Immune abnormalities and treatment resistance in severe mental disorders: the role of polygenic risk, infections and autoimmunity

Dissertation for the degree of Philosophiae Doctor (PhD)

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*Oslo, 2022*
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Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-348-0118-1

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Print production: Graphics Center, University of Oslo.
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Papers I-III
1 ACKNOWLEDGEMENTS

I want to direct a huge thanks to all participants of the TOP study. Their willingness to share from their subjective experiences is the core foundation that enabled the work leading to this thesis. The participants go through a comprehensive assessment and without their patience and courage this thesis would not be possible.

I am exceptionally grateful for my main supervisor, Nils Eiel Steen, for the never-ending support he has given me throughout the process. Nils Eiel has a way of supervising that is engaging, fun and very educational. It has been a true pleasure to get to know him and to collaborate with him from day one, and there is no doubt that my PhD period would have not been the same without him. I also want to direct a huge thanks my co-supervisors Katrine Verena Wirgenes and Ole Andreas Andreassen for excellent supervision, exciting conversations and invaluable support. I am grateful for and inspired by Katrine’s robust overview of clinical and genetic perspective and sharp pen, and by Ole’s remarkable academic wisdom and inventive mind.

I am grateful to the contribution from all co-authors. A huge thanks to Alexey A. Shadrin and Francesco Bettella for help with biostatistics, and Thor Ueland and Pål Aukrust for help with immune markers. I am grateful for the valuable input from Monica B.E.G. Ormerod, Linn N. Rødevand, Synve H. Lunding, Marit Haram, Gabriela Hjell, Ingrid Agartz and Srdjan Djurovic. I want to thank Ingrid Melle for valuable input, extraordinary clinical supervision and general support. I am grateful for the cooperation with Line Gundersen, Eivind Bakken, Marina Herfindal-Engh, Tor Johannes Helleland, Ragnhild B. Storli, Christine L. Brandt, Idun B. Huflätten, Christine Mohn, Therese Markl and Thomas Bjella. I am very happy to have shared office with Stine, Margrethe, Ingrid, Cecilie, Gina, Camilla, Kristin, Linn Sofie, Attila, Trine, Torill, Henrik, Beate, Elina and all other wonderful colleagues at NORMENT.

I want to thank my brother, dad, mom and partner for their patience, support and unconditional love. A huge thanks to all my friends and rest of family for motivation, support and genuine interest in my work. To the ones who have passed away, you will always be my guiding lights.

The work of the current thesis was conducted at Norwegian Centre for Mental Disorders Research (NORMENT), Division of Mental Health and Addiction, Oslo University Hospital, and Institute of Clinical Medicine, University of Oslo. NORMENT is a centre of Excellence funded by the Research Council of Norway. Thank you for giving me this opportunity.
2 LIST OF PUBLICATIONS

Paper I

Limited association between infections, autoimmune disease and genetic risk and immune activation in severe mental disorders

Maren Caroline Frogner Werner, Katrine Verena Wirgenes, Alexey A. Shadrin, Synve Hoffart Lunding, Linn Rødevand, Gabriela Hjell, Monica Bettina Elkjaer Greenwood Ormerod, Marit Haram, Ingrid Agartz, Srdjan Djurovic, Ingrid Melle, Pål Aukrust, Thor Ueland, Ole A. Andreassen, Nils Eiel Steen


Paper II

Immune marker levels in severe mental disorders: associations with polygenic risk scores of related mental phenotypes and psoriasis

Maren Caroline Frogner Werner, Katrine Verena Wirgenes, Alexey Shadrin, Synve Hoffart Lunding, Linn Rødevand, Gabriela Hjell, Monica Bettina Elkjaer Greenwood Ormerod, Marit Haram, Ingrid Agartz, Srdjan Djurovic, Ingrid Melle, Pål Aukrust, Thor Ueland, Ole Andreas Andreassen, Nils Eiel Steen

Translational Psychiatry 12, 38, 2022. https://doi.org/10.1038/s41398-022-01811-6

Paper III

Indicated association between polygenic risk score and treatment-resistance in a naturalistic sample of patients with schizophrenia spectrum disorders

Maren Caroline Frogner Werner, Katrine Verena Wirgenes, Marit Haram, Francesco Bettella, Synve Hoffart Lunding, Linn Rødevand, Gabriela Hjell, Ingrid Agartz, Srdjan Djurovic, Ingrid Melle, Ole Andreas Andreassen, Nils Eiel Steen

3 SUMMARY

Schizophrenia and bipolar spectrum disorders are severe mental disorders with overlapping clinical features and risk factors. The exact underlying biological mechanisms are not clear; however, both disorders have high estimated heritability and ongoing mapping of common genetic risk variants reveal complex genetic mechanisms. Genetic signals from immune loci, associations with previous infections and autoimmune comorbidity indicate a link with the immune system, supported by repeated findings of abnormal levels of peripheral immune markers in these disorders. Standard pharmacological treatment is ineffective for about one third of patients, causing a significant burden. It is suggested that patients with treatment resistance form a subgroup of a more ‘genuine’ schizophrenia. In the current thesis we investigated whether genetic susceptibilities identified for autoimmune diseases, mental disorders, cognition and personality traits, number of previous infections and presence of autoimmune disease could explain immune marker aberrations in patients with severe mental disorders. Additionally, we investigated whether polygenic risk score for schizophrenia is related to treatment resistance to antipsychotics. Participants diagnosed with either bipolar or schizophrenia spectrum disorder and healthy controls were included from the ongoing Thematically Organized Psychosis study, and well-adjusted analysis of covariance and logistic regression were applied to test associations. There were few indicated associations between previous infections or autoimmune disease and immune markers, except for an association between presence of autoimmune disease and soluble interleukin-2 receptor. Further, there was an association between polygenic risk score for schizophrenia and soluble interleukin-2 receptor, and between polygenic risk score for educational attainment and both interleukin-1 receptor antagonist and interleukin-18. Associations explained minor variations in immune marker levels between patients and healthy controls. Moreover, we found an association between higher polygenic risk of schizophrenia and treatment resistance to antipsychotics. Our findings indicate a small role for autoimmune mechanisms and genetic susceptibilities to schizophrenia and related mental phenotypes in low-grade inflammation in severe mental disorders, and for genetic susceptibility to schizophrenia in treatment resistance to antipsychotics. The very little explained variance and few associations suggest that immune marker abnormalities in these disorders are mainly explained by other factors.
Sammendrag

5 ABBREVIATIONS

ADHD Attention-deficit/hyperactivity disorder
APRIL A Proliferation-Inducing Ligand
ASD Autism spectrum disorder
BBB Blood-brain barrier
BD Bipolar spectrum disorder
BDNF Brain derived neurotrophic factor
BMI Body mass index
CIRS Chronic inflammatory response syndrome
CNS Central nervous system
CRP C-reactive protein
DALY Disability-adjusted life years
DAMP Damage associated molecular pattern
DDD Defined daily dose
DNA Deoxyribonucleic acid
DRD2 Dopamine D2 receptor gene
DSM Diagnostic and Statistical Manual of Mental Disorders
EDTA Ethylenediaminetetraacetic acid
EIA Enzyme immunoassays
ELISA Enzyme-linked immunosorbent assay
EEG Electroencephalogram
GAF General Assessment of Functioning Scale
GWAS Genome-Wide Association Studies
HC Healthy controls
HLA Human leukocyte antigen
HRC Haplotype reference consortium
IBD Inflammatory bowel disease
ICAM-1 Intercellular adhesion molecule 1
IE Interaction effect
IFN-γ Interferon gamma
IL Interleukin
IL-1Ra Interleukin-1 receptor antagonist
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IQ</td>
<td>Intelligence quotient</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<tr>
<td>LD</td>
<td>Linkage disequilibrium</td>
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<tr>
<td>MAF</td>
<td>Minor allele frequency</td>
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<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance investigation</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>N</td>
<td>Number</td>
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<tr>
<td>NLRP3</td>
<td>Nod-like receptor (NLR) family, pyrin domain-containing 3</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NOS</td>
<td>Not otherwise specified</td>
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<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
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<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>PGC</td>
<td>Psychiatric Genomics Consortium</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post-traumatic stress disorder</td>
</tr>
<tr>
<td>PRS</td>
<td>Polygenic risk score</td>
</tr>
<tr>
<td>PRS-ADHD</td>
<td>Polygenic risk score for attention-deficit/hyperactivity disorder</td>
</tr>
<tr>
<td>PRS-ANX</td>
<td>Polygenic risk score for anxiety disorders</td>
</tr>
<tr>
<td>PRS-ASD</td>
<td>Polygenic risk score for autism spectrum disorder</td>
</tr>
<tr>
<td>PRS-BD</td>
<td>Polygenic risk score for bipolar disorder</td>
</tr>
<tr>
<td>PRS-CD</td>
<td>Polygenic risk score for celiac disease</td>
</tr>
<tr>
<td>PRS-COG</td>
<td>Polygenic risk score for general intelligence</td>
</tr>
<tr>
<td>PRS-CRP</td>
<td>Polygenic risk score for C-reactive protein</td>
</tr>
<tr>
<td>PRS-EA</td>
<td>Polygenic risk score for educational attainment</td>
</tr>
<tr>
<td>PRS-EXTRA</td>
<td>Polygenic risk score for extraversion</td>
</tr>
<tr>
<td>PRS-IBD</td>
<td>Polygenic risk score for inflammatory bowel disease</td>
</tr>
<tr>
<td>PRS-MDD</td>
<td>Polygenic risk score for major depressive disorder</td>
</tr>
<tr>
<td>PRS-NEURO</td>
<td>Polygenic risk score for neuroticism</td>
</tr>
<tr>
<td>PRS-OPEN</td>
<td>Polygenic risk score for openness to experience</td>
</tr>
<tr>
<td>PRS-PSOR</td>
<td>Polygenic risk score for psoriasis</td>
</tr>
<tr>
<td>PRS-PTSD</td>
<td>Polygenic risk score for post-traumatic stress disorder</td>
</tr>
<tr>
<td>PRS-RA</td>
<td>Polygenic risk score for rheumatoid arthritis</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>PRS-SCZ</td>
<td>Polygenic risk score for schizophrenia</td>
</tr>
<tr>
<td>PRS-SLE</td>
<td>Polygenic risk score for systemic lupus erythematosus</td>
</tr>
<tr>
<td>PRS-T1D</td>
<td>Polygenic risk score for type 1 diabetes mellitus</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SCID-I</td>
<td>Structured Clinical Interview for DSM-IV Axis 1 Disorders</td>
</tr>
<tr>
<td>SCZ</td>
<td>Schizophrenia spectrum disorder</td>
</tr>
<tr>
<td>sgp130</td>
<td>soluble glycoprotein-130</td>
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<tr>
<td>sIL-2R</td>
<td>soluble interleukin-2 receptor</td>
</tr>
<tr>
<td>SMD</td>
<td>Severe mental disorder</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<tr>
<td>sTNFR-1</td>
<td>soluble tumour necrosis factor-1</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td>T1D</td>
<td>Type 1 diabetes mellitus</td>
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<tr>
<td>Th</td>
<td>T-helper</td>
</tr>
<tr>
<td>TOP</td>
<td>Thematically Organized Psychosis Research</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TR</td>
<td>Treatment resistant schizophrenia</td>
</tr>
<tr>
<td>TRRIP</td>
<td>Treatment Response and Resistance in Psychosis working group</td>
</tr>
</tbody>
</table>
6 BACKGROUND

