Increased interleukin 18 activity in adolescents with early-onset psychosis is associated with cortisol and depressive symptoms

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ABSTRACT

Objective: Evidence indicates that the pathophysiology of adult psychosis involves immune dysregulation, but its associations with stress are often not considered. The inflammatory cytokine interleukin (IL)-18, which is elevated in adult schizophrenia, is suggested to be sensitive to stress. We compared the associations of IL-18 with cortisol and clinical variables in adolescents with early-onset psychosis (EOP) aged 12–18 years and age-matched healthy controls (HC).

Method: We measured serum IL-18, IL-18 binding protein (IL-18BP), IL-18 receptor accessory protein (IL-18RAP), IL-18 receptor 1 (IL-18R1) and cortisol, and calculated the IL-18/IL-18BP ratio in patients (n = 31) and HC (n = 60). Psychotic symptoms were assessed using the Positive and Negative Syndrome Scale and depressive symptoms by the Mood and Feelings Questionnaire-Child version (MFQ-C). Bivariate correlation analysis was used to explore relationships between IL-18/IL-18BP ratio and cortisol, depression and other clinical characteristics. Hierarchical multiple linear regression analysis was used to assess their individual contributions to the variance of the IL-18/IL-18BP ratio.

Results: Patients had significantly higher IL-18 levels and IL-18/IL-18BP ratios than HC, but similar IL-18BP, IL-18RAP and IL-18R1. Both cortisol (R^2 change = 0.05) and the MFQ-C score (R^2 change = 0.09) contributed significantly to the variance in IL-18/IL-18BP ratios after controlling for confounders.

Conclusion: We found increased IL-18 system activity in adolescents with EOP. Cortisol and depressive symptoms each contributed to the variance in the IL-18/IL-18BP ratio. Our findings support activation of inflammatory pathways in adolescent psychosis and suggest interactions between stress, inflammation and depressive symptoms in EOP.

1. Introduction

Immune system dysregulation is suggested to play a role in the pathogenesis of psychosis-spectrum disorders, as evaluated by genetic (Sekar et al., 2016; Stefansson et al., 2009), epidemiological (Benros et al., 2011; Brown and Derkits, 2010) and clinical studies of inflammatory markers in blood (Goldsmith et al., 2016) and cerebrospinal fluid (Wang and Miller, 2018). However, most of the evidence of a pro-inflammatory status in people with psychosis has been obtained in adults, and even in meta-analyses of adult medication-naïve first episode psychosis (FEP) heterogeneity among included studies introduce limitations (Fraguas et al., 2019). There are also indications that adolescent-onset FEP features a higher pro-inflammatory status than adult-onset FEP (Moreno et al., 2019). Further, few studies

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have explored immune dysregulation in psychosis and relationship to psychological stress and activation of hypothalamic-pituitary-adrenal (HPA) axis (Rodrigues-Amorim et al., 2017).

Psychological stress, a common risk factor for many physical and psychiatric illnesses, is known to influence the immune system (Fagundes et al., 2013). Apart from its involvement with the sympathetic nervous system and the HPA axis, the molecular basis of how psychological stress influence activation of immunological and inflammatory pathways is not clear (Deak et al., 2017). In patients with schizophrenia (SCZ), a disrupted cortisol-immune interaction has been described, with HPA-axis impairment and increased immune activation and inflammation in experimental psychological stress paradigms (Chiappelli et al., 2016; Glassman et al., 2018). Recent studies indicate that the cytokine interleukin (IL)-18 might be involved in these processes (Sugama and Conti, 2008). IL-18 is a major product of the nucleotide-binding domain and leucine-rich repeat containing family member pyrin domain-containing (NLRP)3 inflammasome, which is suggested to be responsible for translating endogenous and exogenous stress signals into inflammatory responses (Herman and Pasinetti, 2018). In major depression, a stress-associated psychiatric disorder, it was reported that the NLRP3 inflammasome is activated in blood cells from patients (Alcocer-Gomez et al., 2014).

