-brain structure relationship:

Across all participants, we found no significant main effect of IL6 level on brain structural volume in the ACC ROI or across the whole brain that survived FWE correction. The main effect of group generated a marginal effect in the rostral part of the ACC ROI (locus-of-maxima = -15, 47, 0; maximal t = 3.80, P_{FWE}= 0.055, voxel number = 31), as the asymptomatic offspring group showed increased ACC grey

matter volume than the symptomatic offspring. No other main effect of group was observed at the whole-brain level.

Critically, significant interactive effect of group and IL6 level was observed in the rostral ACC, characterized by more positive (or less negative) association of the IL6 level with the ACC volume in the symptomatic offspring group compared to the asymptomatic group (locus-of-maxima = -9, 42, 9; maximal t = 4.23, P_{FWE}=0.017, voxel number = 102) (Figure 1). When the two groups were combined, no significant relationship was observed between the IL6 level and volume of this ACC region (controlling for age, sex and TIV, t = -1.54, p>.1). Follow-up analysis revealed that the grey matter volume of a very similar rostral ACC region showed significantly negative association with the IL6 level in the asymptomatic offspring group (locus-of-maxima = -8, 44, 9; maximal t = 4.16, P_{FWE}=0.021, voxel number = 192), while no such association was detected in the symptomatic offspring.

The ACC cluster in which the GM volume showed significant group × IL6 effect fell entirely in the rostral ACC mask (locus-of-maxima = -9, 42, 9; maximal t = 4.23, P_{FWE}=0.008, voxel number = 102, note that the peak coordinates and voxel number match perfectly with those of the original ACC cluster). In contrast, no significant voxels were observed in the dorsal ACC or subgenual ACC masks. Furthermore, no significant dorsal ACC or subgenual ACC voxels were observed when examining the effect of IL6 in the asymptomatic group. Thus, we are very convinced that our results specifically concerned the rostral portion of the ACC.

IL-6, brain and cognition

After establishing the association between the IL-6 levels and ACC structural volume in the symptomatic and asymptomatic offspring groups, we then tested whether the ACC volume mediated any relationship between the IL6 level and attention performance on the CPT-IP task. First, we examined whether the IL6 level predicted individual participant's attention performance. In the combined sample, linear regression analysis controlling for age and sex indeed revealed that IL6 level significantly and negatively predicted CPT-IP score (β=-1.195, bootstrapping standard error = 0.588, p=0.046). Mediation analysis was then conducted, with the mediator being the mean grey matter values of the significant rostral ACC voxels that were significantly affected by the group × IL6 interaction. This analysis revealed that the mediating effect was significant and negative (bootstrapping CI = -0.9854 to -0.04). Specifically, IL6 level had negative effect on the rostral ACC volume (bootstrapping CI = -0.1588 to -0.0083), which in turn had positive effect on CPT-IP score (bootstrapping CI = 0.6685 to 8.7897) (Figure 2). We also tested the mediation model in which the IL6 level was the mediator, and the rostral ACC volume was the predictor. This model generated no significant mediation effect (bootstrapping CI = -0.785 to 1.6302), but significant direct effect (bootstrapping CI = 0.6685 to 8.7897). Thus, the evidence was in favour of a model that the IL6 influenced attention performance via its effect on the rostral ACC structure.

Given we observed that the IL6-ACC volume relationship was moderated by offspring group, we then tested whether the ACC volume differentially mediated the IL6-cognition relationship in the asymptomatic and symptomatic groups. To this end a moderated mediation model was implemented, with offspring group as the moderator. Indeed, a significant positive moderating effect was observed (bootstrapping CI = 0.3616 to 3.8885) (Figure 2). Follow-up analysis revealed that the mediating effect of ACC volume was significant and negative in the asymptomatic group (bootstrapping CI = -6.0432 to -0.0731), but not in the symptomatic group (bootstrapping CI = -0.3197 to 1.3423). Similar to the pattern in the combined sample, in the asymptomatic offspring, IL6 level showed negative effect on the ACC volume (bootstrapping CI = -0.4535 to -0.1676), which in turn had positive effect on the attention performance (bootstrapping CI = 2.4694 to 15.7104). The significant moderating effect of group remained significant even controlling for all the clinical variables simultaneously (bootstrapping CI = 0.3762 to 4.1294). In this model, the BPRS score showed significant negative relationship with the CPT-IP score (bootstrapping CI = -0.2175 to -0.013).