6.1 Mental health and severe mental disorders

Mental health is defined by the World Health Organization (WHO) as “a state of well-being in which an individual realizes her or his own abilities, can cope with the normal stresses of life, can work productively and is able to make a contribution to her or his community” (1). We all have a mental health. Mental disorders, however, occur when this state is disrupted, and can be described roughly as conditions with abnormal thought, behaviour, relations and/or emotions (2). Mental disorders rank amongst the leading causes of health loss, accounting for 7.4% of global disability-adjusted life years (DALYs) (3). They comprise a broad spectrum of conditions with varying severity, characteristics and outcome. Severe mental disorders (SMD) have no universal operational definition, but usually include mental illnesses with more serious functional outcome, longer duration of health service contact and often psychotic features (4). Schizophrenia and bipolar disorder are SMDs with shared clinical, genetic and pathophysiological features. The Diagnostic and Statistical Manual of Mental Disorders (DSM) (5) contains the classification of all conditions regarded as mental disorders and is the basis of most research literature in the field. In the current thesis DSM-IV (6) is used for diagnosis, but the changes made for the most recent version, DSM-5, will be described in brief.

6.2 Schizophrenia spectrum disorders

Schizophrenia is a SMD with a median estimated lifetime prevalence of 4.00-6.35 per 1000 inhabitants (7, 8). Despite relatively low prevalence, schizophrenia is one of the leading causes of disability worldwide (9, 10). Patients with schizophrenia present with symptoms commonly classified as positive and negative symptoms. Positive symptoms reflect an excess or distortion of normal behaviour or experience and refers to delusions, hallucinations and disorganized speech and/or behaviour. Negative symptoms refer to a reduction or absence of normal behaviours, and includes among other emotional withdrawal, deficits in experiencing pleasure, blunted affect, reduced social interaction seeking and deficits in verbal communication. Further, cognitive abilities are often reduced in schizophrenia, with lowered IQ, reduced executive functioning, reduced attention, poorer language and affected memory compared with healthy controls (11). Schizophrenia is associated with major societal costs and a drastically reduced life expectancy (12). Individuals with schizophrenia more often develop somatic disorders, such as cardiovascular disease and diabetes (13). Further, suicide
risk is 22 times higher, and studies show that individuals with schizophrenia smoke more tobacco, misuse alcohol more often, have an increased use of illicit drugs and have poorer dietary habits (12, 14). Individuals with schizophrenia also report a significantly lower quality of life than do healthy controls (15), and finally, they have higher odds of not being employed, living alone and lacking higher education (16, 17).

**Diagnosis and clinical characteristics**

Symptoms are most likely to develop in early adulthood and the clinical course is heterogeneous, ranging from full recovery to chronic disorder (9, 18). Following DSM-IV (6), schizophrenia is diagnosed when a person fulfils criteria A-F defined as (A) two or more of the following symptoms: (1) Delusions, (2) Hallucinations, (3) Disorganized speech, (4) Grossly disorganized or catatonic behaviour, (5) Negative symptoms, (B): Social/occupational dysfunction, (C): duration for at least 6 months, (D)-(E): exclusion of mood disorder, schizoaffective disorder, substance use or general medical conditions, and finally (F) an additional diagnosis of schizophrenia with autism disorder or other communication disorders of childhood onset is only made if prominent delusions or hallucinations are also present for at least 1 month. Only one of the five A criterion symptoms are required if 1) delusions are bizarre or 2) auditory hallucinations consists of two or more voices conversing or consist of a voice keeping up a running commentary on the person’s behaviour or thoughts. The latter criterion is changed in DSM-5 where it is required to fulfil two A criteria regardless of content of delusions or hallucinations. Schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder and psychosis NOS are related to schizophrenia with varying degrees of overlapping symptomatology, diagnostic characteristics and treatment. Schizophreniform disorder is diagnosed with the same criteria as schizophrenia when symptoms have been present for at least one month to maximum six months.

Schizoaffective disorder is characterized by overlapping mood and psychotic symptoms, and according to DSM-IV, schizoaffective disorder is diagnosed when A) there is an uninterrupted period of illness during which, at some time, there is either a major depressive episode, a manic episode, or a mixed episode concurrent with symptoms that meet criterion A for schizophrenia, B) during the same period of illness, there have been delusions or hallucinations for at least 2 weeks in the absence of prominent mood symptoms, C) symptoms that meet criteria for a mood episode are present for a substantial portion of the total duration of the active and residual periods of the illness and D) the disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical
condition. In the DSM-5, the criterion C is adjusted so that the diagnosis can be made only if full mood disorder episodes have been present for the majority of the total active and residual course of illness, from the onset of psychotic symptoms up until the current diagnosis (19). Delusional disorder is diagnosed if there is ongoing delusions and criterion A for schizophrenia is not met, brief psychotic disorder is diagnosed when psychotic symptoms are present for less than one month and psychosis NOS can be used when there are psychotic symptoms present, but a specific diagnosis cannot be made due to lacking or contradictory information.

*Aetiology and pathophysiology*

The aetiology and pathophysiology of schizophrenia is multifactorial and complex and the causes of the disorder are for the most part unknown. The dopamine hypothesis formulated by Van Rossum in the 1960s introduced disturbance of the dopamine signalling system as a possible mechanism (20). Leading to this hypothesis was the observation that the antipsychotic effect of chlorpromazine resulted from dopamine receptor antagonism (20), and that the antipsychotic effect of antipsychotic drugs correlates with dopamine D2 receptor affinity (21). Further, animal, post-mortem and PET studies have supported a role for dopamine in schizophrenia (22). However, a limitation to this hypothesis is that hyperactivity of the dopamine system is not alone sufficient to cause schizophrenia. For example, dopamine-antagonism does not seem to be effective in treating negative and cognitive symptoms, and clozapine, an atypical antipsychotic drug that is effective in patients lacking effect from other antipsychotics, have low dopamine D2 receptor affinity (22). Another important neurotransmitter indicated in schizophrenia is glutamate, the main excitatory neurotransmitter of the central nervous system (CNS). After observing the psychosis-inducing effects following phencyclidine-induced antagonism of the glutamate receptor, N-methyl-D-aspartate (NMDA), glutamate dysfunction was considered a potential mechanism in schizophrenia (23). Later, pre-clinical and post-mortem studies have supported a role for glutamate, and more recently enrichment of glutamate pathway genes in schizophrenia associated loci is discovered (24). The inhibitory neurotransmitter GABA and serotonin, a modulator of a broad spectrum of functions, is also suggested a role in schizophrenia pathophysiology (25, 26). Altogether these findings nuance the single neurotransmitter hypotheses and suggest a disturbed excitatory and inhibitory balance involving several neurotransmitters in schizophrenia (27).
Heritability estimates for schizophrenia are up to 80%, indicating that genetics have a central role in the pathophysiology of the disorder (9). The knowledge of schizophrenia genetic risk loci and their related biological pathways are continuously expanding, revealing information of potential underlying disease mechanisms. The most recent genome-wide association study (GWAS) of cases with schizophrenia and schizoaffective disorder found 270 disorder associated genetic loci, with the strongest signal coming from the human leukocyte antigen (HLA) locus on chromosome 6, which codes for the major histocompatibility complex (MHC) (28-30). Further, the dopamine D2 receptor gene (DRD2) region ranked high among schizophrenia related genes (31). Functional annotation and enrichment analyses of schizophrenia associated alleles indicates that the most important pathological factor is involved in neuronal development, structure, excitability and synaptic activity and that the affected neuronal functioning is linked with almost all brain regions, reflecting the diversity in clinical representation of schizophrenia (30). However, the current detected genetic variants explain little of the total estimated heritability and a significant amount of the total genetic picture in schizophrenia is still “missing” (32). It should also be mentioned that in addition to the contribution from common genetic variants, rare disrupting single nucleotide variants and copy number variants have also been discovered for schizophrenia, reflecting the genetic complexity (33, 34).

Importantly, there are many non-genetic factors that have been associated with development of schizophrenia. These include growing up in urban areas, belonging to an immigrant group versus being native-born, exposure to cannabis, childhood trauma, obstetric complications and high paternal age (9, 35-37), although the latter is discussed to be partly genetic through increased number of de novo DNA mutations (38). Further, morphological and biochemical characteristics associated with schizophrenia include lower grey matter volumes, larger ventricles, abnormal prefrontal dopamine synthesis (not in treatment resistant patients) and glutaminergic abnormalities (39-41). Finally, there are several indications of a central role for the immune system in schizophrenia, including associations with infections during pregnancy, number of lifetime hospital infections, autoimmune diseases and abnormal levels of peripheral immune markers (42-44).

6.3 Bipolar spectrum disorder

Bipolar disorder is an affective disorder characterized by abnormal fluctuations in the mood with the appearance of manic or hypomanic episodes and depressive episodes (45). Diagnostically, bipolar disorder is subclassified into two major subtypes; bipolar I disorder
and bipolar II disorder, and additionally a “not otherwise specified” (NOS) used when criteria for one of the two main subtypes are not met. The different subtypes each have a lifetime prevalence of between 0.4-1.4% and bipolar I disorder tend to affect men more often than woman, while bipolar II disorder tend to affect women more often than men (46). The clinical course is heterogeneous, but on a population level it is a severe and often chronic mental illness with a reduced life expectancy of 11 – 16 years (47, 48). Suicide risk is up to 30-fold increased, with attempts occurring primarily in depressed or mixed phases of the disorder (49, 50). Suicide risk in bipolar disorder seems to increase with male sex, living alone, being divorced and without children, unemployment, previous suicide attempts, comorbid mental and substance use disorders, recent affective episodes, and inpatient care (51). It is not clear whether there is a difference in risk between type I and type II (50). Another major cause of death in bipolar disorder is cardiovascular disease and an estimated one third of patients die from this (52). Other contributors to decreased life expectancy in bipolar disorder is diabetes mellitus, chronic obstructive pulmonary disease, injuries and respiratory infections (53). Like schizophrenia, bipolar disorder is one of the leading causes of disability in young people worldwide, with early age at onset and disabling symptoms (54), resulting in a large economic cost to society (55). Reduced neurocognitive functioning and socio-occupational difficulties leads to reduced quality of life (56, 57), and although symptoms appear episodic, reduced psychosocial functioning is also often present in euthymic phases (58, 59).