The stress-sensitive NLRP3 inflammasome is proposed as a central player in priming of microglial cells, inducing lowered threshold for activation and amplified release of IL-18 and IL-1β in the CNS (Herman and Pasinetti, 2018). As a brain resident macrophage, microglia surveys and elicits immune responses, in addition to serving homeostatic functions. If persistently activated microglia can promote neuroinflammation, unregulated synaptic pruning, loss of synaptic architecture and neurodegeneration (Salter and Stevens, 2017), which may be relevant to psychiatric disorders. The central nervous system (CNS) is particularly sensitive to the effects of IL-18 due to widespread expression of receptors, which includes regions involved in mood regulation (Alboni et al., 2010). In homeostatic concentrations, IL-18 support neurons and enhance long-term potentiation, but in abundance IL-18 can alter synaptic activity and cytoarchitecture in the brain (Herman and Pasinetti, 2018).

Although genetic (Liu et al., 2011; Shirts et al., 2008), clinical (Orhan et al., 2018; Tanaka et al., 2000; Zhang et al., 2016, 2013) and functional studies (Reale et al., 2011) support a potential role of IL-18 in adult patients with SCZ, alterations in the IL-18 system are poorly described in adolescents with early-onset psychosis (EOP) (defined as psychosis with onset prior to 18 years of age). A small recent study of genetic alterations of the cytokine system in adolescents with EOP reported increased expression of IL-18 mRNA, but a greater decrease in mRNA expression of IL-18 receptor type 1 (IL-18R1), suggesting reduced overall IL-18 signaling (Xu et al., 2016). However, the youngest age groups of adults ($\leq$ 30 years) with SCZ were reported to have elevated IL-18/IL-18BP ratios, reflecting unbound bioactive IL-18, compared to older adults ($\geq$ 50 years) (Palladino et al., 2012). While IL-18 is a potent inflammatory cytokine, its activity can be attenuated by IL-18 binding protein (IL-18BP) and modulated by the soluble forms of IL-18R1 and IL-18 Receptor Accessory Protein (IL-18-RAP), although the impact of these latter molecules on IL-18 activity is uncertain. Thus, more studies of the IL-18 system in EOP that include pediatric patients who have few confounders and that evaluate circulating levels of all IL-18-related proteins are warranted.

Whether alterations in the IL-18 system are part of an intrinsic immune dysregulation in psychosis-spectrum disorders or occur as a consequence of confounders or psychosocial stress associated with these disorders is largely unknown. Previous studies have shown that inflammatory cytokines may be linked to negative symptom severity in adults with psychosis, but such relationships have not been explored in adolescents with EOP (Goldsmith et al., 2018). We hypothesized that patients with EOP exhibits increased IL-18 system activity, and that the activity is associated with levels of cortisol, perceived depressive

symptoms and negative symptoms. Therefore, in the present study we investigated (i) whether adolescents with EOP exhibit alterations in levels of IL-18-related proteins compared with healthy controls (HC), (ii) whether unadjusted and adjusted analysis indicate that the potential bioactive proportion of IL-18, as reflected by the IL-18/IL-18BP ratio, is significantly related to stress as estimated by cortisol levels and self-rated depressive symptoms, (iii) if having EOP contributes significantly to the variance in IL-18/IL-18BP ratio, and whether the ratio is related to the severity of negative symptoms.

2. Materials and methods

2.1. Study design

All participants were part of the ongoing longitudinal case-controlled Thematically Organized-Psychosis Study for Youth (Youth-TOP), at the University of Oslo and Oslo University Hospital, Norway. Inclusion criteria were: (1) meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnostic criteria of a SCZ spectrum disorder (SCZ, schizophreniform disorder, schizoaffective disorder), an affective psychotic disorder (bipolar spectrum disorder, major depressive disorder with psychosis) or other psychotic disorders (psychosis not otherwise specified [NOS], delusional and brief psychotic disorders); (2) aged between 12 and 18 years; (3) being able to provide written consent; (4) being able to communicate in Norwegian.

Exclusion criteria were: (1) an intelligence quotient (IQ) < 70; (2) previous moderate/severe head injury; (3) a diagnosis of substance-induced psychotic disorder; or (4) having organic psychosis.

HC aged 12–18 years from the same catchment area as the patients were randomly selected from the national population registry (www.ssb.no) and invited by letter to participate. HC were excluded if they: (1) currently met the criteria for, or had previously received treatment for, any Axis I diagnosis; (2) had an IQ < 70; (3) had a history of organic brain disease; or (4) had a previous moderate/severe head injury.

