



## Interleukin-18 signaling system links to agitation in severe mental disorders

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### ARTICLE INFO

#### Keywords:

Inflammasome  
Interleukin-1  
Interleukin-18  
Aggression  
Schizophrenia  
Bipolar disorder

### ABSTRACT

**Objective:** Agitation is a challenging clinical feature in severe mental disorders, but its biological correlates are largely unknown. Inflammasome-related abnormalities have been linked to severe mental disorders and implicated in animal models of agitation. We investigated if levels of circulating inflammasome-related immune markers were associated with agitation in severe mental disorders.

**Methods:** Individuals with a psychotic or affective disorder (N = 660) underwent blood sampling and clinical characterization. Plasma levels of interleukin (IL)-18, IL-18 binding protein (IL-18BP), IL-18 receptor 1 (IL-18R1), IL-18 receptor accessory protein (IL-18RAP), and IL-1 receptor antagonist (IL-1RA) were measured. Agitation levels were estimated with the Positive and Negative Syndrome Scale Excited Component. Multiple linear- and logistic regression were used to investigate the associations between agitation and the immune markers, while controlling for confounders. The influence of psychotic and affective symptoms was assessed in follow-up analyses.

**Results:** Agitation was positively associated with IL-18BP ( $\beta = 0.13$ ,  $t = 3.41$ ,  $p = 0.0007$ ) after controlling for multiple confounders, including BMI, smoking, medication, and substance use. Adjustment for psychotic, manic, and depressive symptoms did not affect the results. There were no significant associations between agitation and the other investigated immune markers (IL-1RA ( $\beta = 0.06$ ,  $t = 1.27$ ,  $p = 0.20$ ), IL-18 ( $\beta = 0.05$ ,  $t = 1.25$ ,  $p = 0.21$ ), IL-18R1 ( $\beta = 0.04$ ,  $t = 1.01$ ,  $p = 0.31$ ), IL-18RAP (odds ratio = 0.96,  $p = 0.30$ )). In a subsample (N = 463), we also adjusted for cortisol levels, which yielded unaltered results.

**Conclusion:** Our findings add to the accumulating evidence of immune system disturbances in severe mental disorders and suggest the IL-18 system as a part of the biological correlate of agitation independent of affective and psychotic symptoms.

### 1. Introduction

Severe mental disorders such as schizophrenia and bipolar disorder

are among the leading causes of morbidity (GBD 2019 Diseases and Injuries Collaborators, 2020), with a profound influence on affected individuals and high societal costs (Owen et al., 2016). These disorders

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<https://doi.org/10.1016/j.psyneuen.2022.105721>

Received 25 November 2021; Received in revised form 5 February 2022; Accepted 9 March 2022

Available online 12 March 2022

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have heterogeneous and overlapping clinical presentation, which can involve alterations of perception, thought, emotion, and behavior, including agitation. Agitation is a challenging clinical feature (Volicer et al., 2017) closely related to aggression (Volavka and Citrome, 2011) and linked to the self-reported quality of life (Gardsjord et al., 2018). Despite the extensive impacts of agitation, therapeutic options are limited (Paris et al., 2021), and the biological correlates are largely unknown.

The complex etiology of severe mental disorders involves overlapping polygenic architectures (Smeland et al., 2020) in interplay with environmental factors (Guloksuz et al., 2019). The immune system has emerged as a pathophysiological candidate supported by genetic (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Mullins et al., 2021) and epidemiological studies (Benros et al., 2011, 2013). Moreover, immune activation with dysregulation of pro-inflammatory signaling such as the interleukin (IL)-1, IL-18, tumor necrosis factor (TNF), and IL-6 pathways has been observed across psychotic, manic, and depressive symptoms (Goldsmith et al., 2016; Wedervang-Resell et al., 2020). Intriguingly, similar dysregulation has been indicated in agitation-related conditions in the general population and across mental disorders. Elevated IL-6 has been linked to hostility in healthy individuals (Marstrand et al., 2008) and to impulsive aggression in intermittent explosive disorder (Coccaro et al., 2014). Elevation of TNF- $\alpha$  has been reported in agitated patients in psychiatric emergency settings (Larsen et al., 2019) and elevation of IL-1 $\beta$  has been observed in agitated individuals with dementia (Higuchi et al., 2010). Furthermore, experimental animal models of agitation have suggested a role of IL-1 $\beta$  and IL-18 (Bhatt et al., 2008; Lisboa et al., 2018). The NLRP3 inflammasome is an intracellular complex that activates caspase-1, resulting in the subsequent release of IL-1 $\beta$  and IL-18 (Strowig et al., 2012). The inflammasome-related IL-1 $\beta$  and IL-18 pathways have been proposed to link immune activation and behavior-relevant processes such as neurotransmission, neuronal excitability, and synaptic remodeling (Herman and Pasinetti, 2018; Kaufmann et al., 2017). However, our insight into the role of these inflammasome-related pathways in agitation is limited and the relationship between the IL-18 system and human agitation remains unexplored.