**Diagnosis and clinical characteristics**

A diagnosis of bipolar I disorder requires a history of or presence of mania, which is a similar criterion in DSM-IV and DSM-5 (5). Mania is defined as a distinct period of abnormally and persistently elevated, expansive or irritable mood and abnormally and persistently increased goal-directed activity or energy that is present for most of the day, nearly every day, for at least a 1-week period. During the period of mood disturbance, three (or more) of the following symptoms must have persisted (four if the mood is only irritable) and been present to a significant degree and represent a noticeable change from usual behavior: 1) inflated self-esteem or grandiosity, 2) decreased need for sleep, 3) more talkative than usual or pressure to keep talking, 4) flight of ideas or subjective experience that thoughts are racing, 5) distractibility, 6) increase in goal-directed activity or psychomotor agitation or 7) excessive involvement in pleasurable activities that have a high potential for painful consequences. The disturbance must be severe enough to cause marked impairment in social or occupational functioning, to necessitate hospitalization or include psychotic features. It must be excluded
that physiological effects of a substance or any other medical condition cause the episode. Bipolar II disorder is characterized by at least one hypomanic episode and one major depressive episode. Hypomania share characteristics with mania but is distinguished by potentially shorter duration (4 days), lack of psychosis and not being severe enough to cause social or occupational impairment due to the hypomanic episode itself. There have been some changes in the criteria for mania in DSM-5 versus DSM-IV. The most important is that criterion A now requires that the mood changes are accompanied by abnormally and persistently goal-directed behavior or energy. Major depressive disorder (MDD) is characterized by a clear reduction in mood, interest and pleasure, changes in cognitive functions and occurrence of vegetative symptoms lasting at least 2 weeks (60). MDD with psychotic features is diagnosed when mood disturbance fulfilling criteria for MDD is accompanied by psychotic symptoms such as delusions and hallucinations and might be conceptualized together with bipolar disorder (61, 62).

Aetiology and pathophysiology

The aetiology of bipolar disorder is not clear, and underlying biological mechanisms seem to be multifactorial and complex with differences in manic and depressive states (45, 63). There is evidence for hyperdopaminergia in bipolar mania, supported by both pharmacological and imaging studies (64), whereas for bipolar depression and in euthymic phases the role of dopamine is more uncertain. Decreased serotonin transmission is also suggested in mania (63). Changes in neuronal plasticity with involvement of the brain-derived neurotrophic factor (BDNF) might also be part of the pathophysiology as decreased BDNF is associated with depressive and manic episodes and with factors that negatively impact the course of bipolar disorder (65, 66). Further, there is evidence of elevated free intracellular calcium present in both bipolar mania and depression, suggesting a role for altered calcium functioning in bipolar disorder (67). Also, disturbances in circadian rhythm, supported by melatonin abnormalities and genetic signals, have been widely demonstrated and is suggested a role in bipolar disorder (68).

Bipolar disorder heritability is estimated to 60-85 % (69), suggesting a major role for genetics. The latest GWAS from the Psychiatric Genomics Consortium (PGC) bipolar disorder working group identified 64 genome-wide significant loci associated with bipolar disorder (70). Discoveries of genetic loci have provided fruitful information about bipolar disorder aetiology including insight to potential underlying biological mechanisms (70-72). One of the highlighted gene enrichments from the latest GWAS involves genes associated
with calcium channel blockers, building up under the suggested role for calcium signalling in the pathophysiology of bipolar disorder (70). Also, gene targets involving the immune system, neuronal and synaptic pathways, and targets of current main pharmacological agents in treatment of SMD such as antipsychotics and mood stabilizers were indicated (70, 73).

Non-genetic factors that have been associated with increased risk of bipolar disorder include maternal viral infections, lifetime hospital-treated infections and autoimmune disease, cannabis misuse and childhood maltreatment (74). Childhood maltreatment might lead to an earlier age of onset and a worsened clinical course with increased occurrence of psychotic features, number of episodes and suicide attempts (75). However, it seems that a variety of life events can predispose to mania or depression and interfere with onset age and clinical course (75).

There is robust evidence suggesting associations between bipolar disorder and different conditions that involves immune dysfunction (76, 77). As with schizophrenia, presence of autoimmune disease and infections requiring hospital admittance increases risk of bipolar disorder (78) and there is increased comorbidity with some autoimmune diseases (76). Also, there seem to be a bidirectional relationship between bipolar disorder and general medical conditions with low-grade inflammation, like cardiovascular disease and metabolic syndrome (77). Studies show that almost one third of bipolar disorder patients have some form of metabolic disturbance, including higher prevalence of obesity, higher triglyceride levels and lower HDL cholesterol levels (79). Moreover, robust evidence of alterations in different immune markers in bipolar disorder (80-82) support this suggested link between immune activation and mood disorders (83, 84).

6.4 Genetics of severe mental disorders

Genes and genetic risk

The human genetic material, deoxyribonucleic acid (DNA), consist of two polynucleotide chains made of nucleotide subunits in different arrangements, paired together in a double helix shape (85). A “gene” refers to a DNA sequence where the arrangement of nucleotides encodes a functional ribonucleic acid (RNA) or protein (86). The sequencing of the human genetic code was completed in 2003 and have since been the basis of molecular genetic research in many fields, including psychiatry. Humans have twenty-three pairs of chromosomes, twenty-two pairs of autosomes and one pair of sex chromosomes (XX for women and XY for men). Except for the sex chromosomes in men, humans have two copies
of all genes. The relative contribution to variance in a phenotype caused by genetic factors in a population is termed heritability (87). Heritability cannot be directly applied to the individual level; however, it can guide investigation and assessment of genetic risk, i.e., the genetic contribution to the chance of developing a certain illness. There are complex and dynamic gene-environmental interactions at play in the development of most illnesses (88). Mapping of genetic risk loci and subsequent functional annotation gives insight to underlying biological and pathophysiological mechanisms and might also indirectly help understanding the non-genetic contribution (89).

*Genome-wide association studies and polygenic risk scores*

GWAS is currently one of the most recognized approaches for the detection of genetic variants in complex disorders (90). GWAS investigates genotypes from thousands of people simultaneously, searching the genome for loci that are associated with a certain trait or affect the risk for a disease, making possible the detection of trait-associated common genetic risk variants (90). A GWAS is most often conducted as a case-control comparison of common genetic variation revealed by single nucleotide polymorphism (SNP) arrays (91). Determination of common risk loci and measurement of their effect makes possible the calculation of PRS, which represent the weighted sum of genetic variants associated with a phenotype carried by an individual (87). PRS can predict case-control status in SMD with high significance (92, 93) and be applied to investigate genetic overlap that might inform about relationships with potential disease-relevant phenotypes and comorbidities (94, 95). In more common pathologies like coronary heart disease and prostate cancer, PRS is proven useful as a supplementary tool in deciding on preventive treatment and help interpret screening results, respectively (96, 97). Although not yet clinically useful in SMDs, future GWASs with increasing samples are expected to explain more of the phenotypic variance, with future PRSs suggested to be of clinical use also in psychiatry.

*Genetic research in severe mental disorders*

The last decade has seen a tremendous progress in assessment of the genetic architecture of mental disorders (91). In SMD, most of the genetic contribution seems to be composed of several thousands of common genetic risk variants with each a very little associated risk effect. However, rare genetic variants with larger risk effects also confer some risk to SMD and can give important information about disease mechanisms (98, 99). The number of discovered genetic risk loci for schizophrenia and bipolar disorder increases, but the
established heritability from common risk variants explains only a few percent to one third of the total genetic liability (29, 70, 100, 101). PRS for schizophrenia and bipolar disorder is currently not suitable for individual genetic prediction in clinical psychiatry (102, 103). However, these scores seem to be able to identify subgroups within mental disorders and to inform about genetic relationships between different mental disorders and comorbid diseases or related characteristics such as personality traits (80, 95, 104). Efforts are also made to discover biological pathways associated with the detected genetic variation. Most of genetic variants linked with mental disorders are on non-protein coding regions (91); it is therefore a need to understand which genes are impacted by the respective variants through regulatory mechanisms, which might further inform about effects on biological pathways of importance for SMD. Current biological pathways and processes associated with schizophrenia and bipolar disorder through linkage with genetic variation include the immune system, synaptic plasticity, neurotransmitter receptors and calcium channel signalling (29, 70, 105).

**Genetic relationships between mental disorders**

Like schizophrenia and bipolar disorder, most mental disorders have substantial estimated heritability with a polygenic basis (106-108). Although most detected loci seem to be disease-specific, many loci increase risk for several mental disorders (91). Large studies have identified genetic associations including correlations and polygenic overlap between schizophrenia, bipolar disorder, major depressive disorder (MDD), anxiety disorders, autism spectrum disorders (ASD), post-traumatic stress disorder (PTSD) and attention deficit/hyperactivity disorder (ADHD) (109-111). Correspondingly, PRS for mental disorders are associated with increased risk of different other mental disorders (104, 110). Further, pathway analysis of genetic sharing have implicated calcium channel activity as a shared biological association between mental disorders (110). The genetic overlap discovered for mental disorders indicate central commonalities and suggest that many mental disorders are not separate entities, but might rather exist on a continuum (95).