Participation was based on informed consent and conducted in accordance with the Helsinki Declaration, version 2008 (sixth revision). For those aged < 16 years, consent was provided by parents or guardians. The study was approved by the Regional Ethics Committee (South-East) for Medical and Health Research Ethics (2009/691) and the Norwegian Data Protection Authority (2003/2052).

2.2. Participants

We included patients (n = 33) and HC (n = 63) enrolled between January 2013 and October 2017, for whom fasting IL-18 and IL-18BP levels and cortisol values were available. All were somatically healthy, without known autoimmune or endocrine diseases, and with no comorbid substance abuse or dependence. At the time of blood sampling, none had symptoms of an infectious disease.

2.3. Clinical variables

2.3.1. Diagnosis, global functioning, psychotic and depressive symptoms

The diagnostic evaluation was based on the Norwegian version of the semi-structured clinical interview the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (Kaufman et al., 1997). Global functioning was measured by the Children's Global Assessment Scale (Shaffer et al., 1983). Psychotic symptoms were assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). A Norwegian version of the Mood and Feelings Questionnaire-Child version (MFQ-C) (Costello and Angold, 1988) was used to assess pediatric depressive symptomatology, because we wanted to capture perceived distress and negative affect. The MFQ-C is a 34-item, self-rating questionnaire for children and adolescents aged 6–17 years that has acceptable psychometric properties (Daviss et al., 2006). A cutoff of $\geq$ 29 points has been validated to

identify major depressive episodes (Daviss et al., 2006). Two HC had scores > 29, but were not removed, because preliminary analyses showed that they did not influence the results. Among patients there were no significant correlation between the PANSS negative sum scores and the MFQ-C scores (r = 0.21, p = 0.275). HC were assessed using the same psychometric tools as the patients, except for the PANSS.

2.3.2. Clinical and sociodemographic data

Information about smoking habits, duration of untreated psychosis (DUP) and mother's educational level (as a proxy measure of socioeconomic status) was obtained through clinical interviews. Smoking habits were dichotomized as yes/no smoking on a daily basis at the time of blood sampling. DUP was defined as the time interval in weeks with persistent symptoms qualifying for a score of ≥4 on any of the PANSS items—P1, delusions; P3, hallucinatory behavior; P5, grandiosity; P6, suspiciousness; or G9, unusual thought content—before the subject received adequate treatment for psychosis. Participants were weighed on calibrated digital scales under standard conditions, height was measured using standard methods and BMI (kg/m^2) calculated. One HC was neither weighed nor measured. Imputations were made for two patients and three HC with missing height values. In these cases, we used the approximate height of an adolescent for a given age based on the 50th percentile from the Norwegian reference height/age chart (Juliusson et al., 2013).

2.3.3. Medication

Medication data were retrieved from medical records. Each patient's previous and current types and doses of AP were converted to a chlorpromazine (CPZ)-equivalent dose as described by Andreasen et al. (2010). Each type and dose were subsequently converted to CPZ years using the formula (CPZ in mg) × (time on dosage measured in years), and summed to provide a cumulative lifetime measure (CPZ years).