Hence, we investigated the inflammasome-related systems and agitation in a well-characterized sample of patients with severe mental disorders. We focused on circulating soluble inflammasome-related immune markers, including IL-1 receptor antagonist (IL-1RA), IL-18, IL-18 binding protein (IL-18BP), IL-18 receptor 1 (IL-18R1), and IL-18 receptor accessory protein (IL-18RAP). We hypothesized that agitation across the spectrum of severe mental disorders would be associated with immune activation reflected by levels of the inflammasome-related immune markers. Further, we hypothesized that agitation would be associated with the immune marker levels independently of core symptoms of illness exacerbation (i.e., psychotic, manic, and depressive symptoms). Finally, we explored the neuroendocrine influence on the relationship between agitation and immune activation.

## 2. Material and methods

### 2.1. Study setting and participants

Participants in the present study (N = 660) were recruited between the years 2002 and 2018 through the ongoing Thematically Organized Psychosis (TOP) study at the NORMENT research center, Oslo, Norway. An overview of the recruitment years is shown in Fig. S1. The TOP study enrolls patients with severe mental disorders referred from psychiatric inpatient and outpatient clinics. The inclusion criteria of the present study were a psychotic or affective disorder diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), age between 18 and 65 years, and the ability to give informed consent. The exclusion criteria comprised use of any immunomodulatory agents, immunological

disorder or current infection (indicated by medical records, self-report, medication use, or C-reactive protein (CRP) level above 10 mg/L), neurological disorder, history of severe head trauma, and pronounced cognitive deficit (IQ scores below 70). The work was conducted in accordance with the Declaration of Helsinki, and all participants have given written informed consent. The TOP study is approved by the Regional Ethics Committee, the Norwegian Directorate of Health, and the Norwegian Data Protection Authority.

### 2.2. Clinical assessment

All participants underwent thorough assessments with review of medical records, general physical examination, and clinical interviews, including the Structured Clinical Interview for DSM-IV axis I disorders (SCID-I) (First et al., 1995). Following the assessment, participants were assigned one of the diagnoses within psychotic disorders (SCZ, N = 388), including schizophrenia (DSM-IV 295.1, 295.3, 295.6, or 295.9, N = 216), schizophreniform disorder (DSM-IV 295.4, N = 30), schizoaffective disorder (DSM-IV 295.7, N = 51), delusional disorder (DSM-IV 297.1, N = 27), brief psychotic disorder (DSM-IV 298.8, N = 8), and psychotic disorder not otherwise specified (DSM-IV 298.9, N = 56) or affective disorders (BD, N = 272), including bipolar I disorder (DSM-IV 296.0, 296.4, 296.5, 296.6, 296.7, N = 161), bipolar II disorder (DSM-IV 296.89, N = 81), bipolar not otherwise specified (DSM-IV 296.80, N = 12), and major depressive disorder with psychotic features (DSM-IV 296.24, 296.34, N = 18). Level of functioning was quantified using the Global Assessment of Functioning Split Version (GAF-F) (Pedersen et al., 2007). Psychotic symptoms were evaluated according to the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and derived from the positive component (P1-Delusions, P3-Hallucinatory behavior, P5-Grandiosity, G9-Unusual thought content, and G12-Lack of judgment and insight) of a five factor PANSS model (Kay and Sevy, 1990). Affective symptoms were evaluated with the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al., 1990). Current dose of psychopharmacological medication (antipsychotics, antidepressants, anti-convulsants, and lithium) relative to the defined daily dose (DDD) was calculated in line with the guidelines from the World Health Organization Collaborating Center for Drug Statistics Methodology ([https://www.whocc.no/atc\\_ddd\\_index/](https://www.whocc.no/atc_ddd_index/)). Self-reported information about the use of alcohol (number of alcohol units) and illicit substance use during the two-week period prior the assessment was recorded. Information about smoking status was based on a self-report of daily tobacco smoking. BMI (kg/m<sup>2</sup>) was calculated using weight and height measurements.

### 2.3. Agitation scores

A measure of agitation was obtained using the PANSS Excited Component (PANSS-EC), which is calculated as a sum of the following PANSS items: P4-Excitement, P7-Hostility, G4-Tension, G8-Uncooperativeness, and G14-Poor impulse control (Montoya et al., 2011). The PANSS-EC is a part of a five factor PANSS model (Kay and Sevy, 1990) and is commonly used as a primary outcome measure in randomized controlled trials targeted at agitation (Citrome, 2007). The PANSS consists of 30 interviewer-rated items, each evaluating a current symptom on a scale from 1 (no symptom) to 7 (severe symptom). The PANSS assessment was conducted in temporal proximity of the blood sampling (median 9 days, interquartile range 13 days) and with a good inter-rater reliability (intra-class correlation coefficient above 0.7) (Ringen et al., 2008).

