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Full-length Article

Increased circulating IL-18 levels in severe mental disorders indicate systemic inflammasome activation

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ABSTRACT

Background: Schizophrenia (SCZ) and bipolar disorder (BD) are severe mental illnesses (SMI) that are part of a psychosis continuum, and dysregulated innate immune responses have been suggested to be involved in their pathophysiology. However, disease-specific immune mechanisms in SMI are not known yet. Recently, dyslipidemia has been linked to systemic inflammasome activation, and elevated atherogenic lipid ratios have been shown to correlate with circulating levels of inflammatory biomarkers in SMI. It is, however, not yet known if increased systemic cholesterol load leads to inflammasome activation in these patients.

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Cholesterol
Interleukin-18

Methods: We tested the hypothesis that patients with SCZ and BD display higher circulating levels compared to healthy individuals of key members of the IL-18 system using a large patient cohort (n = 1632; including 737 SCZ and 895 BD), and healthy controls (CTRL; n = 1070). In addition, we assessed associations with coronary artery disease risk factors in SMI, focusing on relevant inflammasome-related, neuroendocrine, and lipid markers.
Results: We report higher baseline levels of circulating IL-18 system components (IL-18, IL-18BPA, IL-18R1), and increased expression of inflammasome-related genes (*NLRP3* and *NLRC4*) in the blood of patients relative to CTRL. We demonstrate a cholesterol dyslipidemia pattern in psychotic disorders, and report correlations between levels of blood cholesterol types and the expression of inflammasome system elements in SMI.
Conclusions: Based on these results, we suggest a role for inflammasome activation/dysregulation in SMI. Our findings further the understanding of possible underlying inflammatory mechanisms and may expose important therapeutic targets in SMI.

1. Introduction

Schizophrenia (SCZ) and bipolar disorder (BD) are severe mental disorders with high heritability that adversely impact the individual with large costs to society (Owen et al., 2016; Correll et al., 2015). These psychotic disorders are suggested to be part of a psychosis continuum (Tesli et al., 2014; Möller, 2003; Craddock, 2005), and dysregulated immune responses, inflammation and autoimmunity have been implicated in their pathophysiology (Khandaker et al., 2015; Pollak et al., 2020). Recent genome-wide association studies (GWAS) of both SCZ and BD have reported genetic loci in immune function-related regions (Consortium, 2009; Corvin and Morris, 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Still, the involved specific immune-related mechanisms are not yet clarified. Recent evidence links psychosis to sterile inflammation of the brain or to systemic inflammatory processes that affect the central nervous system (Chen and Nuñez, 2010; SayuriYamagata et al., 2017; Rubartelli, 2014). This is supported by dysregulated systemic markers of inflammation and immune activation, with correlations to clinical indices of disease severity (Mørch et al., 2016; Mørch et al., 2017; Wedervang-Resell et al., 2020).

Host innate immune responses to microorganisms are predominantly based on germline-encoded pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs), which are ancient, evolutionally conserved microbial motifs shared by many phylogenetic microbial taxa (Medzhitov, 2001). PRRs can also be activated by endogenous non-microbial signals, such as damage-associated molecular patterns (DAMPs). The consequent sterile inflammation can either resolve the initial insult or lead to disease (Chen and Nuñez, 2010). A subfamily of PRRs, the nucleotide-binding leucine-rich repeat (LRR)-containing proteins (NLRs; also known as NOD-like receptors) have emerged as a key family of sensors and regulators responding to microbial PAMPs, as well as to endogenous DAMPs produced under nonmicrobial/noninfectious inflammatory conditions by host cells (Strowig et al., 2012). Upon ligand (PAMP/DAMP) binding, NLR proteins assemble with the adaptor protein ASC which then mediates the activation of caspase-1 and the subsequent production of the pro-inflammatory cytokines IL-1β and IL-18 (Martinon et al., 2002; Keller et al., 2008). This process is referred to as *inflammasome* activation, in which other factors, such as the macrophage migration inhibitory factor (MIF) are also critically involved (Lang et al., 2018). Two of the NLRs, NLRP3 and NLRC4, have been showed to mediate sterile inflammation in the brain (targeting astrocytes and microglia) and have been suggested to be involved in psychotic disorders, but also linked to autoimmunity and cardiovascular diseases (Freeman et al., 2017; Kim et al., 2016; Ventura, 2020). However, systemic inflammasome status and potential activation has not yet been explored in SMI.

NLRP3 and NLRC4, as most of the NLR family members, can be triggered by various endogenous signals, such as uric acid or cholesterol crystals (Strowig et al., 2012). Dyslipidemia and high blood cholesterol have been linked to systemic inflammasome activation in circulating immune cells and in the vascular endothelium implicating inflammasome dysregulation in atherosclerosis and increased coronary artery disease (CAD) risk (Tall and Yvan-Charvet, 2015; Liston and Masters, 2017; Westerterp et al., 2017; Le Bras, 2018). Dyslipidemia and elevated atherogenic lipid ratios correlate with circulating levels of inflammatory biomarkers in psychotic disorders, but it is unknown if enhanced systemic cholesterol load leads to inflammasome activation in these patients (Reponen et al., 2020; Misiak et al., 2017).