Discussion

Most individuals with BD manifest sub-syndromal symptoms during the early phases of the illness. Despite not all offspring of bipolar parent who manifest sub-threshold syndromes would later develop BD, sub-threshold syndromes particularly manifested

during the peak age of the illness onset (i.e. adolescence and early adulthood) are the strong predictors for future BD development, which thereby are gaining increasing clinical and research attention (Duffy et al., 2009; Duffy et al., 2014). On the contrary, those genetically at-risk individuals who are free of clinical symptoms may have distinct biological processes relating to resilience and protective factors. In the current study, the participants included were drawn from an ongoing longitudinal project, in which the participants were followed-up for up to five years. We previously reported the symptomatic offspring were at high risk of developing BD with nearly 20% conversion rate over an up-to-4-year follow-up study (Lin et al., 2017; Lin et al., 2018b). The present study emphasized the differences in the relations of the rostral ACC structure with IL-6 levels, and in the mediating effect of the ACC volume on the cytokine-cognition relationship, between the asymptomatic and symptomatic offspring groups.

Consistent with our hypotheses, we found that the GM values of ACC were not only negatively correlated to peripheral IL-6 level but also mediated the effect of IL-6 on attention in the asymptomatic offspring. Such pattern was not observed in the symptomatic group, highlighting distinct neural-immunological-cognitive patterns among individuals with differential levels of BD risk. Ample evidence shows that inflammatory cytokines such as IL-6 play important roles in learning and memory and long-term potentiation (LTP). For instance, significant impairments in learning and memory and long-term LTP were found in IL-1 type 1 receptor knock-out mice (Yirmiya and Goshen, 2011). In some conditions IL-6 may play a protective role.

Administration of IL-6 could partly protect memory deterioration caused by the amnesic drug scopolamine (Bianchi et al., 1997) as well as in animal model of ischemia (Matsuda et al., 1996). In humans, chronic fatigue syndrome (CFS) patients and healthy controls were administered exogenous IL-6 to temporarily activate the acute inflammatory response, and their neurocognitive performance was unexpectedly improved (Arnold et al., 2002). Moreover, elevated IL-6 levels could protect against deterioration in declarative memory caused by surgical stress in humans (Shapira-Lichter et al., 2008), and higher plasma IL-6 level was correlated with less cognitive decline in Lupus patients (Kozora et al., 2001). Relatively fewer studies have investigated the correlation of cytokines and attention. Attention forms the basis for higher cognitive functioning such as executive control and memory, and deficits in attention could lead to memory impairment.

Our finding of the association of IL-6 and ACC volume provides evidence for the involvement of inflammatory cytokine IL-6 in the structural plasticity of this brain region. Our study further delineated a mediating role of the ACC on the relation between IL-6 and attention. Notably, our previous findings showed that in the asymptomatic offspring, attention was not impaired compared to healthy controls. Thus, apart from bipolar genetic vulnerability, there were no difference between the asymptomatic offspring and healthy controls in terms of cognitive function and clinical symptoms. Considering that these individuals were able to maintain a symptom-free status despite the genetic risk, the asymptomatic offspring may possess resilience mechanisms that protect them from disturbances in mood, as we previously

suggested (Lin et al., 2018a). Our current findings suggest that the ACC structure mediated the effect of IL-6 on attention function exclusively in asymptomatic bipolar offspring, which might be the signature of a resilience mechanism. This possibility warranted further investigation.

Besides its protective effect, however, it is now clear that IL-6 could exert detrimental impacts on the brain function and neurogenesis, particularly when it is elevated in greater magnitude and prolonged periods, as in many clinical conditions. For instance, a functional neuroimaging study showed that during depressive state, increased IL-6 and plasma C-reactive protein (CRP) levels (which was positively correlated with the IL-6 level) disrupted the functional connections between the striatum and the ventromedial prefrontal cortex (vmPFC), weakening the control of the prefrontal cortex over striatum, the extent of which correlated with anhedonia and motor slowing (Felger et al., 2016). A recent 10-year longitudinal study (n=4630) showed that participants with chronically increased IL-6 levels had greater likelihood of common mental disorders when compared with those who had low IL-6 levels, even adjusting for acute inflammation, obesity, smoking and drug treatments (Kivimäki et al., 2014). Despite short-term inflammatory environment may enhance the proliferation of newborn neural precursor cells of the hippocampus, long-term elevation of cytokine levels resulted in the progression of neurogenesis depletion and prominent gliogenesis (Giannakopoulou et al., 2016).