2.4. Biochemical variables

Venous blood samples were drawn between 8.10 and 11.20 a.m. after an overnight fast. For hormone and biochemical analyses, serum was separated within two hours. Cortisol was analyzed at the Hormone Laboratory, Department of Medical Biochemistry, Oslo University Hospital, Aker, Norway using a competitive luminescence immunoassay (Immulite 2000xpi kit from Siemens Healthineers, Erlangen, Germany). Intra- and interassay coefficients were < 10 %. We defined cortisol levels > 860 nmol/L as outliers and removed two HC and one patient from the analyses. Fasting serum total cholesterol (TC), high-density cholesterol (HDL-C) and triglycerides (TG) were analyzed at the Department of Clinical Biochemistry, Oslo University Hospital, Oslo, Norway according to standard enzymatic-colorimetric methods (Roche Diagnostics Norge AS, Oslo, Norway), and C-reactive protein (CRP) was analyzed by particle enhanced immunoturbimetry (Roche Diagnostics Norge AS, Oslo, Norway). CRP values below the quantification limit of 0.6 mg/L were treated as 0.6 mg/L in analyses (61 % of patients and 80 % of HC had values < 0.6 mg/L). One patient had CRP = 13.5 mg/L, representing an outlier value in this sample, but was not removed because preliminary analyses showed that inclusion of this patient did not influence the analyses of the IL-18 system. We measured IL-18, its binding protein IL-18BP, and other secreted components of the IL-18 system, IL-18RAP and IL-18R1. IL-18 (Cat# DY318-05) and IL-18BP (Cat# DY119) levels were analyzed using antibodies from R&D Systems (Stillwater, MN), while IL-18R1 (Cat#11102) and IL-18RAP (Cat#SEK10176) were analyzed using antibodies from Sino Biological (Beijing, China). Samples were analyzed in duplicate in a 384-well format using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (BioTek). Intra- and interassay coefficients were < 10 % for all. For immunoassays, blood

was taken using EDTA vials and the plasma was isolated the next working day and stored at −80 °C. We calculated the ratio of IL-18 to IL-18BP (IL-18/IL-18BP ratio), and used this in subsequent analyses because it indicates the potential level of bioactive IL-18 (Novick et al., 1999). IL-18/IL-18BP ratios > 800 were defined as outlier values, and one HC and one patient were removed from the analyses.

2.5. Subgrouping according to cortisol and negative symptoms

Cortisol was chosen as a measurable neurochemical response to stressors, the end product of HPA-axis activation. In subsequent analyses, we grouped participants into low or high cortisol groups based on a cutoff at the 50th percentile of HC (low < 352 nmol/L, high ≥352 nmol/L). We dichotomized the PANSS negative sum scores so that 15 patients with scores ≥21 were grouped as having a high negative score, and 16 patients with scores < 21 as having a low negative score, because a PANSS negative sum score of 21 corresponds to an average negative item score of 3, defined as clearly pathological.

2.6. Statistics

Statistical analyses were performed using IBM SPSS Statistics (version 25; IBM Corp., Armonk, NY, USA). All analyses were two-tailed with the significance level set at p < 0.05. Data normality was assessed with the Kolmogorov–Smirnov test. Independent Student's *t* tests or chi-square tests were used to compare continuous or categorical demographic variables between groups. Because it showed a skewed distribution, CPZ years was transformed to its natural logarithm and the IL-18/IL-18BP ratios were square-root (sqrt) transformed. Independent Student's *t* tests were used to compare levels of IL-18 related proteins between patients and HC. Bivariate correlation analysis was used to explore relationships between sqrtIL-18/IL-18BP ratio and cortisol, depressive symptoms and other clinical characteristics. Hierarchical multiple linear regression analysis was then used to assess the unique contributions of each factor to the explained variance in the sqrtIL-18/IL-18BP ratio. Variables significantly (p < 0.05) correlated with the dependent variable (smoking daily, cortisol, MFQ-C score and having EOP [yes/no]) were introduced into the regression. Independent variables were entered one by one, with smoking daily at step 1, cortisol at step 2, MFQ-C scores at step 3, and having EOP at step 4. Analyses were conducted to check for assumptions of normality, linearity, multicollinearity and homoscedasticity. The hypotheses were prespecified and not data driven, so p-values were not corrected for multiple testing.

3. Results

Demographics and descriptive information on participants are presented in Table 1. There were no significant differences in demographic variables between patients and HC, except that more patients than HC smoked on a daily basis (32 % vs. 7 %, p = 0.001), and patients had significantly higher MFQ scores and lower CGAS scores compared to HC. BMI and CRP did not differ significantly. There was no significant difference in levels of cortisol between patients and HC, but the patients had significantly higher levels of TG and TC/ HDL-C ratios.