### 2.4. Blood sampling, cortisol, and immune markers

Venous blood samples were drawn in the morning after an overnight fast. Serum cortisol level was measured using a competitive

luminescence immunoassay (Immulite 2000xpi, Siemens Healthineers, Erlangen, Germany) at the Hormone Laboratory, Department of Medical Biochemistry, Oslo University Hospital, Norway. Plasma was collected using EDTA vials, isolated the next working day, and stored at  $-80^{\circ}\text{C}$  in the biobank. Information about the cumulative freezer storage time of the sample was recorded (Enroth et al., 2016). Immune markers were analyzed with enzyme-linked immunosorbent assay (ELISA) methods. We used antibodies from R&D Systems (Stillwater, MN, USA) to measure levels of IL-18 (Cat# DY318-05) and IL-18BP (Cat# DY119), antibodies from Sino Biological (Beijing, China) to measure levels of IL-18R1 (Cat#11102) and IL-18RAP (Cat#SEK10176), and antibodies from PeproTech (Cranbury, NJ, USA) to measure IL-1RA (Cat#900K474). Samples were analyzed in duplicate in a 384-well format using a pipetting robot (SELMA, Analytik Jena, Jena, Germany) and a dispenser (BioTek, Winooski, VT, USA). Absorption was read by ELISA plate reader (BioTek, Winooski, VT, USA) at 450 nm with 540 nm wavelength correction. IL-18, IL-18BP, IL-18R1, and IL-18RAP were analyzed in 2018, while IL-1RA was analyzed in a subsample of participants ( $N = 405$ ) in 2013. The assay sensitivity was 22 pg/mL for IL-18, 25 pg/mL for IL-18BP, 10 pg/mL for IL-18RAP, 25 pg/mL for IL-18R1, and 25 pg/mL for IL-1RA. In 9 samples (2%), levels of IL-1RA were under the detection limit and were set to 25 pg/mL. Intra- and inter-assay coefficients of variation were below 10% for all analyses. To ensure compliance with the exclusion criteria, the samples were screened for serum CRP levels above 10 mg/L, using particle-enhanced immunoturbidimetric methods from Roche Diagnostics (Indianapolis, IN, USA) at the Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway.

## 2.5. Statistical analyses

Statistical analyses were performed using R software package version 4.1.1 (www.R-project.org). Descriptive characteristics were compared across diagnostic categories using chi-squared-, Wilcoxon rank-sum-, and independent samples t-tests. Normality was assessed by inspection of Q-Q plots and histograms. Based on inspection of immune marker distributions and standardized residuals, plasma levels of IL-1RA, IL-18, IL-18BP, and IL-18R1 were logarithmically transformed, and outliers outside 3 standard deviations were removed (3.2% of IL-1RA, 0.0% of IL-18, 2.9% of IL-18BP, 0.9% of IL-18R1). The association between agitation and immune activation was analyzed using multiple linear regression, with immune markers (IL-1RA, IL-18, IL-18BP, and IL-18R1) as the dependent variable and agitation scores (PANSS-EC) as the independent variable, while controlling for sex, age, BMI, smoking status (smoking daily versus non-smoker), diagnosis (SCZ versus BD), medication (antipsychotics, antidepressants, anticonvulsants, and lithium quantified as DDDs), alcohol use (number of alcohol units), illicit substance use (cannabis and stimulants as dichotomous variables), and freezer storage time. Stability of regression coefficients was checked using bootstrapping approach with 2 000 replicates. Due to a highly skewed distribution, IL-18RAP was dichotomized based on the median value and analyzed with logistic regression. Interaction between agitation and diagnosis was tested using the same models. The influence of psychotic and affective symptoms on any significant association between agitation and the immune marker was investigated in follow-up analyses. We used the same model as in the main analysis and additionally adjusted for psychotic (sum of PANSS Positive Component items: P1, P3, P5, G9, G12), manic (YMRS scores), and depressive symptoms (CDSS scores). To explore the influence of stress hormone levels, main analyses were repeated in a subsample with available cortisol measurements ( $N = 463$  for the IL-18 system markers,  $N = 356$  for IL-1RA), followed by an additional adjustment for serum cortisol level. Inspection of standardized residuals, Cook's distances, and variation inflation factors was used to ensure no violation of the test assumptions. We applied the Bonferroni method to correct for multiple testing (5 consecutive analyses with IL-1RA, IL-18, IL-18BP, IL-18R1,

and IL-18RAP). All analyses were two-tailed, with a significance level at 0.05 for the descriptives and at 0.01 (0.05/5) for the main, follow-up, and cortisol subsample analyses.

## 3. Results

### 3.1. Descriptive characteristics

Demographic and clinical characteristics are presented in Table 1. The patients in the SCZ group were more frequently male and less frequently of European ethnicity than the patients in the BD group. The patients in the SCZ group had lower level of functioning (GAF-F), higher psychotic symptom load (PANSS positive component), as well as higher level of agitation (PANSS-EC) compared to the BD group. The patients in the SCZ group were also more frequently smokers and used more anti-psychotic medication, less lithium, less anticonvulsants, and less alcohol two weeks before the assessment. The levels of immune markers across patient groups are presented in Table 1. The patients in the SCZ group had higher levels of IL-1RA, IL-18, IL-18BP, and IL-18R1. Descriptive characteristics of the IL-1RA and cortisol subsamples are shown in Tables S1 and S2.