**Genetic relationships with somatic and other related phenotypes**

In addition to the shared genetics with other mental disorders, SMD risk loci seem to be associated with different somatic conditions and SMD-related phenotypes. Potential genetic relationships like these might inform about underlying mechanisms of importance in SMD. Recent studies have identified shared genetic loci between SMD and body mass index (BMI) (112), loneliness, cardiovascular disease (113), autoimmune diseases (114-116), general
cognitive abilities (117) and personality traits (118, 119). It is estimated that genes account for a great amount of the variability in cognition and educational attainment (120, 121) and both phenotypes overlap genetically with SMD (122). Cognitive impairment is a core feature in SMD, with influence on functional outcome (123). Educational attainment correlates with cognition (124) and there seems to be a mixed directional genetic relationship between the two (124-126). Genetic links are also found between education and personality (127). Personality describes the combination of different recognisable behavioural patterns in humans and one of the most frequently used models for assessing personality is the five-factor model (128). This model categorises five different broad personality dimensions: openness to experience, neuroticism, extraversion, conscientiousness and agreeableness. There is polygenic overlap between schizophrenia and neuroticism, openness, agreeableness and extraversion, and shared genetic loci also shared between openness and bipolar disorder (118, 119). Clinically, bipolar disorder is associated with higher neuroticism and openness, and lower agreeableness, conscientiousness and extraversion (129). Schizophrenia is associated with higher neuroticism and lower levels of the remaining four dimensions as compared to healthy controls (130).

6.5 The immune system and severe mental disorders

Basic immunology

The human immune system is first and foremost known for its vital task of protecting the body from foreign and harmful organisms (131). Additionally, the immune system act against many other types of environmental stress and injury, has overly complex functions and is broadly involved in health and disease. The immune system can be divided into two main parts that are intimately connected: the innate and the adaptive immune system. The innate immune system acts in an unspecific manner with immediate reaction against pathogens (132). This is necessary awaiting an eventual response from the adaptive part of the immune system as producing enough lymphocytes can take up to 5 days. The innate immune system involves all bodily tissues and consists of macrophages, mast cells, neutrophils, eosinophils, basophils, dendritic cells and natural killer cells, as well as complement proteins (132, 133). Foreign organisms are recognized by their pathogen-associated molecular patterns (PAMPs), damage associated molecular patterns (DAMPs) or downregulated or altered expression of molecules normally presented by healthy cells (missing-self) (132). A key recognizing receptor is the Toll-like receptor (TLR) family, consisting of 10 different receptors altogether able to recognize most microbes (132). TLR allows for recognition and response, as well as
discrimination between, groups of microbial pathogens, further inducing the appropriate innate immune response (134). This initial recognition allows for activation of different cells and factors leading to production of messenger molecules such as cytokines. Cytokines are secreted proteins that via signalling in the local microenvironment or sometimes systemically initiate and regulate immune activity, cell growth, cell differentiation and trafficking (135). Chemokines, interleukins and interferons are different types of cytokines that can be further subdivided into groups depending on predominant area of involvement. Proinflammatory cytokines produced by the innate immune system then stimulate adaptive immune cell responses. Another mediator and marker crucial to the immune response is the cell adhesion molecule, as these are important for migration of immune cells from the blood stream to the tissue (136).

The adaptive part of the immune system consists of B (Bursa and Bone marrow derived) and T (Thymus derived) lymphocytes, further divided into subgroups of specialized lymphocytes (137). Upon recognition of antigens presented by different antigen-presenting cells or microbial surface proteins, patrolling lymphocytes get activated. One central group of antigen-presenting molecules are those encompassed by the MHC, divided in two main classes: class I and class II (138). Antigen presentation by these MHC molecules makes mature T lymphocytes evolve into different effector cells that can provide direct elimination of microbes, signal other B and T lymphocyte responses or activate mechanisms with regulatory effects on the immune response (137). B lymphocytes provide humoral immune response which means that they produce antibodies to pathogens (antigens) leading T lymphocytes to eliminate the pathogen. A first encounter to an antigen leads to a slow production of antibodies and memory T and B lymphocytes, whereas the next encounter with the same pathogen will then induce a quicker response with higher affinity (137). With a gigantic repertoire of lymphocytes with unique receptors due to immunologic memory, the adaptive system can produce pathogen-specific responses against the respective antigens upon stimulation signals.

**Immune system in CNS**

The immune privilege of the CNS, although once believed to be so, is not absolute (83). Complex mechanisms allow for regulated communication between CNS and the peripheral immune system; hence the brain can be affected by systemic inflammatory response and vice versa (139). A familiar consequence of peripheral cytokines acting on the CNS is the ‘sickness behaviour’, when we feel depressed, irritable, tired and even mildly cognitively
impaired during viral or bacterial infections (83). The blood-brain barrier (BBB) strictly regulates entry of immune cells and signalling molecules to the brain parenchyma (139) and lymphatic vessels in the dura mater drains to peripheral lymph nodes (140), together maintaining fluid homeostasis and immune system communication. Most likely there are several ways of which peripheral cytokines and PAMPs communicate with the brain and thus might play a role in sickness behaviour and development of SMD (83, 84, 141). Roughly described, these pathways include a cellular route through the recruitment of monocytes over the BBB into the brain, a neuronal route via afferent nerves and a humoral route involving the so-called circumventricular organs, which are areas that lack BBB (141-143). Communication from peripheral cytokines to the central nervous system will ultimately lead to activation of the microglia, which are the brain's own immune cells (84). Microglia can act out phagocytosis, antigen presentation and synapse modelling, as well as produce both pro-inflammatory and anti-inflammatory cytokines to regulate local inflammatory response (144). Microglia play a significant role for the developing brain and chronically activated microglia are hypothesized to be involved in SMD pathophysiology through interference with neurodevelopment (144). Evidence from both genetic, epidemiologic and clinical studies suggest a complicated relationship between the immune system and the CNS, including the involvement of inflammation in the pathophysiology of SMD (43, 145).

**Immune hypotheses in schizophrenia and bipolar spectrum disorder**

It is well established that blocking of the NMDA-receptor can cause symptoms resembling both positive and negative symptoms of psychosis and studies show that some patients with schizophrenia have decreased NMDA-receptor functioning (146). Thus, an immune-based NMDA-receptor hypofunction caused by attack from auto-antibodies is suggested to be a disease mechanism involved with schizophrenia (146). Related to this is the kynurenic acid hypothesis which builds on the finding that kynurenic acid, a NMDA-receptor antagonist, is elevated in the prefrontal cortex of schizophrenia patients (147). Elevated kynurenic acid comes from cytokine induced breakdown of tryptophan and can potentially influence both glutamate and dopaminergic neurotransmission, resulting in psychotic and cognitive symptoms (148). The kynurenic pathway is also suspected a role in bipolar disorder pathophysiology, potentially involving glutaminergic, immune and monoaminergic systems (149). Macrophage and T lymphocyte imbalance is hypothesised in both schizophrenia and bipolar disorder (28, 150). In schizophrenia, the macrophage T lymphocyte hypothesis builds on the observations that administration of IL-2 can induce schizophrenia-like symptoms in
people without mental disorders, suggesting that one of the basic biological mechanisms in schizophrenia is chronically activated macrophages failing to control T lymphocyte secretion of IL-2 and IL-2R (151). Similarly, a role for T-helper type 17 (Th17) cells have been suggested, derived from the fact that patients have altered number and functioning T lymphocytes (152) and that cytokines of the Th17 pathway correlates with symptom measures of psychosis (153).

Neuroinflammation and priming of microglia might play a part in SMD pathophysiology. BBB permeability seems to be increased by injury or from sustained release of inflammatory factors (154), leaving the brain more exposed from inflammatory factors induced by for example systemic infectious diseases and stressful events. Further, this can induce exacerbated microglial response to subsequent inflammatory events, leading to abnormal wiring of brain circuits with consequences for mood stability, behaviour and cognition (150, 155, 156). Complement component 4 (C4) was recently discovered and demonstrated to be a key driver of the association between schizophrenia and the MHC locus (157, 158). C4 can locate synapses, axons, dendrites and neuronal cell bodies, which makes it a potential mediator of synaptic pruning in humans with possible implications for SMD development through postnatal synaptic degradation (158).

Infections and severe mental disorder

A link between infectious diseases and psychosis was suspected already back in the early 1900, when ‘psychosis of influenza’ was a recognized phenomenon (159, 160). In later years this has been supported by epidemiologic (42, 78) and genetic (29, 70) studies, although we do not yet know the exact mechanisms underlying these potential relations. Maternal infections, particularly during, but also pre- and post-pregnancy, as well as paternal infections, have been associated with increased risk of schizophrenia (145, 161-163). The most studied infective agents include toxoplasma gondii (164), herpes virus type 2 (165), rubella (166), influenza virus (167, 168) and bacterial infections (169). There is evidence for associations between maternal influenza during gestation and bipolar disorder, although the role for maternal infections in bipolar disorder is a bit more uncertain (170, 171). Also, infections during childhood seems to increase risk of schizophrenia (172), and further, large registry studies of over 3.5 million individuals have shown a dose-dependent association between number of hospital-treated infections and risk for both schizophrenia and bipolar disorder (42, 78). The risk was increased with nearer proximity to the infection; however, significantly increased risk was true also for infections as old as fifteen years ahead of
diagnose. Furthermore, a large population-based study reported findings that suggest a synergistic effect between maternal mental disorder and gestational infections on risk of psychosis development in the offspring (163). Taken together, infectious conditions might have a role in SMD, however, inconsistencies in previous findings regarding directions and effect sizes remains to be clarified.

**Autoimmunity and severe mental disorders**

A weakened immune system is vulnerable to intruders and even mild infections can cause serious harm to a person with immune deficiency. On the contrary, autoimmune mechanisms, through over responding to minor threats or attacking the body’s own cells and organs without exterior threat, can cause damage locally or systemically. Autoimmunity occurs when elimination and/or control of self-reactive lymphocytes fails, leading the immune system to attack the body’s own cells and tissues, potentially resulting in damage and disease (173). Autoimmune diseases occur in 4.5% of the general population with a female overrepresentation and average age at onset at 40-50 years of age (174). There is great variability concerning organs affected, clinical manifestations and degree of severity, with over 80 different autoimmune diagnosis identified (174). Often, symptoms debut a long time after first occurrence of immune disturbance, thus it can be difficult to determine the initiating cause (173). Like SMD, autoimmune diseases are thought to have a complex pathophysiology including interplay between genetic and non-genetic factors.