3.1. IL-18 related protein levels in patients with EOP

Both levels of IL-18 and IL-18/IL-18BP ratios were significantly higher in patients than in HC (Fig.1). In contrast, there were no significant differences between patients and HC in levels of IL-18BP, IL-18RAP or IL-18R1, see Table1. Similar patterns were seen when comparing AP-naïve patients (n = 11) vs HC (IL-18, p = 0.016; IL-18/IL-18BP ratio, p = 0.005; IL-18BP, IL-18R1, IL-18RAP, not significant), and nonsmoking patients (n = 21) vs. HC (IL-18, p = 0.011; IL-18/IL-18BP ratio, p = 0.010; IL-18BP, IL-18R1, IL-18RAP, not significant), and when controlling for sex (IL-18, p = 0.001; IL-18/Il-18-ratio,

Table 1
Demographic and clinical characteristics of patients and healthy controls.

	HC Mean (±SD)	n	Patients Mean (±SD)	n	T-test or X^2 p-value
Demographics					
Age	15.9 (1.4)	60	16.3 (1.4)	31	n.s
Male sex[1]	31/ 52	60	11/35	31	n.s
BMI	21.1 (3.1)	59	22.5 (4.9)	31	n.s
IQ	104.6 (12.9)	51	100.2 (13.4)	29	n.s
Mother's education	15.3 (2.3)	53	15.1 (2.8)	31	n.s
Smoking daily[1]	4/7	60	10/32	31	0.001
Blood measures					
IL-18/IL-18BP ratio	134.4 (125.6)	60	261.9 (193.0)	31	0.002
IL-18 (pg/mL)	768.6 (681.5)	60	1356.9 (798.8)	31	0.001
IL-18BPa (pg/mL)	6.3 (2.5)	60	5.8 (1.4)	31	n.s
IL-18RAP (pg/mL)	58.0 (27.6)	59	50.9 (13.2)	30	n.s
IL-18R1 (pg/mL)	0.97 (0.51)	60	0.92 (0.40)	31	n.s
Cortisol (nmol/L)	350.2 (123.5)	60	357.2 (140.3)	31	n.s
TC/HDL-C ratio	2.6 (0.7)	60	3.3 (1.0)	31	0.001
TG (mmol/L)	0.6 (0.3)	60	0.9 (0.5)	31	0.001
CRP	0.83 (0.79)	59	1.25 (2.32)	31	n.s
Clinical measures					
CGAS	90.6 (6.1)	51	44.7 (9.6)	31	< 0.001
MFQ-C	6.7 (7.1)	50	26.9 (14.1)	29	< 0.001
DUP [2]			15 (226)	31	
Illness duration [2]			1.2 (6.0)	26	
PANSS					
-pos			16.3 (4.7)	31	
-neg			17.7 (6.9)	31	
-gen			34.5 (8.5)	30	
CPZ			146.6 (178.2)	31	
CPZ years			8.9 (15.6)	31	
AP-naïve [1]			11/35	31	
No AP [1]			13/42	31	
Medication			Aripiprazole	7	
			Quetiapine	5	
			Risperidone	3	
			Olanzapine	2	
			Clozapine	1	
Diagnosis			SCZ spectrum	15	
			Affective psychosis	2	
			Psychosis NOS	14	

Key: HC, healthy controls; SD, standard deviation; BMI, body mass index; IQ, Intelligence Quotient; TC/HDL-C ratio, total cholesterol/high-density lipoprotein cholesterol ratio; TG, triglycerides; CRP, C-reactive protein; CGAS, Children's Global Assessment Scale; MFQ-C, Mood and Feelings Questionnaire; DUP, duration of untreated psychosis in weeks; Illness duration in years; PANSS, Positive and Negative Symptom Scale; -pos, total positive score; -neg, total negative score; -gen, total general score; CPZ, chlorpromazine equivalents; CPZ years, lifetime exposure in chlorpromazine equivalents; AP-naïve, Antipsychotic medication-naïve; No AP, no use of antipsychotic medication at time of blood sampling; SCZ, schizophrenia; NOS, psychosis not otherwise specified. [1] *n* /%. [2] median (range).

p < 0.001; IL-18BP, IL-18R1,IL-18RAP, not significant).