In the present study, we tested the hypothesis that patients with SCZ and BD have higher circulating levels of key members of the IL-18 system using a large SMI cohort (n=1632) including SCZ (n=737) and BD (n=895), relative to healthy controls (CTRL) (n=1070). Furthermore, we assessed associations with CAD risk factors in SCZ and BD, focusing on relevant inflammasome-related, neuroendocrine, and lipid markers.

2. Methods

2.1. Sample characteristics

The study sample (n = 2702) consisted of 1070 healthy controls (CTRL), 737 SCZ spectrum disorder patients (544 schizophrenia, 153 schizoaffective, 40 schizophreniform), or patients with BD (n=895; 487 BD type I, 354 BD type II, 54 BD not otherwise specified). Details regarding demographic and clinical variables are shown in Table 1. All patients were diagnosed according to the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) or the Mini-International Neuropsychiatric Interview (MINI). The recruitment procedure and clinical evaluation for the study sample is described in detail in previous reports (Dieset et al., 2012; Simonsen et al., 2011). The main exclusion criteria were clinically significant brain injury, neurological disorder, any ongoing infection or cancer. All participants gave written informed consent and the study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All procedures and methods were carried out in accordance with relevant guidelines and regulations.

2.2. Assessing the levels of circulating cytokines and lipids

All patients were subjected to a physical examination by a physician at inclusion into the study. Before the physical examination, blood samples were drawn after over-night fasting and analyzed for fasting plasma glucose, TG, HDL-C, LDL-C, TC and C-reactive protein (CRP). All serum analyses were performed at the Department of Clinical Chemistry, Oslo University Hospital, Oslo, Norway, on an Integra 800 (Roche Diagnostics, IN, USA), using standard methods. In addition, patients were

asked about their smoking habits. To obtain normally distributed variables, all outcome measures besides LDL-C and TC were log transformed. For more information, see Birkenaes *et al.* (Birkenaes et al., 2008).

We measured IL-18, its binding protein IL-18BPA, and other secreted components of the IL-18 system, IL-18RAP and IL-18R1. IL-18 (Cat# DY318-05) and IL-18BPA (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.

2.3. RNA microarray analysis and quality control

Blood samples were collected in Tempus Blood RNA Tubes (Life Technologies Corporation). Total RNA was extracted with the TEMPUS 12-Port RNA Isolation Kit (Applied Biosystems) and ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems) according to manufacturer's protocol. Global gene expression analyses were performed with Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc.) consisting of ~47,000 probes. Multidimensional scaling and hierarchical clustering were used for regular quality control, including sample quality measurements and removal of outliers, as well as removal of multiple batch effects (RNA extraction batch, RNA extraction method, DNase treatment batch, cRNA labelling batch, and chip hybridization). This was followed by log2-transformation. More details on microarray preprocessing and quality control have been published elsewhere (Akkouh et al., 2020). Probes showing zero expression in more than 90% of the samples were ignored, leaving 23,476 markers left for examination. All genome-wide expression analyses were performed on the batch-adjusted log2-transformed data.

2.4. Statistics

Statistical analyses were performed in R using the base statistical package (R Core Team. R, 2020). Differences in sample characteristics were assessed using analysis of variance and subsequent Tukey's honest significant difference post-hoc tests or t-tests and chi-squared or Fisher's exact tests, for continuous and categorical data respectively. A p-value threshold of $p<0.05$ was applied.

Correlations between measures of circulating IL-18 system components and cholesterol, as well as, mRNA levels of IL-18 system components were investigated. Associations between diagnosis (SCZ, BD, SCZ+BD) and circulating levels of IL-18 system components were assessed using linear regression, adjusting for age, sex, BMI, circulating levels of CRP (where relevant), and the length of time that blood samples were stored after collection. Additional adjustments were also made for smoking status. Similarly, associations between diagnosis and mRNA levels of IL-18 system components and levels of circulating cholesterol (TC, HDL and LDL) were also assessed using linear regression with these same covariates. To correct for multiple testing we applied a Bonferroni-corrected significance level of $p<9.259 \times 10^{-4}$ (Four IL-18 system components, three cholesterol levels and 11 probes across three diagnostic groupings = 54 tests; 0.05/54).

Table 1
Demographic and clinical characteristics of the sample.