No relation between IL-6 levels and the GM volume of the ACC was found in the symptomatic offspring. One possibility is that it may take prolonged periods for the

brain structure to be changed by increased IL-6 levels. Many studies showed that the ACC structure was normal in the early stage of BD, but was reduced in BD patients whose illness course lasted for years (Bora et al., 2010). Another possibility is that during this stage of increasing risk of developing BD, the impacts of inflammatory cytokines might be more prominent in the regions such as the dorsolateral prefrontal cortex, amygdala, and hippocampus, regions that might be more significantly affected during a stage closer to BD onset (Poletti et al., 2017; Savitz et al., 2013). In our study, symptomatic offspring, compared to their asymptomatic counterparts, had higher plasma IL-6 levels and worse sustained attention performance. Given the nature of this high-risk stage, the lack of correlations observed in the symptomatic offspring may reflect vulnerabilities that predispose the individuals to illness onset. The findings of this study supported our hypothesis that the two subgroups of 'unaffected' bipolar offspring—asymptomatic and symptomatic offspring—had distinct biological underpinnings, consistent with previous studies including ours (Lin et al., 2017; Lin et al., 2015; Paillère et al., 2014).

The results of this study should be considered in light of some limitations. First, this study did not include healthy controls and BD groups for comparisons, though the groups we included were congruent with our *a priori* hypotheses. Second, in this cross-sectional study of bipolar offspring whose clinical outcomes were assessed for up to 4 years, only baseline measurements of plasma IL-6 and attention cognition were included. Third, the sample size was modest. Fourth, since the asymptomatic offspring were still at the peak age of onset, it remains possible that these individuals

would progress to the symptomatic stage and to full illness development. However, our preliminary follow-up data (up to 4 years) indicate that no asymptomatic individual had developed full-blown hypo/mania. Finally, future study could investigate correlations between inflammatory cytokines and more extended brain networks connected with the ACC, such as the lateral prefrontal cortex and limbic areas.

The long term clinical outcomes and cognitive functions of bipolar offspring and patients are heterogeneous (Van Rheenen et al., 2019). Thus Identifying resilience mechanisms that protect against the development of the illness is as critical as identifying biomarkers that reflect the early etiological process of BD and that predict future BD onset. We obtained evidence for distinct mechanisms by which the ACC structure interacts with inflammatory cytokine IL-6 and attention, supporting phase-specific changes in the two subgroups of "unaffected" BD offspring. Future follow-up studies are warranted to confirm whether the distinct cytokine-brain-cognitive mechanisms may help predict the clinical outcomes of the two bipolar offspring groups. Towards the goal of pre-emption and prevention of bipolar disorder, if IL-6 plays a relevant mechanistic role, rather than serving as an epiphenomena, then perhaps targeting IL-6 pharmacologically may have benefits and represent a novel treatment avenue (Zhou et al., 2017).

Disclosures

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Partial correlation plots showing significant group × interleukin (IL) 6 effect on the rostral anterior cingulated cortex (ACC) grey matter (GM) volume. While IL6-optical density (OD) level was significantly and negatively associated with rostral ACC volume in the asymptomatic offspring group, a non-significant positive association was found for the symptomatic offspring. Left panel: the significant ACC cluster (in red) is overlain on the grey matter DARTEL template generated from the combined participant sample. Right panel: scatter plot of the relationship between the IL6 level (log-transformed) and the ACC volume, separately for the symptomatic and asymptomatic groups. Both variables were corrected for nuisance variables including age, sex and Total intracranial volume (TIV). *: significant at $p<0.05$.

Figure 2. Mediation and moderated mediation models of the IL6-ACC volume-attention relationship. In the combined sample, the rostral ACC (rACC) grey matter volume significantly mediated the negative association between the IL6 level and performance on the Continuous Performance Test-Identical Pair test (CPT-IP) attention task. This mediation effect, however, was further moderated by offspring group, such that the effect was detected in the asymptomatic, but not symptomatic, offspring. Confident-interval (CI): bootstrapping confidence interval. *: significant at $p<0.05$.