3.2. IL-18 system activity and relationship to cortisol, depressive symptoms and other clinical characteristics

Because the IL-18/IL-18BP ratio indicates the potentially bioactive proportion of IL-18, this ratio was used in subsequent analyses. The IL-18/IL-18BP ratio was significantly correlated with the level of cortisol (r = 0.31, p = 0.003) and with depressive symptoms as measured by MFQ-C score (r = 0.48, p < 0.001), and smoking daily (r = 0.38, p < 0.001). In split file analysis, there was a significant correlation between cortisol and IL18/IL-18BP-ratio in patients (r = 0.52, p = 0.003), but not in HC (r = 0.18, p = 0.176). Lipid levels (TC/HDL-C: r = 0.14, p = 0.137; TG: r = 0.17, p = 0.117) and AP medication (r = 0.09, p = 0.475) were not significantly related to the IL-18/IL-

18BP ratio. All correlations are listed in Supplementary Table1. In adjusted analysis, both cortisol (R^2 change = 0.05, p = 0.030) and MFQ-C score (R^2 change = 0.09, p = 0.001) were shown to contribute specifically and significantly to the explained variance in IL-18-ratio, after controlling for smoking (see Table 2). Controlling for time at blood draw and BMI in the regression model did not change the results. In fact, although BMI has been shown many times to be associated with inflammation (Lumeng and Saltiel, 2011), BMI was not correlated with the outcome variable neither in the complete sample (r = 0.10, p = 0.385), nor when patients (r = 0.002, p = 0.907) and HCs (r = 0.004, p = 0.778) were analyzed separately.

3.3. Role of EOP and relationship with negative symptoms

After controlling for smoking, level of cortisol and MFQ-C, having EOP did not provide a significant additional contribution to the explained variance (see Table 2). Among patients, total PANSS negative score was not significantly correlated with IL-18/IL-18BP ratio (r = -0.17, p = 0.363), and there was no significant difference in levels of IL-18/IL-18BP ratio between patients having a low negative symptom score compared to those having a high score (p = 0.355).

4. Discussion

Our main finding was that adolescents with EOP had elevated plasma levels of IL-18 and IL-18/IL-18BP ratios, without changes in IL-18BP, IL-18RAP and IL-18R1. Serum cortisol and depressive symptoms independently contributed to the explained variance in IL-18/IL-18BP ratio, suggesting that stress, depressive symptoms and IL-18-pathway activation may interact in adolescents with EOP.

Our findings in adolescents with EOP support findings in adults with psychosis that showed elevated IL-18 levels (Orhan et al., 2018; Tanaka et al., 2000; Wu et al., 2016; Xiu et al., 2012) and an imbalance in the IL-18/IL-18BP ratio among younger adult patients (Palladino et al., 2012). An increased IL-18/IL-18BP ratio may induce a pro inflammatory state because elevated levels of free IL-18 stimulate nuclear factor-κB-dependent transcription of inflammatory cytokines, chemokines, adhesion molecules and inflammatory enzymes (Barnes and Karin, 1997). Further, elevated plasma IL-18 levels appear to promote the development of several diseases including atherosclerosis (Whitman et al., 2002) and diabetes mellitus (Oikawa et al., 2003), which occur with increased prevalence in people with psychosis-spectrum disorders (Olfson et al., 2015). Because such disorders may be confounders when examining IL-18 and related inflammatory cytokines in adults with psychosis, it is important to note that this is not the case in this population of adolescents with psychosis: we found no impact of BMI or lipid levels (TG and TC/HDL-C-ratio) on the IL-18/IL-18BP ratio in our patients.