### 3.2. Agitation and immune markers

The results are summarized in Table 2. The association between agitation and IL-18BP is shown in Fig. 1. As presented in Table 3, agitation was significantly positively associated with IL-18BP after controlling for confounders. Adjustment for psychotic, manic, and depressive symptoms did not affect the results (outlined in Tables 4 and S3). We found no significant associations between agitation and the other investigated immune markers and there were no significant

**Table 1**  
Descriptive characteristics of participants.

Total N = 660	SCZ N = 388	BD N = 272
<b>Demographic and clinical characteristics</b>		
Male (%)	59.5*	41.2
Age (median (IQR))	27 (12)	29 (16)
European ethnicity (%)	79.1*	86.0
GAF-F (mean $\pm$ SD)	46.4 $\pm$ 12.7*	55.0 $\pm$ 13.1
CDSS (median (IQR))	4 (7)	4 (7)
YMRS (median (IQR))	2 (8)	2 (4)
PANSS Positive Component (median (IQR))	12 (6)*	6.5 (4)
PANSS-EC (median (IQR))	7 (3)*	6 (3)
BMI (mean $\pm$ SD)	26.1 $\pm$ 5.1	25.7 $\pm$ 4.6
Smoking (%)	46.4*	36.8
Antipsychotics (%)	83.2*	49.3
Antidepressants (%)	26.0	31.6
Lithium (%)	2.3*	17.3
Anticonvulsants (%)	7.2*	32.4
Alcohol units last 2 weeks (median (IQR))	0 (4)*	2 (8)
Cannabis (%)	6.4	8.8
Stimulants (%)	2.8	2.9
<b>Immune markers</b>		
IL-1RA** (median (IQR))	234 (292)*	174 (272)
IL-18 (median (IQR))	1 088 (1 641)*	819 (1 383)
IL-18BP (median (IQR))	6 433 (3 029)*	5 806 (2 534)
IL-18R1 (median (IQR))	898 (473)*	838 (420)
IL-18RAP (median (IQR))	46 (18)	48 (17)

SCZ, Psychotic disorders; BD, Affective disorders. CDSS, Calgary Depression Scale for Schizophrenia; GAF-F, Global Assessment of Functioning Split Version; PANSS, Positive and Negative Syndrome Scale; PANSS-EC, Positive and Negative Syndrome Scale Excited Component; YMRS, Young Mania Rating Scale. IL-1RA, Interleukin-1 receptor antagonist (pg/mL); IL-18, Interleukin-18 (pg/mL); IL-18BP, Interleukin-18 binding protein (pg/mL); IL-18R1, Interleukin-18 receptor 1 (pg/mL); IL-18RAP, Interleukin-18 receptor accessory protein (pg/mL). SD, Standard deviation; IQR, Interquartile range.

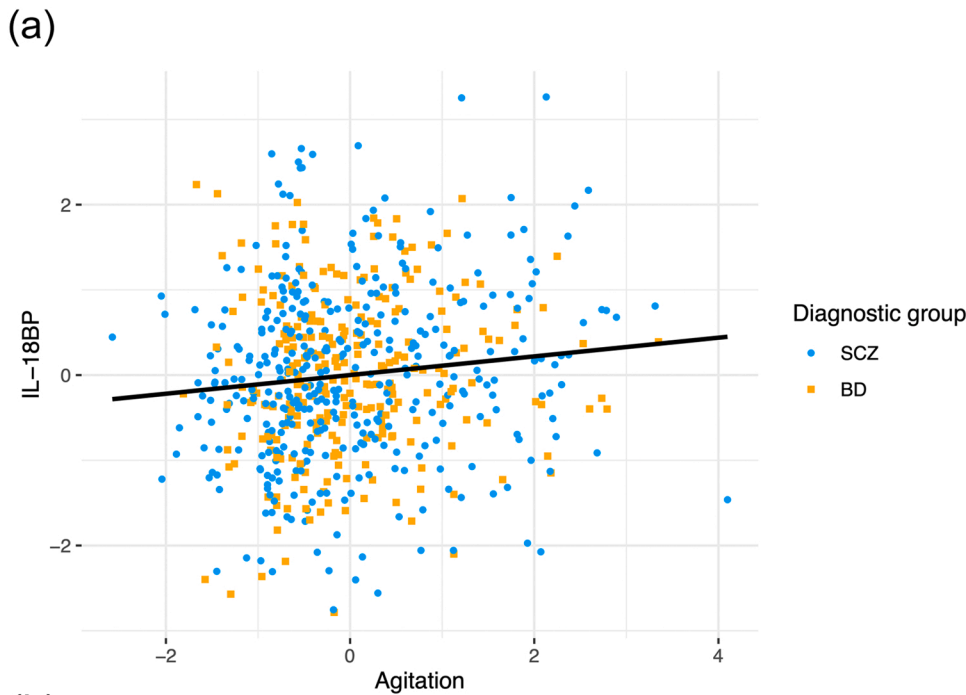
\*  $p < 0.05$ .

\*\* IL-1RA subsample ( $N = 405$ ,  $N_{\text{SCZ}}=264$ ,  $N_{\text{BD}}=141$ ).