		Age	BMI	PANSS	YMRS	HDL	LDL	TC	CRP	ddd_AP
SCZ	n = 737	n = 737	n = 455	n = 555	n = 472	n = 528	n = 528	n = 528	n = 509	n = 525
	Mean	32.75	25.97	63.55	5.18	1.32	3.18	5.08	3.54	1.18
	SD	13.28	5.27	16.56	5.11	0.41	0.94	1.05	3.55	0.82
BD	n = 895	n = 895	n = 779	n = 421	n = 416	n = 419	n = 419	n = 419	n = 376	n = 223
	Mean	38.60	25.92	45.36	3.09	1.47	3.06	5.01	3.15	0.87
	SD	13.69	4.80	10.21	4.39	0.43	0.93	1.07	3.44	0.64
CTRL	n = 1070	n = 1070	n = 849	–	–	n = 1035	n = 1035	n = 1035	n = 968	–
	Mean	32.51	24.70	–	–	1.53	2.87	4.83	2.25	–
	SD	10.00	3.90	–	–	0.45	0.89	1.00	2.62	–
	SCZ vs BD vs CTRL	<2e-16	1.060E-08	–	–	<2e-16	6.770E-10	3.990E-05	1.280E-14	–
	SCZ vs BD	<2e-16	9.773E-01	<2e-16	1.560E-10	<2e-16	9.627E-02	6.565E-01	1.433E-01	9.130E-07
	SCZ vs CTRL	9.160E-01	5.100E-06	–	–	<2e-16	<2e-16	7.850E-05	<2e-16	–
	BD vs CTRL	<2e-16	3.000E-07	–	–	1.634E-02	1.320E-03	1.167E-02	5.300E-06	–
		Sex (Male)	AP use (Yes)	Smoke regularly (Yes)	Smoke regularly (No)	Smoke regularly (Total)				
SCZ	n = 737	n = 425	n = 525	n = 275	n = 276	n = 551				
	Percentage	57.67%	71.23%	37.31%	37.45%	74.76%				
BD	n = 895	n = 363	n = 223	n = 179	n = 247	n = 426				
	Percentage	40.56%	24.92%	20.00%	27.60%	47.60%				
CTRL	n = 1070	n = 559	n = 0	n = 122	n = 571	n = 693				
	Percentage	52.24%	0.00%	11.40%	53.36%	64.77%				
	SCZ vs BD vs CTRL	7.010E-12	–	<2e-16						
	SCZ vs BD	8.320E-12	<2e-16	1.696E-02						
	SCZ vs CTRL	2.600E-02	–	<2e-16						
	BD vs CTRL	3.002E-07	–	<2e-16						

BMI = Body mass index; PANSS = The Positive and Negative Syndrome Scale; YMRS = The Young Mania Rating Scale; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; CRP = C-reactive protein; DDD_AP = defined daily dose of antipsychotics (AP).

3. Results

3.1. Circulating levels of IL-18 system components in SMI

Circulating IL-18 is a readily detectable signature cytokine of systemic inflammasome activation. We thus first examined whether there were any associations between diagnosis and plasma levels of four critical components of the IL-18 system in a large cohort of SCZ (n=737) and BD patients (n=895), and CTRL (n=1070) controlling for age, sex, and BMI. Indeed, patients with SMI (SCZ+BD) displayed significantly higher levels of IL-18 ($p<0.0001$), the IL-18-binding protein IL-18BPA ($p<0.0001$), and IL-18R1 ($p=0.03$) relative to controls (Figure 1). No significant alterations were found in the level of IL-18 receptor accessory protein (IL-18RAP; $p=0.969$) (Figure 1). Evaluated as diagnostic subgroups revealed similar patterns in patients with SCZ (IL-18 $p<0.0001$; IL-18BPA $p=0.013$; IL-18R1 $p=0.31$; IL-18RAP $p= 0.956$) and BD (IL-18 $p<0.0001$; IL-18BPA $p= 0.001$; IL-18R1 $p= 0.035$; IL-18RAP $p= 0.72$) (Figure 1).

Since MIF is a critical component in inflammasome activation (Lang et al., 2018), we next evaluated plasma levels of this factor in our clinical sample. Patients with SMI displayed trending, but not significant increases in plasma MIF ($p=0.205$), with similar patterns in the SCZ ($p=0.432$) or BD ($p=0.179$) subcohorts (Figure 1). Further, since BD type I. and type II. represent two, potentially different disease entities with regards to their biological underpinnings, we tested whether plasma levels of IL-18, IL-18BPA, and IL-18R1 showed any difference relative to controls in these subcategories. When testing cytokine levels in BD subtypes, we found elevated circulating IL-18 in both subgroups with similar effect sizes and p-values as compared to healthy controls (Supplementary Figure S1). This suggests no outstanding difference between BD subcategories with regards to their IL-18 system-related inflammatory status, and further supports the psychosis continuum model.

We also tested if the immune-activation of the IL-18 system was distinct, or merely a result of the enhanced low-grade inflammation as reflected by CRP. However, after controlling for CRP, elevated IL-18 system component levels remained significant in the SMI (SCZ+BD) and BD, but not in the SCZ cohort (Supplementary Figure S2). This suggests that IL-18 upregulation is beyond CRP related immune-activation in SMI and BD, while it seems more dependent on subclinical inflammation in SCZ.

Since smoking has been suggested to influence circulating inflammatory cytokine levels in clinical studies (Reponen et al., 2020; Misiak et al., 2017), we also performed additional analyses focusing on those plasma markers that displayed significant alterations in SMI vs controls with large effect sizes (IL-18 and IL-18BPA) controlling for smoking status. We found that only IL-18 levels were significantly affected by smoking in the SCZ and SMI cohorts, but smoking did not influence IL-18 levels neither in patients with BD nor in healthy controls. Additional adjustments for smoking status did not affect the results regarding IL-18BPA levels in any of the diagnostic groups (Supplementary Table S1). Interestingly, when comparing circulating levels of IL-18 and IL-18BPA only in the non-smoker populations we found that, similarly to our results above, SMI patients displayed significantly elevated levels relative to controls (CTRL vs SCZ, and CTRL vs BD; Supplementary Table S1). This suggests that higher plasma levels of IL-18 system elements in SMI is most likely a biological component of the psychosis continuum that is independent of smoking status.