Already in 1930 it was suggested that development of schizophrenia could result from autoantibodies attacking brain tissue (175) and later it was discovered that some autoimmune diseases, like Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), and particularly Autoimmune Encephalitis, can present with neuropsychiatric symptoms (176). There is a substantial degree of comorbidity between autoimmune diseases and SMD (177-179), and interestingly, autoimmune diseases with suspected brain-reactive antibodies increase risk of bipolar disorder and schizophrenia even more than other autoimmune diseases (42, 78). Subjects diagnosed with an autoimmune disease have higher risk of subsequent diagnose with SMD and vice versa (43, 179, 180), and presence of autoimmune disease seems to act synergistically with hospital-treated infections on both bipolar disorder and schizophrenia risk (42, 78). Bipolar disorder have repeatedly been associated with autoimmune thyroid disease and MS, but also ulcerative colitis, psoriasis, rheumatoid arthritis (RA), Guillain-Barré syndrome and autoimmune hepatitis seems to increase risk for the disorder (181). Patients with schizophrenia have an increased risk of comorbid psoriasis, pernicious anaemia, Graves
diseases, celiac disease and hypersensitivity vasculitis (177), and there is an increased prevalence of thyrotoxicosis, celiac disease, autoimmune haemolytic anaemia and Sjogren’s syndrome prior to a diagnose of schizophrenia (179). A much-discussed observation is the repeated finding of a negative relationship between RA and schizophrenia, which is at odds with the positive direction of most autoimmune-SMD associations (182). The underlying mechanisms explaining this relationship remain to be completely understood, however, genetic factors and common biological pathways are suggested (183, 184).

*Genetic evidence for immune system involvement in severe mental disorders*

The suggested role for immune mechanisms in SMD is supported by immune signals from common genetic risk variants (29, 70, 116, 180), of which the MHC locus have gained particular attention in schizophrenia (28, 185) and more recently in bipolar disorder (70, 186, 187). The MHC is a gene dense region at chromosome 6 containing the HLA gene cluster. HLA is crucial for the inflammatory process in which HLA-mediated expression of different peptides on the cell surface can induce, regulate and select appropriate immune response (188). HLA genes are associated with autoimmune disease (189), susceptibility to infections (190) and other mental disorders (187). Also, several non-MHC immune related loci have been associated with schizophrenia, among these are genes expressed in B and T lymphocytes (116). Some of these non-MHC immune candidate gene regions have also been associated with bipolar disorder and cognitive abilities (116). Studies have shown that risk for mental disorder in the offspring is associated with infections occurring both during and outside of the pregnancy period, and with infections in both the mother and father, which could suggest shared genetic susceptibility to infections and mental disorders (145, 162). Moreover, there is robust evidence for genetic correlation between mental disorders, including bipolar disorder and schizophrenia, and immune-related phenotypes (115). Regarding the role of PRS, findings are more mixed. PRS for schizophrenia seem to predict risk for some autoimmune diseases (43), while no such association was found between PRS for schizophrenia and risk of serious infection (191).

*Low-grade inflammation and immune markers in severe mental disorders*

Repeated findings of abnormal levels of peripheral immune markers in SMD indicate a low-grade inflammation and support a role for the immune system in these disorders (44, 76, 192). Cluster analyses indicates shared inflammatory changes between related mental disorders (192). C-reactive protein (CRP), a marker for general inflammation, have been robustly
positively associated with SMD, indicating general inflammation in disease mechanisms (193). There is evidence for some similarities in immune marker patterns for schizophrenia and bipolar disorder, with receptor antagonist of interleukin-1 (IL-1Ra), soluble interleukin-2 receptor (sIL-2R), tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) found elevated in acutely ill patients with schizophrenia and bipolar disorder, and interleukin-1 beta (IL-1β) and sIL-2R found elevated in chronic schizophrenia and euthymic bipolar disorder (194). Other markers that have been repeatedly found elevated in schizophrenia are soluble receptor 1 of TNF-α (sTNFR-1), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-18 (IL-18), interferon gamma (IFN-γ) and transforming growth factor beta (TGF-β) (44). Soluble glycoprotein 130 (sgp130) and a proliferation-inducing ligand (APRIL) are found decreased in both patients with schizophrenia and bipolar disorder compared with healthy controls (195, 196). Additionally, sTNFR-1, sTNFR-2, TNF-α, IL-2, interleukin-4 (IL-4), IL-10, IL-18 and interleukin-33 (IL-33) are found elevated in bipolar disorder, some of which seem to vary with affective symptom state (197-201). Cell adhesion molecules, and particularly intercellular adhesion molecule 1 (ICAM-1), have been associated with both schizophrenia and bipolar disorder, possibly linked to medication (44, 202, 203). Based on their solid associations with schizophrenia and bipolar disorder, we chose the following seven markers for further investigation in the current thesis: IL-1Ra, sIL-2R, IL-18, sgp130, sTNFR-1, APRIL and ICAM-1.

The **IL-1 family** include a large collection of cytokines and receptors with important functions primarily associated with innate immunity (204). Two of the cytokines included in the current thesis, IL-1Ra and IL-18, are both part of the IL-1 family. IL-1Ra block IL-1α and IL-1β action by binding to IL-1R1, a similar mechanism to that of Anakinra, a recombinant IL-1Ra drug used to treat autoimmune diseases such as RA (205). IL-1Ra have been linked with schizophrenia through associations with symptom severity (206) and IL-1Ra gene polymorphism (207). IL-18 is vital in the T-helper type 1 (Th1) response and has been found increased in both schizophrenia and bipolar disorder (201). IL-18 synthesized in the CNS is thought to influence brain development, function and degeneration (208), further supporting a role in SMD.

**Gp130** is an IL-6 co-receptor of which all IL-6 cytokines transduce their signals (209, 210). IL-6 is a major pro-inflammatory cytokine with mediating effects on neural activities as well as in general inflammation (211). The soluble form of gp130 (sgp130) circulate at relatively high levels and since it reflects activation of several IL-6 cytokines it can be considered as a
marker for IL-6-involved inflammatory activity (209). Abnormal levels of IL-6 and sgp130 are linked with several pathological conditions including schizophrenia and bipolar disorder (194, 212-214). Further, it seems that levels of IL-6 might be linked with states of disorder and subgroups of patients, with decreased levels during treatment (194) and associations with cannabis use in schizophrenia (215).

The *TNF* and *TNF-R* superfamily encompass almost 30 different ligands and receptors with important roles for modulation of cellular functions and involvement in both innate and adaptive immunity (216). TNF-α is a major pro-inflammatory cytokine involved in several processes, including adherence of circulating leukocytes to endothelial cells and fever-induction by hypothalamic centres (217). TNFR-1 is one of the two receptors of TNF-α, distributed in a range of cells and tissues with main functions in cell death and inflammation. The TNF-family have been associated with autoimmune diseases (217) and mental disorders (218), with indications of enhanced activity in TNF pathways in both bipolar disorder and schizophrenia, also related to symptom severity (219).

*APRIL* is a member of the TNF-superfamily produced by myeloid cells and with a particular role in B lymphocyte maturation and functioning (216). APRIL have been associated with different autoimmune diseases, for example it is found decreased in SLE patients with CNS affection suggesting involvement in CNS pathology (220). Recently, lower levels of APRIL were found significantly associated with both bipolar disorder and schizophrenia (195).

*sIL-2R*, a stable and reliable marker of T lymphocyte activation, is found increased in both patients with bipolar disorder and schizophrenia (44, 221). sIL-2R is elevated in a range of autoimmune diseases, particularly those with T lymphocyte involvement such as MS, inflammatory bowel disease (IBD) and primary biliary cirrhosis (222-224), of which all have been associated with SMD. sIL-2R is also suggested to be involved in the T lymphocyte macrophage theory of schizophrenia (151) and more recently the compensatory immune-regulatory reflex system (CIRS) implicated in both schizophrenia and bipolar disorder (225, 226).

*ICAM-1* have been robustly associated with SMD (44, 202, 203). This cell adhesion molecule facilitates leukocyte migration through the endothelium (227) and is important for BBB integrity by regulating movement of molecules into and out of the CNS (203). ICAM-1 levels are low in healthy individuals and upregulated in many different pathologies, such as cardiovascular disease, cancer and autoimmune conditions (203, 227, 228). Due to its key
function in regulatory mechanisms in BBB and overexpression during inflammation, ICAM-1 is of interest in mental disorder research and proposed both as a possible trait and state marker in schizophrenia and bipolar disorder, potentially with links to medication (203).

6.6 Antipsychotic drug treatment

Antipsychotic drugs are the main psychopharmacological treatment for schizophrenia, and together with lithium and antiepileptics they are also important in the treatment of bipolar disorder (229, 230). The current selection of antipsychotic drugs consists of a variety of types with different biochemical characteristics and mechanisms of actions through binding to multiple classes of neurotransmitter receptors including adrenergic, cholinergic, dopaminergic, histaminergic, and serotoninergic receptors (231). However, they all seem to share the ability to act on central dopamine D2 receptors, which is considered the key mechanism of action responsible for the antipsychotic effect on positive symptoms (232). Traditionally, antipsychotics are classified as either first- or second-generation. First-generation antipsychotics have increased risk of serious and troublesome side effects from their effect on the extrapyramidal system, such as motor restlessness, tremor and irregular movements (231). Second-generation antipsychotics impose lesser risk for extrapyramidal side effects, but often produce weight gain and sedation (233). There are only minimal differences between the drugs in terms of efficacy on overall favourable symptom change in schizophrenia, however, clozapine seems to be the most effective of all (233). In patients with bipolar disorder, antipsychotic drugs might be more potent than lithium and antiepileptics in treatment of acute mania, but the long-term role for antipsychotic treatment in bipolar disorder is less certain (229).

Treatment resistance

About one third of patients with schizophrenia do not experience sufficient effect from antipsychotic drug treatment (234), being so called treatment-resistant (TR). Recent findings suggest TR patients might belong to a categorically distinct subgroup with different pathophysiology (235). Imaging studies show differences in brain morphology, with grey matter reduction in many cortex areas in patients with TR compared to both patients with schizophrenia responding to treatment and healthy controls (236). Also, dopamine synthesis capacity is found to be lower in TR, alike that in healthy controls (39). Further, brain glutamate levels have been found to be higher in TR individuals compared to treatment-responsive patients (237). Also, there are clinical and demographic factors associated with TR
in schizophrenia, including living in less urban areas, poorer premorbid social adjustment, drug abuse and earlier age at onset (238, 239).

Three elements are considered as key in defining the concept of TR: (1) the correct diagnosis has been made, (2) adequate treatment has been given and (3) there has been inadequate response to this antipsychotic treatment (240). However, there are currently several different guidelines applied in TR research with considerable variation in how they define TR (240). Following the consensus criteria made by Treatment Response and Resistance in Psychosis working group (TRRIP), inadequate response to antipsychotic treatment in schizophrenia is defined as having minimal or no improvement in symptoms and functioning after appliance of at least two different antipsychotics with minimum dose equivalent 600 mg chlorpromazine and at least 80% of doses taken, over a period of at least six weeks with each drug (240).