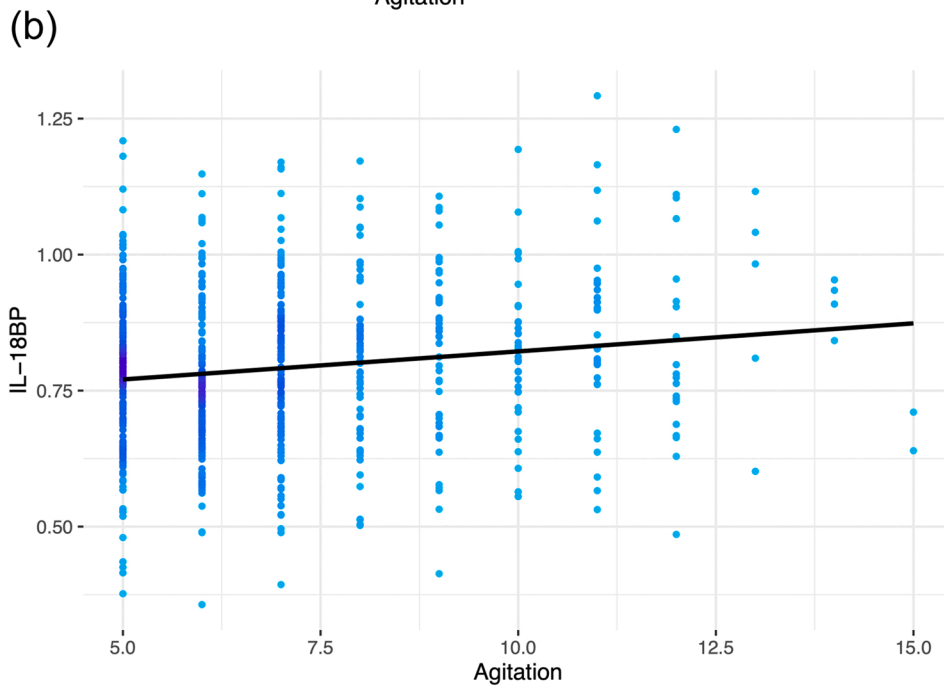
**Table 2**  
Associations between agitation and inflammasome-related immune markers.

	Coefficient (95% confidence interval)	Standardized coefficient $\beta$	t	p
IL-1RA	0.010 (-0.006 to 0.026)	0.06	1.27	0.20
IL-18	0.009 (-0.005 to 0.024)	0.05	1.25	0.21
IL-18BP	0.009 (0.004 – 0.015)	0.13	3.41	0.0007
IL-18R1	0.003 (-0.003 to 0.009)	0.04	1.01	0.31
	<b>Odds ratio (95% confidence interval)</b>			<b>p</b>
IL-18RAP	0.96 (0.89 – 1.03)			0.30

IL-1RA, Interleukin-1 receptor antagonist; IL-18, Interleukin-18; IL-18BP, Interleukin-18 binding protein; IL-18R1, Interleukin-18 receptor 1; IL-18RAP, Interleukin-18 receptor accessory protein.



**Fig. 1.** Association between agitation and IL-18BP. a) After correction for multiple confounders and symptom dimensions. X axis: Z-scores (standardized residuals) of PANSS-EC (Positive and Negative Syndrome Scale Excited Component) obtained from a regression model with PANSS-EC as dependent and sex, age, BMI, smoking, diagnosis, medication, alcohol use, substance use, freezer storage time, psychosis, mania, and depression as independent variables, Y axis: Z-scores (standardized residuals) of log-transformed interleukin-18 binding protein (IL-18BP) levels (ng/mL) obtained from a regression model with IL-18BP as dependent and sex, age, BMI, smoking, diagnosis, medication, alcohol use, substance use, freezer storage time, psychosis, mania, and depression as independent variables. BD, Affective disorders; SCZ, Psychotic disorders. b) Raw data. PANSS-EC (Positive and Negative Syndrome Scale Excited Component), Y axis: Log-transformed interleukin 18 binding protein (IL-18BP) plasma levels (ng/mL). Shading represents the density of observations.



**Table 3**  
Association between agitation and IL-18BP in a multiple linear regression model.

	Coefficient (95% confidence interval)	Standardized coefficient $\beta$	t	p	R <sup>2</sup>
Agitation	0.009 (0.004 – 0.015)	0.13	3.41	0.0007	0.135
Sex <sup>a</sup>	0.051 (0.028 – 0.074)	0.17	4.43	0.00001	
Age	0.002 (0.001 – 0.004)	0.17	4.21	0.00003	
BMI	0.003 (0.001 – 0.006)	0.11	2.88	0.004	
Smoking	0.0004 (–0.023 to 0.024)	0.001	0.03	0.97	
Diagnosis <sup>b</sup>	0.039 (0.012 – 0.066)	0.13	2.88	0.004	
Freezer time	0.002 (–0.002 to 0.005)	0.03	0.90	0.37	
Antipsychotics	0.002 (–0.012 to 0.016)	0.01	0.30	0.77	
Antidepressants	0.015 (0.002 – 0.028)	0.08	2.21	0.03	
Lithium	0.020 (–0.009 to 0.049)	0.05	1.35	0.18	
Anticonvulsants	0.005 (–0.027 to 0.037)	0.01	0.31	0.76	
Alcohol	–0.0003 (–0.001 to 0.001)	–0.03	–0.69	0.49	
Cannabis	0.020 (–0.024 to 0.065)	0.04	0.90	0.37	
Stimulants	0.042 (–0.026 to 0.110)	0.05	1.20	0.23	

<sup>a</sup> Female coded as 0, Male coded as 1.

<sup>b</sup> Affective disorders coded as 0, Psychotic disorders coded as 1.