3.2. IL-18 system dysregulation and circulating immune cells in SMI

To test for possible involvement of peripheral immune cells in the observed dysregulation of the IL-18 system in SCZ and BD, we also analyzed the gene expression of IL-18 system components (*IL18*,

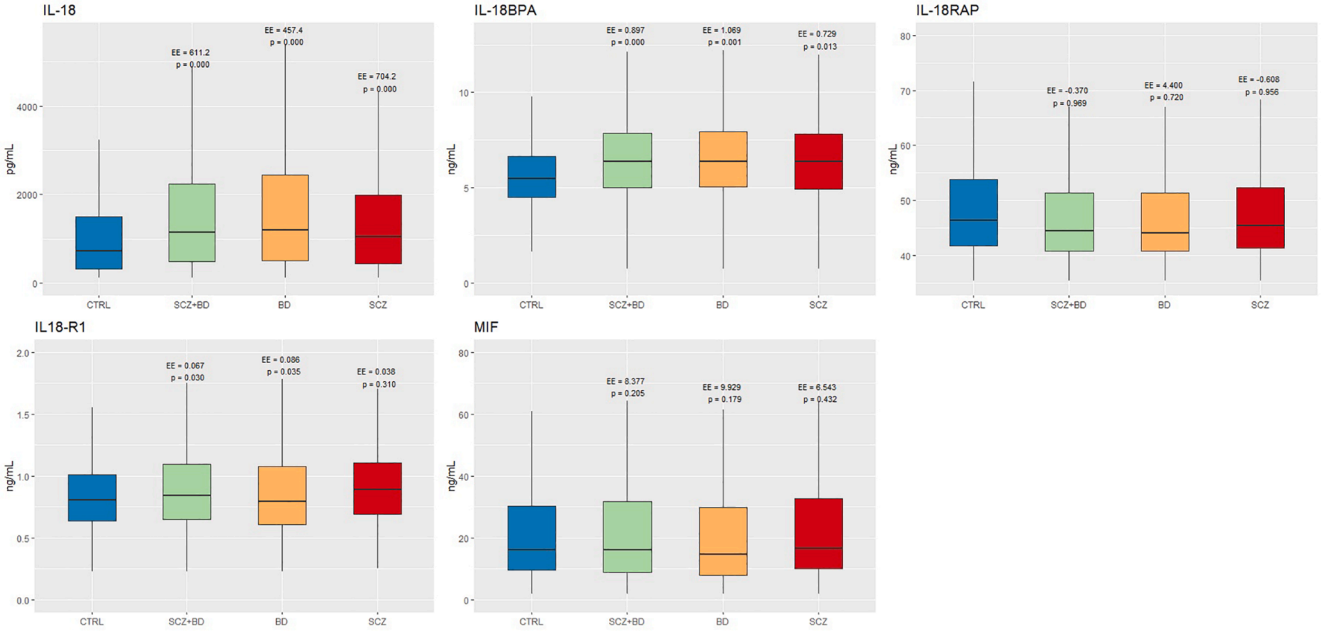

Fig. 1. Plasma levels of IL-18 family cytokines and MIF are elevated in patients with SMI relative to healthy controls. Circulating levels of IL-18 (A), IL-18BPA (B), IL-18RAP (C), IL-18R1 (D), and MIF (E) are shown in patients with SMI (SCZ + BD), with SCZ, BD, or in controls (CTRL), controlling for age, sex, and BMI. Boxplots show median (line at 50% quantile) and interquartile ranges (bottom of boxplot at 25% quantile, top at 75% quantile). p values and effect estimates (EE) are presented on top of each bar relative to CTRL.