Instead, we found that the level of plasma cortisol explained 5 % of the variance in levels of IL-18/IL-18BP ratio after controlling for smoking, a finding that seems to be driven by the strong relationship between cortisol and IL-18 activity shown in patients only. Several clinical studies have suggested that acute and chronic psychosocial stress affects the immune system in a pro-inflammatory direction, although specific data on the IL-18 pathway are lacking (Bierhaus et al., 2003; Marsland et al., 2017). However, our results are consistent with those of experimental studies demonstrating that psychosocial stress and HPA-axis activation augment IL-18 plasma levels and expression in the adrenal gland (Sekiyama et al., 2006; Sugama et al., 2006). Although IL-18 cannot cross the BBB, unless its integrity is compromised, peripheral IL-18 could also reflect centrally increased levels associated with NLRP3 inflammasome activity related to psychological stress, in line with our finding that there is a strong relationship between IL-18 activity and levels of cortisol in patients with EOP. Interaction between intrinsic vulnerability and external stressors is an established hypothesis for the cause of SCZ (van Winkel et al., 2008), and individual
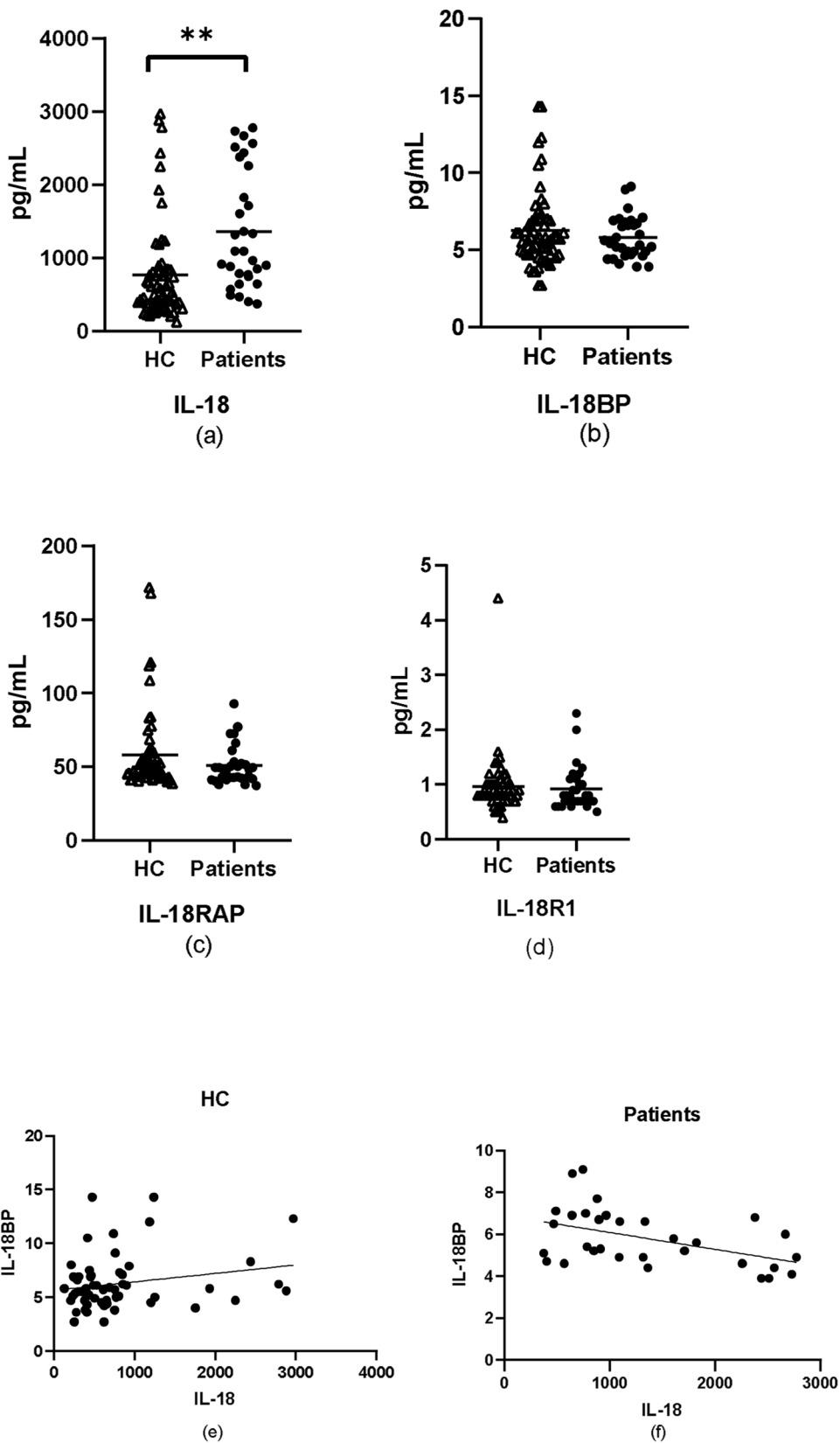
Fig. 1. (a-d) Group differences between patients and healthy controls in levels of (a) IL-18; (b) IL-18BP; (c) IL-18RAP; (d) IL-18R1. (e-f) Scatter plots shows associations between levels of IL-18 and IL-18 BP in (e) healthy controls and (f) patients, with a significant negative correlation in patients only (r = -0.48, p = 0.007). Solid lines represent linear regressions.