**Table 4**  
Association between agitation and IL-18BP after adjustment for symptom dimensions.

	Coefficient (95% confidence interval)	Standardized coefficient $\beta$	t	p	R <sup>2</sup>
Agitation, corrected for psychosis <sup>a</sup>	0.008 (0.002 – 0.014)	0.12	2.82	0.005	0.137
Agitation, corrected for mania <sup>a</sup>	0.009 (0.004 – 0.015)	0.13	3.34	0.001	0.135
Agitation, corrected for depression <sup>a</sup>	0.009 (0.004 – 0.015)	0.13	3.34	0.001	0.135
Agitation, corrected for psychosis, mania, and depression <sup>a</sup>	0.008 (0.002 – 0.014)	0.11	2.78	0.006	0.137

<sup>a</sup> Also controlled for sex, age, BMI, smoking, diagnosis, medication, alcohol use, substance use, and freezer storage time.

interactions between agitation and diagnosis. The bootstrapping approach confirmed stability of regression coefficients, converging on 95% confidence interval of 0.004 – 0.015 for association between

agitation and IL-18BP. The cortisol subsample yielded the same results, apart from subtle changes in effect sizes (association between agitation and IL-18BP ( $\beta = 0.17$ ,  $t = 3.73$ ,  $p = 0.0002$ ), IL-1RA ( $\beta = 0.05$ ,  $t = 1.04$ ,  $p = 0.30$ ), IL-18 ( $\beta = 0.08$ ,  $t = 1.85$ ,  $p = 0.07$ ), IL-18R1 ( $\beta = 0.06$ ,  $t = 1.29$ ,  $p = 0.20$ ), IL-18RAP (odds ratio = 0.95,  $p = 0.25$ )), and the results remained unaltered after the additional adjustment for cortisol (association between agitation and IL-18BP ( $\beta = 0.17$ ,  $t = 3.69$ ,  $p = 0.0003$ ), presented in Table S4).

#### 4. Discussion

We conducted a comprehensive study of circulating inflammasome-related immune markers and agitation in a well-characterized sample of patients with severe mental disorders. The main finding of the study was a significant positive association between agitation and IL-18BP levels, which was independent of psychotic, manic, and depressive symptoms. The current findings add to the accumulating evidence of immune system disturbances in severe mental disorders.

Our finding of an association between agitation and IL-18BP levels was not driven by depressive, manic, or psychotic symptoms. Further, we also controlled for other factors known to affect the immune system such as sex, age, BMI, smoking, medication, and substance use (Baumeister et al., 2016; Haack et al., 1999; Lippai et al., 2013). Thus, agitation seems to be a clinical phenomenon linked to immune disturbances independently of psychotic and affective symptoms, in line with previous findings from the general population (Marsland et al., 2008).

Components of the inflammasome-related pathways interact in a complex way to ensure coordinated immune responses (Dinarello, 2018). IL-18BP is a major signaling protein within the IL-18 system and is regarded as a stable marker of immune activation (Dinarello et al., 2013). Specifically, upregulation of IL-18BP following elevations of IL-18 forms a negative feedback loop and, as such, serves as a part of the regulatory signaling system. Given the regulatory role of IL-18BP, our findings may suggest a compensatory upregulation of IL-18BP reflective of a state of immune activation. This is consistent with previous indices of immune activation in agitated states (Barzilay et al., 2016; Higuchi et al., 2010; Larsen et al., 2019) and agitation-related conditions (Coccaro et al., 2014). The present findings support a link between altered IL-18 system signaling and clinical features in severe mental disorders. A compensatory alteration of systemic IL-18 signaling has previously been indicated in individuals with schizophrenia (Palladino et al., 2012), which is also in line with previously reported case-control differences based on a subject sample overlapping with the current (Szabo et al., 2022). In contrast, we found no association between agitation and circulating levels of IL-18, which raises the question about the specific source of the immune signal underlying IL-18BP elevation.

Of note, IL-18BP elevations can be a sum of multiple immune signals. Besides the negative feedback loop with IL-18, IL-18BP can be also upregulated by inflammatory factors such as interferon (IFN)- $\gamma$  or IFN- $\alpha$  (Dinarello et al., 2013). Interestingly, elevation of circulating IL-18BP has been described as a part of the response to IFN- $\alpha$  treatment (Kaser et al., 2002), a treatment regime linked with agitation (Renault et al., 1987). Substantially higher rates of side effects in form of agitation following IFN- $\alpha$  treatment of hepatitis as compared to IFN- $\alpha$ -free treatment provide an indication of a causal involvement of the immune system in human agitation (Lawitz et al., 2013). Upregulated transcriptional activity within the interferon signaling pathway has been observed in impulsive aggression (Coccaro et al., 2021). Moreover, a potentiating effect of inflammatory stimulation with lipopolysaccharide on aggression in pigs has also been reported, with somewhat conflicting evidence regarding effect on IFN- $\gamma$  levels in a porcine brain (Veit et al., 2020). Taken together, this may point toward some possible mechanisms underpinning the observed association between agitation and IL-18BP.

No significant associations were found between agitation and levels of IL-1RA. This could represent a true null finding, in line with findings

of no differences in levels of circulating IL-1 $\beta$  in a mouse model (Takahashi et al., 2021). Given the previous findings implicating a role of the IL-1 system in agitation (Bhatt et al., 2008; Friedman et al., 1996; Higuchi et al., 2010), further investigation of agitation and the IL-1 signaling pathway may be warranted.