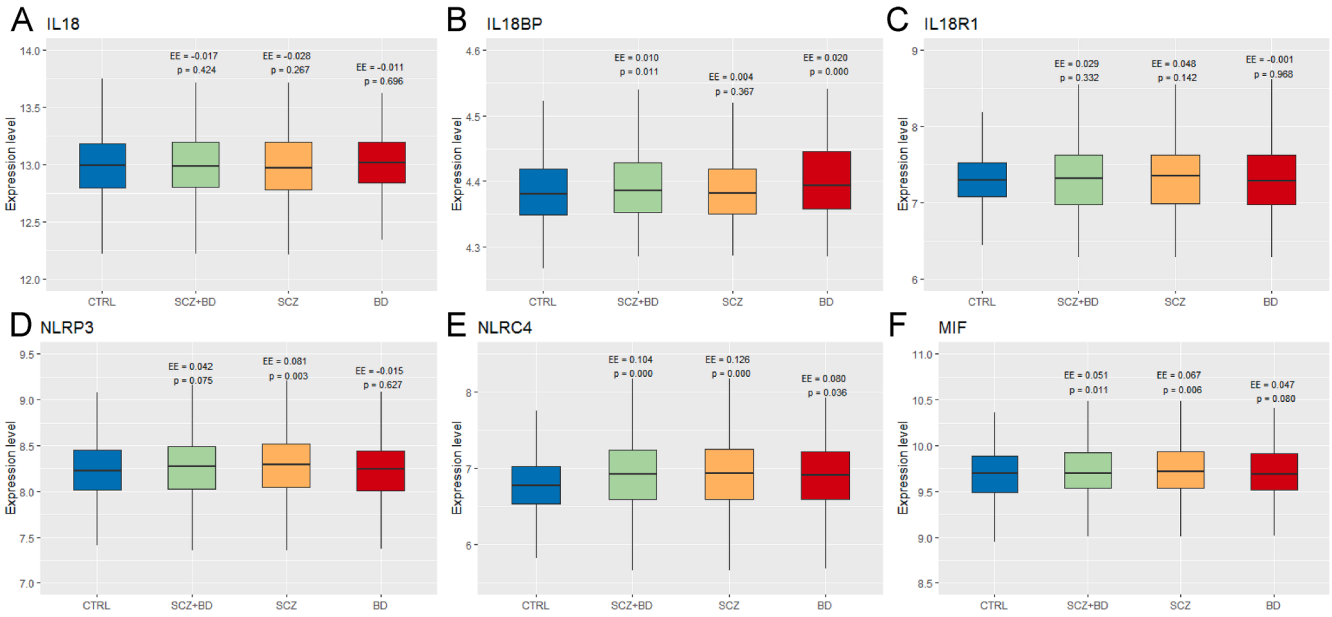

Fig. 2. Relative mRNA expression of IL-18 system elements and inflammasome genes in circulating immune cells of patients with SMI, and in healthy controls. Relative expression of IL-18 family cytokine (A-C), inflammasome (D-E), and MIF (F) genes in patients with SMI (SCZ + BD), in SCZ, BD, or in healthy controls (CTRL) are presented, controlling for age, sex, and BMI. Boxplots show median and interquartile ranges as in Fig. 1. p values and effect estimates (EE) are presented on top of each bar relative to CTRL.

IL18BPA, *IL18R1*, *IL18RAP*), *MIF*, and inflammasome elements (*NLRP3* and *NLRC4*) in circulating leukocytes from SMI patients and CTRLs.

Patients with SMI displayed higher expression of *IL-18BPA* (p= 0.01) and *NLRC4* (p= 0.0004) relative to controls (Figure 2). In the SCZ cohort we only found significant differences in the expression of *NLRP3* (p=0.0031) and *NLRC4* (p=0.0001), but not in IL-18 system-related genes when compared to CTRLs (Figure 2). Both IL-18BPA (p= 0.0001) and NLRC4 (p= 0.036) mRNA levels were similarly increased in BD (Figures 2B and 2E). No significant differences were found in the gene expression levels of other IL-18 pathway members or inflammasome elements in SMI or BD versus controls (Figure 2A, 2C, and 2D). Additionally, we found increased MIF mRNA expression in SMI (p= 0.012) and SCZ (p=0.006), but not in BD (p=0.08; Figure 2F). No correlation was observed between plasma levels of IL-18 and IL-18BPA and their corresponding mRNA expression in leukocytes in SCZ, BD or controls.

3.3. Cholesterol levels are positively correlated with the expression of inflammasome-IL-18 system elements in SCZ, but not in BD and CTRLs

Cholesterol crystals may activate the NLRP3 inflammasome and experimental studies suggest that hyperlipidemia may promote production of these crystals in endothelial cells (Baumer et al., 2017). Based on the established dyslipidemia observed in SMI and, as also shown in our patients (Table 1), we next examined associations between dysregulated IL-18 members, cholesterol levels and mRNA levels of inflammasome components in SCZ and BD.

We found negative correlations between the elevated total cholesterol levels and mRNA expression of *MIF* in BD (p=0.038; Figure 3, Supplementary Table S2), but not in SCZ or controls. LDL levels were also negatively correlated with *IL18* (p=0.049) in SCZ, but not in BD or in CTRL (Figure 3, Suppl. Table S2). Furthermore, we found negative

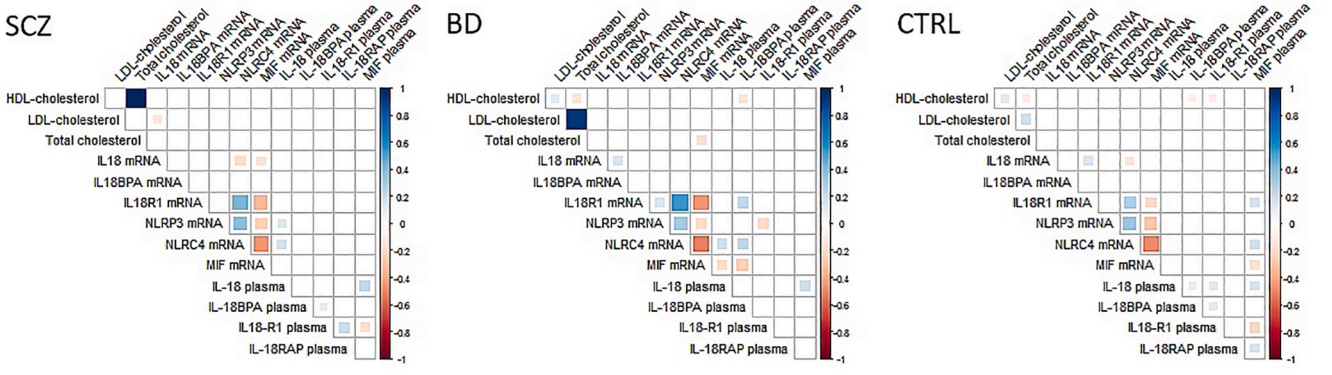

Fig. 3. Correlations between diagnosis, immune status and blood lipid levels. Positive (blue) and negative (red) correlations are shown in patients with SCZ, BD, and in healthy controls (CTRL). Only significant correlations are plotted ($p < 9.259 \times 10^{-4}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correlations between HDL and IL-18BPA plasma levels in the BD cohort (p=0.025) and in healthy controls (p=0.026; Suppl. Table S2), but not in SCZ (Figure 3).