Adding to the burden of schizophrenia in general, individuals with TR seem to have even more impaired functioning, poorer psychosocial adjustment, higher rates of hospitalization and represent a higher cost to society relative to antipsychotic responsive patients (235, 241, 242). Further, the trial-and-error testing of medication results in troublesome side-effects (243). Currently, clozapine is the only antipsychotic drug licensed for TR monotherapy (244). Relatives of patients with TR are more often diagnosed with schizophrenia (245, 246), suggesting genetic susceptibility for schizophrenia might be implicated in TR. However, research investigating associations between PRS for schizophrenia and TR have heterogenous and mixed results. Two studies indicated associations between PRS for schizophrenia and a history of clozapine treatment (247) and lack of response to antipsychotics (248), while three other studies did not find support for this (239, 249, 250).

6.7 Knowledge gaps

The evidence that supports central roles for genetic factors and immune mechanisms involved with SMD is substantial. Larger GWAS sample sizes enable identification of an increasing number of genetic risk variants and annotation studies indicates possible biological pathways. Comorbidity with autoimmune disease, increased risk from previous infections and altered levels of peripheral immune markers are replicated findings. Still, little is understood and established concerning causality, exact underlying biological mechanisms and clinical implications. We do not know what causes low-grade inflammation in SMD or whether genetic factors, autoimmune mechanisms or previous infections play a part in this. First, we do not know to what extent polygenic risk of schizophrenia or bipolar disorder is associated with or can explain parts of this immune activation. Further, exploring potential roles for PRS
for autoimmune diseases and SMD-related phenotypes in low-grade inflammation in SMD could provide fruitful information. Second, more evidence is needed to support immune hypotheses in SMD including the suggested potential links between previous infections and autoimmunity and immune marker abnormalities. Finally, based on the opportunities from the large genetic studies, we are now able to investigate the potential role of genetics of SMD in key clinical areas such as treatment response and prognosis. TR is a major issue in schizophrenia; thus, studies are needed to identify whether the genetic predisposition play a role in treatment response, and ultimately whether this can inform about underlying mechanisms to help improve treatment.
7 AIMS OF CURRENT THESIS

The main aim was to explore possible mechanisms of 1) immune abnormalities in bipolar disorder and schizophrenia and 2) pharmacological TR in schizophrenia. To achieve this, we tested associations between PRS for SMD and immune abnormalities and pharmacological TR, and for immune abnormalities we also tested associations with PRS for autoimmune diseases, PRS for SMD-related mental phenotypes with immune associations, and history of infections and autoimmune disease.

Aim 1

Investigate whether low-grade systemic inflammation in bipolar disorder and schizophrenia is associated with PRS for schizophrenia or bipolar disorder, previous infections and presence of autoimmune disease.

Hypothesis: Systemic immune abnormalities in SMD are associated with higher genetic risk for the respective disorders, higher number of infections and presence of autoimmune diseases.

Genetic data of large international consortia have recently made possible calculation of PRS for schizophrenia and bipolar disorder, explaining some of the genetic variance of these disorders (29, 70). There is substantial genetic evidence for immunological mechanisms in SMD (116, 251) in line with findings of altered inflammatory markers (44, 194). Additionally, infections and autoimmune diseases seems to increase risk of SMD (42, 78). We analysed PRS, infections and autoimmune diseases (registry-based) together with relevant immune markers in patients with schizophrenia spectrum disorders (N = 732) or bipolar spectrum disorders (N = 460) and healthy controls (N = 938), adjusting for confounders, to examine whether immune marker abnormalities in SMD can be explained by genetic susceptibility, infections and autoimmunity.

Aim 2

Investigate whether low-grade systemic inflammation in bipolar disorder and schizophrenia is associated with PRS for autoimmune diseases, PRS for CRP and PRS for SMD-related mental phenotypes with immune association, including other mental disorders, personality traits and cognition.

Hypothesis: Systemic immune abnormalities are associated with higher PRS for autoimmune disease, CRP and SMD-related mental phenotypes with immune associations.
There is evidence of genetic overlap between SMD and cognitive traits, personality and various mental phenotypes (109, 117, 119) with immune links (252-256). We analysed PRS for autoimmune diseases, CRP, mental disorders, personality traits and cognitive abilities together with plasma immune markers in SMD (N = 1004) and healthy controls (N = 947), adjusting for relevant demographic and clinical variables, to examine whether increased genetic susceptibility of these phenotypes were associated with immune abnormalities in SMD.

**Aim 3**

Investigate whether genetic susceptibility for schizophrenia is related to pharmacological TR in schizophrenia.

Hypothesis: Higher PRS for schizophrenia is associated with TR to antipsychotics.

TR to antipsychotics is a major issue in schizophrenia (235). Patients experiencing TR may represent a specific subgroup of the disorder related to genetic factors (235). We used consensus-defined criteria to determine TR in individuals with schizophrenia spectrum disorders (N = 321) and investigated possible associations with PRS for schizophrenia. Analyses were adjusted for relevant confounders and patients with first time illness were excluded as being in the initial phase of exploring treatment regime. Additionally, we tested associations between TR and other variables related to risk of developing schizophrenia, such as winter birth. Finally, we investigated the potential role of dose/serum ratio of antipsychotics.
8 MATERIALS AND METHODS

8.1 Study setting

The current thesis is a part of the Thematically Organized Psychosis study (TOP) at the Norwegian Centre for Mental Disorders Research (NORMENT), a Centre of Excellence established in 2013. The main goal for NORMENT is to find answers to why some people develop SMD. TOP focuses on SMD and includes patients with schizophrenia spectrum or bipolar spectrum disorders from the major hospitals in the Oslo region, and healthy controls matched by age from the same catchment area. As of the entrance of 2022 over 1900 patients and almost 1250 healthy controls were included in the TOP study.

8.2 Ethics

All participants received a written and oral description of the study, before giving a written informed consent to participate. All studies were approved by The Regional Ethics Committee and The Norwegian Data Inspectorate, and the Norwegian Directorate of Health approved the biobank.

8.3 Participants

In the current thesis the following diagnoses were included as schizophrenia spectrum disorders (‘SCZ’): schizophrenia, schizoaffective disorder, schizophreniform disorder, brief psychotic disorder, delusional disorder and psychosis not otherwise specified (psychosis NOS), and as bipolar spectrum disorders (‘BD’): bipolar I disorder and bipolar II disorder, bipolar disorder not otherwise specified (bipolar disorder NOS) and depressive disorder with psychotic symptoms. Patients were referred from in- and outpatient mental health clinics, and healthy controls (HC) were randomly selected from statistical records in the same geographical catchment area as patients. The participants were between 18 and 65 years old at inclusion, and both patients with first time episode and multiple episodes were included in paper I and II, while first time episode patients were excluded from paper III. Competency to give informed consent was a prerequisite to participate, and individuals with a severe somatic illness, head trauma or IQ below 70 were excluded to increase specificity of diagnostics and symptom measures. HCs were not included if they or a close family member had been diagnosed with a SMD. All participants must master a Scandinavian language. Most often patients were referred from their therapist, but sometimes from their general practitioner. Participation is entirely voluntary and may be withdrawn at any time without further
questioning. The inclusion process is continuously on-going, enabling a large and growing database that can be used by different scientists for different sub-studies. The research protocol is comprehensive, including semi-structured interviews and self-report questionnaires for assessment of demographic and clinical data. Additionally, participants undergo a neurocognitive assessment, blood sampling and magnetic resonance investigation (MRI) and electroencephalogram (EEG). Demographic and clinical data, peripheral immune marker measure from blood plasma and genetic data obtained from blood and saliva samples were used in the current thesis.

8.4 Clinical assessment

Diagnostics were made by using the Structured Clinical Interview (SCID-1) (257) for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (6). Diagnostic interviews were performed by psychologists and physicians supervised by a senior professor in psychiatry comprehensively trained for the interviews based on a UCLA training program (258). The diagnostic reliability has been found to be very good, with Cohen’s kappa for diagnosis ranging between 0.92 and 0.99 (259). Symptoms of psychosis were assessed using the Positive and Negative Syndrome Scale (PANSS) (260) and function was assessed using the Global Assessment of Functioning (GAF-F) scale (261). Previous psychiatric history was recorded based on interviews and medical records.

8.5 Polygenic risk score

DNA was extracted from blood and saliva samples collected in the clinic. Genotyping was performed on Human Omni Express-24 v.1.1 (Illumina Inc., San Diego, CA, USA) and deCODE Genetics (Reykjavik, Iceland). The following describes how polygenic risk scores were calculated by the biostatistical group at NORMENT.

Pre-imputation quality control was performed using PLINK 1.9 (262). Briefly, variants were excluded if they had low coverage (95%), had low minor allele frequency (MAF) (<0.01), deviated from Hardy-Weinberg equilibrium (p<10^-4), or occurred at significantly different frequencies in different genotyping batches (FDR<0.5). Whole individual genotypes were excluded if they had low coverage (<80-95%) or high likelihood of contamination (heterozygosity above mean + 5 standard deviations). Samples with mismatch between genetic and reported sex were excluded. MaCH software (263, 264) was used to impute the genotypes of all participants onto reference haplotypes derived from haplotype reference consortium (HRC) trans-ethnic reference panel (version 1.1) (265) in paper I and II, and
European ancestry in the 1000 Genome Project (genomic build GRCh37) in paper III. The summary statistics were quality controlled by removing variants that met any of the following conditions: MAF<0.01 (paper I and II) MAF <0.05 (paper III); imputation quality (ratio between observed and expected allelic variance) < 0.8; not present in more than half of the sub-studies. The high linkage disequilibrium (LD)-regions MHC region (chr6:25119106–33854733) and 8p23.1 (chr8:7200000–12500000) were excluded for computation of PRS for SCZ and BD. PRSs were computed following the method described by Purcell et al. (92, 266) using PRSice-2 (267) with default clumping parameters (250kb clumping window, 0.1 LD r2 threshold using target sample for LD estimation) for the PRSs in paper I and II. In paper III, variants surviving quality control were separately clumped into independent regions on the basis of the LD structure of the 1000 Genomes Phase III European population, PLINK v1.9 was used with the following parameters: –clump-p1 1.0 –clump-p2 1.0 –clump-r2 0.2 – clump-kb 500 and the allelic dosage coefficients (or logarithms of the odds ratios) of the variants with minimum p-values from all independent regions were used in constructing the PRS for SCZ.