Table 2
Total explained variance in sqrtIL-18/IL-18BP ratio.

Adjusted R^2					
	Model 1	Model 2	Model 3	Model 4	p-value R^2change
Smoking daily	**0.13**				**0.001**
Cortisol		**0.18**			**0.030**
MFQ-C			**0.27**		**0.001**
Having EOP				0.28	0.242

Key: MFQ-C, Mood and Feelings Questionnaire; Having EOP, having a diagnosis of early-onset psychosis. *Significant results in bold.

differences in immune reactivity to stress serve as predictors of future depression (Pace et al., 2006). Our finding that cortisol contributes to IL-18/IL-18BP ratio variability in EOP supports that psychosocial stress may be linked to low-grade inflammation in psychosis (Howes and McCutcheon, 2017).

Enhanced systemic inflammation is also associated with depression in adults (Kohler et al., 2017) and adolescents (Mills et al., 2013). In addition, IL-18-deficient mice exhibited resilience to depression-like behavior promoted by chronic stress (Kim et al., 2017), indicating that IL-18 plays a role in the development of stress-induced depression. Moreover, most clinical studies have found a positive correlation between levels of IL-18 and depressive symptoms in patients with depression (Inserra et al., 2019) and psychosis (Xiu et al., 2012), even though one study reported a negative correlation between IL-18 and depressive symptoms in middle aged patients with chronic psychosis (Bossu et al., 2015). Our finding of a positive correlation between IL-18/IL-18BP ratio and MFQ-C score may further support a role for IL-18 in the pathophysiology of depression. However, because depression is also associated with bidirectional dysregulation of the HPA axis and immune system alterations, longitudinal studies are needed to dissect these relationships.

Although a positive relationship between negative symptoms and tumor necrosis factor, IL-6 and CRP has been demonstrated in patients with psychosis (Garcia-Rizo et al., 2012; Goldsmith et al., 2018), in the present study the IL-18/IL-18BP ratio did not correlate significantly with negative symptoms among adolescents with EOP. This is consistent with Zhang's observation that there was no association between serum IL-18 and negative symptoms in patients with chronic SCZ (Zhang et al., 2016).

The present study has several strengths, including a young and clinically well-characterized sample with few confounders, and a proportion of 35 % AP medication-naïve patients. In addition, all participants were from the same catchment area with similar ethnicity and duration of mother's education (a proxy measure for sociodemographic status). However, the study has some limitations. Its cross-sectional nature did not allow exploration of cause and effect. Blood for measuring plasma cortisol was drawn at different time points between 08.10 and 11.20 a.m. However, timing effects were not found to influence analyses. Patients with psychosis have dysregulations in circadian rhythmicity (Seney et al., 2019), including alterations in sleep-wake cycle and rhythmic hormonal profiles, such as cortisol, in addition to increased subjective reactivity to external stressors. Consequently, it is unclear to what extent levels of cortisol in our study can be interpreted as a function of psychological stress, and our data have to be interpreted with caution. We therefore need longitudinal studies with better measures of perceived psychological stress and more objective measures of stress response in relation to the IL-18 system. Lastly, while we here focused on the IL-18 system, there is a need for studies of other parts of the immune system in EOP, and the current findings suggest that cytokine network studies would be of great value.

5. Conclusion

We found elevated levels of plasma IL-18 and elevated IL-18/IL-18BP ratios in adolescents with EOP. Cortisol and depressive symptoms independently contributed to the explained variance in the IL-18/IL-18BP ratio. Our findings indicate that inflammatory pathways are already activated at an early age in patients with psychosis, with IL-18 as a potentially important player. Our findings also suggest an interaction between stress, inflammation and depressive symptoms in adolescents with EOP.

Declaration of Competing Interest

SF has received an honorarium as a data consultant for RAND Corporation for a project sponsored by the Janssen-Cilag pharmaceutical company. OAA has received a speaker's honorarium from Lundbeck. Others authors; none.

Appendix A. Supplementary data

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