Inflammatory signaling pathways are known to be upregulated following acute psychological stress, which is also accompanied by activation of hypothalamic-pituitary-adrenal (HPA) axis (Marland et al., 2017). Similarly, anger (Pesce et al., 2013) and hostility (Kiecolt-Glaser et al., 2005) have been reported to lead to activation of the immune system. Activation of the HPA axis with elevations of cortisol constitutes a part of the complex network of neuro-immune signaling pathways that engage in a bidirectional crosstalk (Dantzer, 2018). There was no influence of the adjustment for cortisol levels in our analysis, which indicates that other mechanisms than acute psychological stress may underlie the association between agitation and levels of circulating IL-18BP. Interestingly, immune alterations distinct from those linked to stress-related disorders have been observed in impulsive aggression (Coccaro et al., 2021). However, it is unknown how agitation symptoms relate to immune alterations following chronic psychological stress (Weber et al., 2017).

The main strength of our study lies in the novelty of addressing the relationship between elements of the inflammasome-related signaling pathways and human agitation. Moreover, the well-characterized clinical sample allowed us to control for relevant potential confounding factors and the large sample size enabled us to target even small effect sizes. However, the study also has some limitations. Due to the relatively comprehensive study protocol and, implicitly, the participant enrollment mainly in non-acute phase, there is a sparsity of high agitation levels in our sample. Thus, efforts to complement our findings with an investigation of the phenomenon in acute psychiatric settings might be warranted. Moreover, IL-1RA and cortisol were analyzed in subsamples. Furthermore, since IL-1 $\beta$ , in contrast to IL-1RA, often circulates at levels just above the detection limit of commercially available assays (Arend, 2002), the IL-1 system's activity was assessed solely by IL-1RA in our study. In general, our analyses were also restricted to the secreted circulating elements of the inflammasome-related signaling systems. Finally, despite adjustments for a wide range of potential confounders, residual confounding cannot be ruled out and the cross-sectional observational design prevents inferences about causal directions.

## 5. Conclusions

Taken together, our results suggest the IL-18 system as a significant, yet modest, part of the biological correlate of agitation in severe mental disorders, independent of affective and psychotic symptoms. The findings add to the growing body of evidence that implicates immune system disturbances in severe mental disorders. Future clinical studies should address the source and the temporal dynamics of the immune signal underlying IL-18BP elevation linked to agitation and related clinical states.

## Declaration of interest

Ole A. Andreassen is a consultant for HealthLytix and received speaker's honorarium from Lundbeck and Sunovion. All other authors report no conflict of interest.

## Acknowledgments

The authors would like to thank the study participants for their valuable contribution, the staff members at NORMENT for data acquisition, and Department of Medical Biochemistry at Oslo University Hospital for the serum analyses.

## Funding

This work was supported by the Research Council of Norway (Grant Number 223273), South-Eastern Norway Health Authorities (Grant Number 2020100), and Østfold Hospital (Grant Number AB3461).

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psychneuen.2022.105721.