In paper I and III, we calculated PRS for SCZ, ‘PRS-SCZ’, based on the newest meta-analysis of all Schizophrenia Working Group of the Psychiatric Genomics Consortium’s genome-wide association sub-studies except TOP cohorts (29, 30), with participants diagnosed with schizophrenia and schizoaffective disorder. Paper I was conducted after paper III, therefore the GWAS used in paper I is newer than in paper III. In paper I, we calculated PRS for bipolar disorder, ‘PRS-BD’, based on the latest meta-analysis except the TOP cohorts, with participants meeting international consensus criteria (DSM-IV, ICD-9, or ICD-10) for a lifetime diagnosis of bipolar disorder (70). In paper II, we calculated PRS using GWAS summary statistics for the following phenotypes: 1) the autoimmune diseases rheumatoid arthritis (PRS-RA) (268), systemic lupus erythematosus (PRS-SLE) (269), inflammatory bowel disease (PRS-IBD) (270), psoriasis (PRS-PSOR) (271), celiac disease (PRS-CD) (272), type 1 diabetes mellitus (PRS-T1D) (273), as well as C-reactive protein (PRS-CRP) (274), 2) the mental disorders major depressive disorder (PRS-MDD) (108), anxiety disorders (PRS-ANX) (107), autism spectrum disorders (PRS-ASD) (275), attention-deficit/hyperactivity disorder (PRS-ADHD) (106), post-traumatic stress disorder (PRS-PTSD) (276), 3) the cognitive traits general intelligence (PRS-COG) (277) and the cognitive ability proxy educational attainment (PRS-EA) (121) and 4) the personality traits neuroticism (PRS-NEURO), openness to experience (PRS-OPEN) and extraversion (PRS-EXTRA) (118). PRSs
were computed using a p-value threshold of 0.05 for selection of SNPs in the immune marker analyses in paper I and II, while for the TR analyses in paper III six different thresholds were tested. PRSs were standardised before analysis.

8.6 Registry data

In paper I we used registry data of infections and autoimmune diseases from 2006 (earliest available registry data) until 2018 from the Norwegian Patient Registry (NPR) and Norway Control and Payment of Health Reimbursement (KUHR) database. These registries cover all diagnosis from both primary (KUHR) and specialist health care (NPR) for all Norwegian citizens.

Rate of infections, i.e. number of infections from the 12-year period 2006-2018 retrieved from NPR, included the following diagnosis; all diagnoses from the general infection chapters A and B, except B20-24 (human immunodeficiency virus and acquired immunodeficiency syndrome), further, inflammatory diseases of the CNS (G00-G08), hordeolum and chalazion (H00), keratitis (H16), suppurative and unspecified otitis media, otitis media in diseases classified elsewhere (H66-67), acute rheumatic fever (I00-I02), acute pericarditis (I30), phlebitis and thrombophlebitis (I80), acute upper respiratory infections, influenza and pneumonia, other acute lower respiratory infections (J00-J22), chronic sinusitis (J32), peritonsillar abscess (J36), suppurative and necrotic conditions of lower respiratory tract (J85-86), acute appendicitis (K35), unspecified appendicitis (K37), abscess of anal and rectal regions (K61), peritonitis (K65), cholecystitis (K81), infections of the skin and subcutaneous tissues (L00-L08), infectious arthropathies (M00-M03), acute tubule-interstitial nephritis (N10), cystitis (N30), urethritis and urethral syndrome (N34), inflammatory diseases of prostate (N41), orchitis and epididymitis (N45), inflammatory disorders of the breast (N61), salpingitis and oophoritis, inflammatory disease of uterus, except cervix, inflammatory disease of cervix uteri, other female pelvic inflammatory disease (N70-73), infections of genitourinary tract in pregnancy (O23), puerperal sepsis, other puerperal infections (O85-86), infections of breast associated with childbirth (O91) and maternal infectious and parasitic diseases classifiable elsewhere but complicating pregnancy, childbirth and the puerperium (O98). From KUHR, general infections (A70-78), lymphadenitis (B70), gastrointestinal infections (D70-73), appendicitis (D88), worms/other parasites (D96), infections conjunctivitis (F70), blepharitis/style/chalazion, eye infection (F72-73), trachoma (F86), ear infections (H70-74), infection of circulatory system (K70), infections of musculoskeletal system (L70), neurological infections (N70-73), respiratory infections (R71-78, R80-83), skin
infections (S70-73, S76, S84), endocrine infections (T70), urological infections (U70-72), puerperal infections and infections complicating pregnancy (W70-71, W94), female genital infections (X70-74, X84, X90-92) and male genital infections (Y70-75) were included as episodes of infection. Equivalent diagnoses overlapping in time between the registers were registered as the same episode. Repeated diagnosis of infections was coded as the same or different episodes using all available information including length of interval between diagnoses. If not possible to determine otherwise, infections were coded as separate diagnosis if more than two months apart; however, if the same diagnose was registered repeatedly with a constant time interval they were considered checkups and coded as one infection episode.

Presence of autoimmune diseases was retrieved from KUHR and NPR for the 2006-2018 period for all groups as an estimate of differences prior to immune assessments; that is, autoimmune disease was coded as present or absent for each participant and used as a dichotomous variable. From NPR, sarcoidosis (D86), thyrotoxicosis (E05), type 1 diabetes mellitus (E10), multiple sclerosis (G35), other demyelinating diseases of CNS (G37), iridocyclitis (H20), optic neuritis (H46), Crohn’s disease and ulcerative colitis (K50-51), intestinal malabsorption (K90), psoriasis (L40), alopecia areata (L63), vitiligo (L80), lupus erythematosus (L93), seropositive rheumatoid arthritis (M05), juvenile arthritis (M08), Sicca syndrome (M35) and ankylosing spondylitis (M45) were included as diagnoses of autoimmune disease. From KUHR, chronic enteritis/ulcerative colitis (D94), rheumatoid/seropositive arthritis (L88), multiple sclerosis (N86), psoriasis (S91) and insulin dependent diabetes (T89) were included as diagnoses of autoimmune disease.

8.7 Blood sampling and immune measurements

Blood was withdrawn from the antecubital vein in the morning within two weeks of symptom assessment. A majority of participants were fasting (N = 67%) and did not take morning medication prior to blood drawing. Following the same methods as described in Mørch et al. 2019 (278) plasma levels of the immune markers were measured in duplicate by enzyme immunoassays (EIA) by using commercially available antibodies (R&D Systems, Minneapolis, MN, USA) in a 384 format using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT, USA) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an Enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad, Hercules, CA, USA). Intra- and inter-assay coefficients of variation were <10% for all EIAs. For immunoassays, blood was drawn using EDTA vials and the plasma was isolated within the next working day and stored at −80 °C. Blood
sampling was performed between 8:00 and 17:00 hours with some variations between patients and HC. The immune markers included in the current thesis (sIL-2R, IL-1Ra, IL-18, sgp130, APRIL, sTNFR-1 and ICAM-1) were selected based on previous association with SCZ, BD or neuropsychiatric symptomatology. Patient vs HC differences of these immune markers are described more thoroughly in other studies from the centre (195, 201, 202, 279).

8.8 Classification of treatment resistant (TR) schizophrenia

In paper III, patients with SCZ were classified as being TR or non-TR. The TR classification was adapted for retrospective data based on the TRRIP working group's consensus criteria (240). A patient was defined as being TR from data of the five years prior to inclusion (available data) based on either 1) history of treatment with clozapine or 2) two or more failed trials of antipsychotic treatment, each of at least six weeks duration and with therapeutic dosage. At least one of the antipsychotics had to be a second-generation antipsychotic. Previous trials were classified as failed if there were changes in antipsychotic agents. The current trial was classified as failed if the patient had significant symptoms (a few patients had frequent psychotic episodes [five or more during the five years] and were thus classified as TR regardless of current symptoms). Current significant symptoms were defined as at least one score of at least moderate severity on the PANSS positive subscale together with a score of 60 or less on the GAF functioning scale (GAF-F), indicating at least moderately impaired functioning (240). The antipsychotic treatment was not counted as a failed trial if the medication was stopped due to adverse effects. Antipsychotic drugs used for indications other than psychosis, typically as needed for sleep or anxiety, did not count as a trial of antipsychotic treatment. Patients having used a third, or more, antipsychotic drugs during the last five years after the two initial trials, were classified as TR regardless of ongoing symptoms. We did not apply any limitation on the maximum duration of an antipsychotic trial. Based on the criteria, both patients currently in remission and patients with psychotic symptoms could be classified as TR. Patients not fulfilling the criteria for TR were classified as non-TR.

8.9 Assessment of serum levels of medication

Antipsychotic drug serum levels were assessed by methods previously described in Steen et al., 2017 (280). Standardised relationships between dose and serum level of antipsychotics were calculated to enable the comparison of several antipsychotic drugs: First, the dose of each participant's primary antipsychotic drug was divided by the Defined Daily Dose (DDD) (281) of the drug to obtain a standardised dose for each participant. Second, the measured
serum level of this antipsychotic drug was divided by the median of the reference range for this antipsychotic drug (282). Finally, the standardised dose was divided by the standardised serum level, to obtain a relationship between dose and serum level comparable across antipsychotics indicating the turnover of the antipsychotic drug.

8.10 Statistics
All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago II, version 24 and 26). To investigate differences in demographic and clinical variables we used chi-square tests for the categorical variables, independent sample t-test for normally distributed continuous variables and Mann Whitney U or Kruskal Wallis test for non-parametric distributions. We used histograms and Q-Q plots to carefully inspect distributions of residuals. To meet assumptions for normal distribution, all immune markers were logarithmically transformed and, depending on the degree of deviation, residuals outlying more than 3 x interquartile range (sgp130, STNFR-1, IL-18 and ICAM-1) or 1.5 x interquartile range (IL-1Ra, sIL-2R and APRIL) above or below the first and third quartile were removed.