IL-18 plasma levels were positively correlated with *NLRP3* (p=0.0012) and *NLRC4* (p=0.003) mRNA levels in SCZ and with *NLRC4* in BD, but not in CTRLs (Figure 3 and Suppl. Table S2). Interestingly, the plasma levels of IL-18BPA were also positively correlated with *NRLC4* mRNA levels in BD, but not in SCZ or CTRL (Figure 3 and Suppl. Table S2). In addition, MIF plasma levels were positively correlated with IL-18 plasma levels in all cohorts (SCZ p<0.0001; BD p=0.008; CTRL p=0.0014; Figure 3 and Suppl. Table S2) that may imply an intrinsic regulatory connection principle between the inflammasome-MIF and IL-18 systems independent of SMI.

Finally, we found similar negative correlations of gene expression patterns between *MIF* and inflammasome system-related genes (*IL-18R1*, *NLRP3, NRLC4*) in all groups (Figure 3 and Suppl. Table S2) suggesting a physiological constellation that is not unique to psychotic disorders. No other significant associations were found between diagnoses, cholesterol levels, and any of the other elements of the inflammasome-IL-18 system (Suppl. Table S2).

4. Discussion

In this large population of patients with SMI and healthy controls we found i) higher plasma levels of IL-18, IL-18BPA, and IL-18R1 in SMI, ii) which was independent of CRP levels in BD, but not in SCZ; iii) elevated mRNA levels of inflammasome genes (*NLRP3* and *NLRC4*), but not IL-18 family members in immune cells isolated from whole blood from patients with SMI relative to controls. Finally, iv) we observed correlations between blood lipids and inflammasome system elements in psychotic disorder patients, but not in controls. These results support potential inflammasome activation in severe mental disorders, with different patterns across the psychosis continuum which may be linked to dyslipidemia-related processes in BD and SCZs.

Since elevation in the levels of IL-18 system components in blood is a hallmark signature of systemic inflammasome activation (Strowig et al., 2012), our results showing significantly higher plasma levels of IL-18, IL-18BPA, and IL-18R1 in SMI relative to controls suggest dysregulation of the inflammasome system in patients. The phenomenon is more prominent in BD where the pattern persisted after controlling for CRP levels in our cohorts. Simultaneous increases of IL-18BPA, a natural endogenous inhibitor of IL-18 receptor signaling (Kim et al., 2000), and the receptor-adaptor signaling module IL-18R1 with IL-18, together with no alterations in the circulating levels of the enhancer IL-18RAP may indicate a systemic compensatory mechanism due to chronic inflammasome overactivation. We also observed trending elevated plasma levels of MIF in patients with SMI relative to healthy controls, which was independent of circulating CRP as well. Interestingly, while increases in MIF plasma concentrations were also trending in the SCZ and BD cohorts, they were not significant which is suggestive of a less disease and more continuum-specific phenomenon (Tesli et al., 2014; Möller, 2003). This was confirmed by significantly elevated *MIF* mRNA levels in circulating immune cells in SMI and also in patients with SCZ, but not in BD. However, the observed very small effect sizes suggest that the major source of circulating MIF is probably not immune cells in SMI. Since MIF is indispensable in inflammasome activation and IL-18 release (Lang et al., 2018), our results suggest a pathological dysregulation of the entire MIF-inflammasome-IL-18 axis in patients with SMI, which is possibly more pronounced in SCZ.

Evaluation of mRNA levels of IL-18 system components from leukocytes revealed no dysregulation as seen for circulating protein levels in SMI and there was no correlation between mRNA expression and circulating protein levels. Although this may suggest that circulating immune cells are not the major source of the enhanced plasma levels of IL-18 and IL-18BPA in our study, inflammasome activation only induces the cleavage and release of the mature IL-18 protein, but not IL-18 mRNA synthesis. Thus, leukocytes from SMI patients could still release enhanced levels of IL-18 as we did observe increased *NLRP3* and *NLRC4* mRNA levels in patients with SCZ, suggesting inflammasome upregulation in these patients. Furthermore, plasma IL-18 was positively correlated with *NLRC4* expression in SMI. Nonetheless, other tissues could contribute to the abnormal production of IL-18 and IL-18BPA in SMI. Besides circulating immune cells, systemic source of IL-18 and IL-1β can also be the liver (Szabo and Csak, 2012) or the vascular endothelium (Grebe et al., 2018; Krishnan, 2014) following tissue-specific inflammasome activation. Chronic triggering of inflammasomes in these tissues may lead to elevated levels of IL-18 family cytokines which, in turn, can affect circulating immune cells and thereby modulate the expression of *MIF* mRNA in leukocytes. This is also in line with the observed increased in mRNA levels of the inflammasome genes in SCZ (*NLRP3* and *NLRC4*) and in BD (*NLRC4*), but not in CTRL which raises the possibility of a primed inflammasome system in immune cells in SMI. Further associations were found between increased IL-18 plasma levels and elevated *NLRC4* levels in blood in both SCZ and BD, but not in controls, which suggest a disorder-specific, chronic inflammatory background and abnormal inflammasome upregulation in psychotic disorders. In the context of psychoneuroimmunology, partial overlap in the observed associations supports the validity of the psychosis continuum model, which suggests a floating bio-psychological continuum in SCZ-BD rather than distinct diagnostic entities (Tesli et al., 2014; Möller, 2003; Craddock, 2005).