## References

- Addington, D., Addington, J., Schissel, B., 1990. A depression rating scale for schizophrenics. *Schizophr. Res.* 3 (4), 247–251.
- Arend, W.P., 2002. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* 13 (4–5), 323–340. [https://doi.org/10.1016/s1359-6101\(02\)0020-5](https://doi.org/10.1016/s1359-6101(02)0020-5).
- American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV, 4th ed.*, American Psychiatric Association.
- Barzilay, R., Lobel, T., Krivoy, A., Shlosberg, D., Weizman, A., Katz, N., 2016. Elevated C-reactive protein levels in schizophrenia inpatients is associated with aggressive behavior. *Eur. Psychiatry* 31, 8–12. <https://doi.org/10.1016/j.eurpsy.2015.09.461>.
- Baumeister, D., Ciufolini, S., Mondelli, V., 2016. Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? *Psychopharmacology* 233 (9), 1575–1589. <https://doi.org/10.1007/s00213-015-4044-5>.
- Benros, M.E., Nielsen, P.R., Nordentoft, M., Eaton, W.W., Dalton, S.O., Mortensen, P.B., 2011. Autoimmune diseases and severe infections as risk factors for schizophrenia: a 30-year population-based register study. *Am. J. Psychiatry* 168 (12), 1303–1310. <https://doi.org/10.1176/appi.ajp.2011.11030516>.
- Benros, M.E., Waltoft, B.L., Nordentoft, M., Ostergaard, S.D., Eaton, W.W., Krogh, J., Mortensen, P.B., 2013. Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. *JAMA Psychiatry* 70 (8), 812–820. <https://doi.org/10.1001/jamapsychiatry.2013.1111>.
- Bhatt, S., Bhatt, R., Zalcman, S.S., Siegel, A., 2008. Role of IL-1 beta and 5-HT2 receptors in midbrain periaqueductal gray (PAG) in potentiating defensive rage behavior in cat. *Brain, Behav., Immun.* 22 (2), 224–233. <https://doi.org/10.1016/j.bbi.2007.07.011>.
- Citrome, L., 2007. Comparison of intramuscular ziprasidone, olanzapine, or aripiprazole for agitation: a quantitative review of efficacy and safety. *J. Clin. Psychiatry* 68 (12), 1876–1885.
- Coccaro, E.F., Irwin, M., Arevalo, J.M.G., Dizon, T., Cole, S., 2021. Gene expression in peripheral blood mononuclear cells in impulsive aggression: intermittent explosive disorder compared with non-aggressive healthy and psychiatric controls. *Psychoneuroendocrinology*, 105453. <https://doi.org/10.1016/j.psychneuen.2021.105453>.
- Coccaro, E.F., Lee, R., Coussons-Read, M., 2014. Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans. *JAMA Psychiatry* 71 (2), 158–165. <https://doi.org/10.1001/jamapsychiatry.2013.3297>.
- Dantzer, R., 2018. Neuroimmune Interactions: From the Brain to the Immune System and Vice Versa. *Physiol. Rev.* 98 (1), 477–504. <https://doi.org/10.1152/physrev.00039.2016>.
- Dinarello, C.A., 2018. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol. Rev.* 281 (1), 8–27. <https://doi.org/10.1111/imr.12621>.
- Dinarello, C.A., Novick, D., Kim, S., Kaplanski, G., 2013. Interleukin-18 and IL-18 binding protein. *Front. Immunol.* 4, 289. <https://doi.org/10.3389/fimmu.2013.00289>.
- Enroth, S., Hallmans, G., Grankvist, K., Gyllensten, U., 2016. Effects of long-term storage time and original sampling month on biobank plasma protein concentrations. *EBioMedicine* 12, 309–314. <https://doi.org/10.1016/j.ebiom.2016.08.038>.
- First, M.B., Spitzer, R., Gibbon, M., Williams, J.B.W., 1995. *Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition, Version 2.0*. New York State Psychiatric Institute.
- Friedman, E.M., Reyes, T.M., Coe, C.L., 1996. Context-dependent behavioral effects of interleukin-1 in the rhesus monkey (*Macaca mulatta*). *Psychoneuroendocrinology* 21 (5), 455–468. [https://doi.org/10.1016/0306-4530\(96\)00010-8](https://doi.org/10.1016/0306-4530(96)00010-8).
- Gardsjord, E.S., Romm, K.L., Røssberg, J.I., Friis, S., Barder, H.E., Evensen, J., Haahr, U., Ten Velden Hegelstad, W., Joa, I., Johannessen, J.O., Langeveld, J., Larsen, T.K., Opjordsmoen, S., Rund, B.R., Simonsen, E., Vaglum, P., McGlashan, T., Melle, I., 2018. Is going into stable symptomatic remission associated with a more positive development of life satisfaction? A 10-year follow-up study of first episode psychosis. *Schizophr. Res.* 193, 364–369. <https://doi.org/10.1016/j.schres.2017.07.006>.
- GBD, 2020. Diseases and Injuries Collaborators. (2020, Oct 17). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 396 (10258), 1204–1222. [https://doi.org/10.1016/s0140-6736\(20\)30925-9](https://doi.org/10.1016/s0140-6736(20)30925-9).
- Goldsmith, D.R., Rapaport, M.H., Miller, B.J., 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol. Psychiatry* 21 (12), 1696–1709. <https://doi.org/10.1038/mp.2016.3>.



- Russo, S.J., 2021. Neuromodulatory effect of interleukin 1 $\beta$  in the dorsal raphe nucleus on individual differences in aggression. *Mol. Psychiatry*. <https://doi.org/10.1038/s41380-021-01110-4>.
- Veit, C., Janczak, A.M., Ranheim, B., Vas, J., Valros, A., Sandercock, D.A., Piepponen, P., Dulgheriu, D., Nordgreen, J., 2020. The Effect of LPS and Ketoprofen on Cytokines, Brain Monoamines, and Social Behavior in Group-Housed Pigs. *Front Vet. Sci.* 7, 617634 <https://doi.org/10.3389/fvets.2020.617634>.
- Volavka, J., Citrome, L., 2011. Pathways to aggression in schizophrenia affect results of treatment. *Schizophr. Bull.* 37 (5), 921–929. <https://doi.org/10.1093/schbul/sbr041>.
- Volicer, L., Citrome, L., Volavka, J., 2017. Measurement of agitation and aggression in adult and aged neuropsychiatric patients: review of definitions and frequently used measurement scales. *CNS Spectr.* 22 (5), 407–414. <https://doi.org/10.1017/s1092852917000050>.
- Weber, M.D., Godbout, J.P., Sheridan, J.F., 2017. Repeated social defeat, neuroinflammation, and behavior: monocytes carry the signal. *Neuropsychopharmacology* 42 (1), 46–61. <https://doi.org/10.1038/npp.2016.102>.
- Wedervang-Resell, K., Friis, S., Lonning, V., Smelror, R.E., Johannessen, C., Reponen, E. J., Lyngstad, S.H., Lekva, T., Aukrust, P., Ueland, T., Andreassen, O.A., Agartz, I., Myhre, A.M., 2020. Increased interleukin 18 activity in adolescents with early-onset psychosis is associated with cortisol and depressive symptoms. *Psychoneuroendocrinology* 112, 104513. <https://doi.org/10.1016/j.psyneuen.2019.104513>.
- Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. *Br. J. Psychiatry* 133, 429–435. <https://doi.org/10.1192/bjp.133.5.429>.