Systemic inflammasome activation can be triggered by a myriad of exogenous and endogenous ligands. However, systemic and chronic stimulation presupposes naturally occurring and circulating inflammasome ligands, such as abnormally produced but otherwise innocuous metabolic products or physiological agents, for example cholesterol (Strowig et al., 2012) or uric acid (Martinon et al., 2006). Abnormally high levels of circulating uric acid has been detected in patients with SCZ (Solberg et al., 2019). Furthermore, preclinical studies have also suggested inflammasome activation in an overlapping domain of brain sterile inflammation, increased cardiovascular risk and psychosis (Freeman et al., 2017; Kim et al., 2016; Ventura, 2020). Our data are also in good agreement with previous clinical studies reporting elevated levels of IL-18 (Xiu et al., 2012; Fillman et al., 2016; Hylén et al., 2020) and IL-18BPA in SCZ (Wedervang-Resell et al., 2020; Palladino et al., 2012). However, systemic inflammasome activation has not been investigated in the context of SMI in large clinical cohorts. In addition to cytokine measures, in the present study we found correlations between LDL-cholesterol and the expression of IL-18 gene in SCZ. We also found negative correlations between total cholesterol and *MIF* mRNA, and HDL and IL-18BPA plasma in BD. Since circulating total cholesterol can form crystals that can cause systemic inflammasome activation (by serving as DAMPs) and thereby the systemic release of IL-18 (Tall and Yvan-Charvet, 2015; Liston and Masters, 2017; Westerterp et al., 2017; Le Bras, 2018), while HDL-cholesterol has been shown to inhibit inflammasome activation and to lower total cholesterol (Grebe and Latz, 2013; Thacker et al., 2016), our results raise the possibility that chronic dyslipidemia may contribute to a lipid-driven sterile inflammation at least in BD. However, the observed correlations between blood cholesterol and IL-18 system elements are weak, and evaluation of triggers for

inflammasome activation in SMI will have to be further evaluated in forthcoming studies.

The present work also has limitations. Despite the careful adjustments for a comprehensive range of variables, residual confounding factors cannot be ruled out. Furthermore, the cross-sectional nature of the study did not allow exploration of cause and effect. Furthermore, since chronic medication with certain neuroleptics are known to influence circulating cholesterol levels, another issue is the lack of data on the potential effect of antipsychotic medication on cholesterol levels in our SCZ and BD subgroups. Although statistically significant, correlations between blood cholesterol levels and the expression of inflammasome-related genes are relatively weak. Consequently, it is unclear to what extent levels of cholesterol types in our study can be interpreted as causative agents regarding IL-18-mediated inflammatory dysregulation, and our data have to be interpreted with caution. We therefore need longitudinal studies with a focus on the emergence, symptom evolution and severity of psychosis in relation to blood cholesterol and the IL-18 system. Finally, although the present study focused on inflammasomes and the IL-18 system, there is a need for investigation of other parts of the innate immune system in SMI, and we propose that cytokine network studies would be of great importance.

Contemporary psychiatry research addresses questions on the pathobiological underpinnings of severe mental disorders. Complex brain-immune interactions that involve inflammatory innate immune mechanisms might have causal and therapeutic implications for psychiatric illness (Khandaker et al., 2015). Here we report higher baseline levels of circulating IL-18 system components as well as increased expression of inflammasome-related genes in the blood of patients with SMI. Based on these results, we suggest a role for systemic inflammasome activation/dysregulation in psychotic disorder patients. Our findings further the understanding of possible underlying inflammatory mechanisms in SMI, and may expose important therapeutic targets in severe mental disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.10.017.

References

Akkouh, I.A., et al., 2020. Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: Potential attenuating effects on recruitment of regulatory T cells. Brain Behav. Immun. 87, 634–644.
Baumer, Y., et al., 2017. Hyperlipidemia-induced cholesterol crystal production by endothelial cells promotes atherogenesis. Nat. Commun. 8 https://doi.org/10.1038/s41467-017-01186-z.
Birkenaes, A.B., et al., 2008. Dyslipidemia independent of body mass in antipsychotic-treated patients under real-life conditions. J. Clin. Psychopharmacol. 28, 132–137.
Chen, G.Y., Nuñez, G., 2010. Sterile inflammation: sensing and reacting to damage. Nat. Rev. Immunol. 10, 826–837.
Correll, C.U., et al., 2015. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. World Psychiatry 14, 119–136.
Corvin, A., Morris, D.W., 2014. Genome-wide association studies: findings at the major histocompatibility complex locus in psychosis. Biol. Psychiatry 75, 276–283.
Craddock, N., 2005. The genetics of schizophrenia and bipolar disorder: dissecting psychosis. J. Med. Genet. 42, 193–204.
Dieset, I., et al., 2012. Up-regulation of NOTCH4 gene expression in bipolar disorder. Am. J. Psychiatry 169, 1292–1300.
Fillman, S.G., et al., 2016. Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. Mol. Psychiatry 21, 1090–1098.
Freeman, L., et al., 2017. NLR members NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes. J. Exp. Med. 214, 1351–1370.
Grebe, A., Hoss, F., Latz, E., 2018. NLRP3 Inflammasome and the IL-1 Pathway in Atherosclerosis. Circ. Res. 122, 1722–1740.
Grebe, A., Latz, E., 2013. Cholesterol crystals and inflammation. Curr. Rheumatol. Rep. 15, 313.
Hylén, U., et al., 2020. Increased inflammasome activity in markedly ill psychiatric patients: An explorative study. J. Neuroimmunol. 339, 577119. https://doi.org/10.1016/j.jneuroim.2019.577119.
International Schizophrenia, Consortium, et al., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460, 748–752.
Keller, M., et al., 2008. Active caspase-1 is a regulator of unconventional protein secretion. Cell 132, 818–831.
Khandaker, G.M., et al., 2015. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. Lancet Psychiatry 2, 258–270.
Kim, S.-H., et al., 2000. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc. Natl. Acad. Sci. USA 97, 1190–1195.
Kim, H.K., et al., 2016. Nod-like receptor pyrin containing 3 (NLRP3) in the post-mortem frontal cortex from patients with bipolar disorder: A potential mediator between mitochondria and immune-activation. J. Psychiatr Res. 72, 43–50.
Krishnan, S.M., et al., 2014. IL-1β and IL-18: inflammatory markers or mediators of hypertension? Br. J. Pharmacol. 171, 5589–5602.
Lang, T., et al., 2018. Macrophage migration inhibitory factor is required for NLRP3 inflammasome activation. Nat. Commun. 9 https://doi.org/10.1038/s41467-018-04581-2.
Le Bras, A., 2018. Cholesterol-dependent inflammasome activation accelerates atherosclerosis. Nat. Rev. Cardiol. 15, 318–319.
Liston, A., Masters, S.L., 2017. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. Nat. Rev. Immunol. 17, 208–214.
Martinon, F., et al., 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol. Cell 10, 417–426.
Martinon, F., et al., 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440, 237–241.
Medzhitov, R., 2001. Toll-like receptors and innate immunity. Nat. Rev. Immunol. 1, 135–145.
Misiak, B., et al., 2017. Lipid profile disturbances in antipsychotic-naive patients with first-episode non-affective psychosis: A systematic review and meta-analysis. Schizophr Res. 190, 18–27.
Möller, H.J., 2003. Bipolar disorder and schizophrenia: distinct illnesses or a continuum? J. Clin. Psychiatry 64 (Suppl 6), 23–27.
Mørch, R.H., et al., 2016. Inflammatory evidence for the psychosis continuum model. Psychoneuroendocrinology 67, 189–197.
Mørch, R.H., et al., 2017. Persistent increase in TNF and IL-1 markers in severe mental disorders suggests trait-related inflammation: a one year follow-up study. Acta Psychiatr Scand. 136, 400–408.
Owen, M.J., et al., 2016. Schizophrenia. Lancet 388, 86–97.
Palladino, I., et al., 2012. Elevated levels of circulating IL-18BP and perturbed regulation of IL-18 in schizophrenia. J. Neuroinflammation 9. https://doi.org/10.1186/1742-2094-9-206.
Pollak, T.A., et al., 2020. Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin. Lancet Psychiatry 7, 93–108.
R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing 2020; Vienna, Austria.

Reponen, E.J., et al., 2020. Atherogenic Lipid Ratios Related to Myeloperoxidase and C-Reactive Protein Levels in Psychotic Disorders. Front Psychiatry 11. https://doi.org/10.3389/fpsyt.2020.00672.
Rubartelli, A., 2014. DAMP-Mediated Activation of NLRP3-Inflammasome in Brain Sterile Inflammation: The Fine Line between Healing and Neurodegeneration. Front Immunol. 5, 99.
SayuriYamagata, A., et al., 2017. Medical comorbidity in bipolar disorder: the link with metabolic-inflammatory systems. J. Affective Disord. 211, 99–106.
Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421–427.
Simonsen, C., et al., 2011. Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. Schizophr Bull 37, 73–83.
Solberg, D.K., et al., 2019. A five-year follow-up study of antioxidants, oxidative stress and polyunsaturated fatty acids in schizophrenia. Acta Neuropsychiatr 31, 202–212.
Strowig, T., et al., 2012. Inflammasomes in health and disease. Nature 481, 278–286.
Szabo, G., Csak, T., 2012. Inflammasomes in liver diseases. J. Hepatol. 57, 642–654.
Tall, A.R., Yvan-Charvet, L., 2015. Cholesterol, inflammation and innate immunity. Nat. Rev. Immunol. 15, 104–116.
Tesli, M., et al., 2014. Polygenic risk score and the psychosis continuum model. Acta Psychiatry Scand. 130, 311–317.
Thacker, S.G., et al., 2016. High-density lipoprotein reduces inflammation from cholesterol crystals by inhibiting inflammasome activation. Immunology 149, 306–319.
Ventura, L., et al., 2020. Ivolvement of NLRP3 inflammasome in Schizophrenia-like behavior in young animals after maternal immune activation. Acta Neuropsychiatr 14, 1–29.
Wedervang-Resell, K., et al., 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.
Westerterp, M., et al., 2017. Cholesterol Accumulation in Dendritic Cells Links the Inflammasome to Acquired Immunity. Cell Metab. 25 (6), 1294–1304.e6.
Xiu, M.H., et al., 2012. Elevated interleukin-18 serum levels in chronic schizophrenia: Association with psychopathology. J. Psychiatr Res. 46, 1093–1098.