In paper I and II, we first analysed group differences in immune markers, PRS, infection rate and presence of autoimmune disease. Secondly, bivariate correlation analyses were performed to identify variables for adjusted main analyses together with immune markers. Correlations at significance level of $p \leq 0.1$ were selected for main analyses. Main analyses were performed using analysis of covariance (ANCOVA) with the immune markers as dependent variable, adjusting for diagnosis variable, sex, age at immune marker assessment and freezer storage time, and additionally ethnicity (paper I), genetic principal component 1 and 2 and genotyping batch when investigating PRS. Number of infections, autoimmune disease and the different PRSs were investigated one at a time as independent variables, and analyses was performed with and without these variables to assess the effect of the respective variable of interest on immune marker level difference between patients (SMD, SCZ or BD) and HC. In paper III, to assess the association between PRS-SCZ and TR, we used binary logistic regression. Patients were coded as either TR or non-TR and this binary variable was set as the dependent variable. PRS-SCZ was set as the predictor variable with adjustments for ancestry (genetic principal components 1-12) and for genotyping batch in addition to age at inclusion and sex. To avoid confounding from population stratification we adjusted for ethnicity in paper I and included only European subjects in paper II and III.
We corrected for multiple testing in all three papers. In *paper I* and *II* we applied a moderate correction of the significance level of $p = 0.025$ (0.05/2) and $p = 0.0125$ (0.05/4), respectively, to correct for the testing of a genetic and non-genetic model (*paper I*) and for the testing of PRS for the four groups autoimmune diseases, mental disorders, cognitive traits and personality traits (*paper II*). In *paper III* we applied a stricter Bonferroni correction of the significance level of $p = 0.01$ (0.05/5) due to the testing of five different SNP significance thresholds.
9 RESULTS

9.1 Paper I

In paper I we investigated whether PRS-SCZ or PRS-BD, previous infections and autoimmune disease and could explain immune marker level abnormalities in SMD. We found that infection rate differed between all groups (BD>HC>SCZ, all p<0.001) whereas autoimmune disease was more frequent in BD compared to SCZ (p = 0.004) and HC (p = 0.003). The main findings were a positive association between presence of autoimmune disease and sIL-2R (p = 0.001) and negative association between PRS-SCZ and sIL-2R (p = 0.006) across SCZ and HC; however, associations represented only minor changes to the difference in plasma levels between SCZ and HC. Overall, this study provided little evidence for a role for PRS-SCZ and PRS-BD, previous infections and autoimmune disease in immune activation in SMD and suggest that this might mainly be explained by other factors.

9.2 Paper II

In paper II we tested whether PRS for autoimmune diseases and SMD-related mental phenotypes with immune associations could explain immune marker level abnormalities in SMD. We found significant negative associations between PRS-EA and IL-1Ra (p = 0.01) and IL-18 (p = 0.01), nominal positive associations between PRS-PSOR and sgp130 (p = 0.02) and PRS-ANX and IL-18 (p = 0.03), and nominal negative associations between PRS-ANX and sIL-2R (p = 0.02) and PRS-EA and sIL-2R (p = 0.03). Associations explained minor amounts of the immune marker plasma level differences between SMD and HC. Different PRS and immune marker associations in the SMD group compared to HC were shown for PRS-EXTRA and IL-1Ra (interaction effect (IE), p = 0.002), and nominally for PRS-OPEN and IL-1Ra (IE, p = 0.02) and sTNFR-1 (IE, p = 0.04). Our findings suggest polygenic susceptibilities to immune marker aberrations in SMD involving genetic overlap with SMD-related mental phenotypes, particularly educational attainment, and psoriasis.

9.3 Paper III

In paper III we investigated potential associations between polygenic susceptibility for SCZ and TR to antipsychotics in a sample of SCZ patients and found significant associations between higher PRS-SCZ and TR (p = 0.003). This was true only for the significance threshold p-value = 0.01 for inclusion of SNPs in the PRS, as PRSs based on other GWAS significance thresholds was not significantly related to TR in the current study. The statistical
model yielded a sensitivity of 29.6% and a specificity of 90.6%, corresponding to a correct classification of 70.1% of cases as TR or non-TR. The positive predictive value was 61.5% (correctly predicted TR) and the negative predictive value was 71.7% (correctly predicted non-TR). The total variance explained by the model with PRS-SCZ, age, sex, batch number and principal components 1–12 was 14.6% (Nagelkerke's pseudo R2). Additionally, season of birth was able to predict TR in the regression model (p = 0.05). Our findings suggest that TR is related to genetic factors that also drive core pathophysiological processes in SCZ, however, the predictive values of the current model are not sufficient for clinical use.
10 DISCUSSION

The main findings of the current thesis were associations between presence of autoimmune disease, PRS-SCZ and PRS for some SMD-related mental phenotypes and immune marker levels in SMD, and an indicated association between PRS-SCZ and TR in SCZ. In paper I and II we found significant associations between sIL-2R, IL-1Ra and IL-18 and presence of autoimmune disease, PRS-SCZ and PRS-EA, each explaining very little of the plasma level difference between patients and HC. These results suggest involvement of autoimmune mechanisms and polygenic factors in immune abnormalities in SMD, involving SCZ risk variants and genetic overlap with educational attainment. However, the lack of other associations indicates that immune abnormalities in SMD are mostly explained by other factors than previous infections, autoimmunity and PRS for SMD and SMD-related mental phenotypes. In paper III the main finding was a significant association between PRS-SCZ and TR to antipsychotic, suggesting that genetic mechanisms might underlie variation in treatment response in SCZ. However, the positive and negative predictive values of the prediction model is not sufficient for clinical utilisation.

10.1 Polygenic risk score and immune abnormalities in severe mental disorders

PRS for SMD and immune abnormalities

We found a negative association between PRS-SCZ and plasma level of sIL-2R, however, the association explained very little of the plasma level difference between patients and HC. Except from this finding, there were no other associations between either PRS-SCZ or PRS-BD with the immune markers, and thus, the current study provided sparse evidence for a link between genetic susceptibility for SMD and immune marker abnormalities in these disorders. Although genetics are involved in immune system activity and common genetic variants indicated for both SCZ and BD are located at immune regions, our findings are in line with the suggestion that most of the immune variation in humans are dependent on non-genetic factors (88). However, the lack of significant associations could have other explanations, such as effects from GWAS characteristics or methods for PRS calculation. We excluded the MHC-region from the current PRS, which is common practice due to the high LD of the region that makes interpretation of eventual findings difficult. However, to speculate, keeping the MHC-region could have resulted in PRSs that would have captured more of the current immune marker levels, since MHC is central to immune responses and have strong links with both SMD and cytokines. Another reason for the lack of significant associations might be
related to current GWAS sizes with relatively low PRS power, which might not yet be sufficient to capture variation in the immune marker levels. Related to this, even though we chose immune markers based on previous association with SCZ and BD, it could be that these specific markers are not related to the genetic loci currently identified.

The significant association between PRS-SCZ and sIL-2R is interesting, although the negative direction of the association is opposite from what was expected. Plasma levels of sIL-2R are higher in SCZ compared with the general population (44), in line with the results in the current study. To speculate, one explanation for the current finding could be that genetic liability to SCZ have moderating or even confining effects on parts of the immune system, while there are other non-genetic factors that causes abnormal immune marker levels. If so, this could mean that genetic risk for SCZ restrains the immune response making individuals with higher genetic risk more vulnerable to acquire infections. This was suggested after finding that the risk increase for SCZ was similar with parental infections occurring during or outside of the pregnancy period, and whether it was the father or mother that was infected (162). We did not find associations between PRS-BD and any of the immune markers in the current study. Participants with BD more frequently had an autoimmune disease and higher rate of previous infections, indicating immune links with the disorder. However, it might be that the PRS-BD captures even less of immune marker variability than do PRS-SCZ, with the current PRS-BD explaining just below 4.6 % of the phenotypic variance, which is less than the current explained variance of PRS-SCZ in SCZ (29, 70).

**PRS for autoimmune disease and immune abnormalities**

Our findings suggest a positive association between PRS-PSOR and sgp130 across patients and HC, although the association did not survive correction for multiple testing. The association explained only minimal of the plasma level difference between the two groups. Psoriasis is a quite common immune mediated chronic skin disorder with potential of developing into psoriasis arthritis (283). Patients with SCZ have a higher risk of developing psoriasis than the general population, suggesting shared disease mechanisms (284). Psoriasis is associated with increased levels of IL-6 (285), well in line with our findings, as abnormal sgp130 reflects activity in the IL-6 pathway (209, 286). However, in the current study we found a nominally lower PRS-PSOR in patients with SMD than HC in contrast with the clinical comorbidity and previously detected positive genetic correlation (116, 287). This could be due to the current use of a newer GWAS for calculation of PRS-PSOR. However,
although the predominant observation is increased prevalence of psoriasis in SMD, this is not the case in all studies (179), indicating some uncertainty to this observation.

There were no other significant associations between PRS for the different autoimmune diseases and immune marker abnormalities in SMD. This is contrary to our hypothesis based on a literature pointing towards both genetic correlation and high clinical comorbidity between SMD and several autoimmune diseases (43, 181). However, there could be other explanations to the lack of findings. The GWAS sample sizes used to calculate the PRSs might be underpowered to capture immune marker level variation, reflected by the limited explained variance in the analysis of the association between PRS-PSOR and sgp130. Also, genetic liability is most likely not sufficient alone for development of an autoimmune disease, thus the lack of association with low-grade inflammation could be that immune marker aberration have stronger links to actual developed autoimmune disease than the polygenic risk.

**PRS for comorbid mental disorders and immune abnormalities**

PRS-ANX was significantly higher in both SCZ and BD compared with HC, in line with previous findings of genetic correlation and clinical comorbidity between anxiety disorders and SMD (109, 288, 289). The main analyses indicated associations between PRS-ANX and IL-18 and sIL-2R, however, these did not survive correction for multiple testing and explained minimal amounts of the plasma level difference between patients and HC. Immunological mechanisms are suggested in anxiety and there is evidence of altered cytokine balance (290). The positive association with IL-18 is in line with previous findings of elevated IL-18 in anxiety and increasing levels with increased severity of the disorder (291). The negative association with sIL-2R is supported by previous research that found decreased levels of sIL-2R in anxiety (292-294) and sIL-2R levels varying with clinical state in BD (295). Interestingly, we found a BD specific association between PRS-ANX and sIL-2R, suggesting involvement of sIL-2R related functions in subgroups of BD with genetic susceptibilities for anxiety. Recently, Almulla et al. 2021 (296) suggested that depression and anxiety in SCZ is associated with cytokines and immune pathways, fitting well with the findings in the current study. To speculate, it could be that immune abnormalities are linked to higher genetic liability to anxiety in subgroups of patients with SMD. This could be tested in future studies with larger sample sizes.
There were no other associations between PRS for comorbid mental disorders and immune marker levels in the main analyses. However, in addition to PRS-ANX we found higher PRS-ADHD, PRS-PTSD and PRS-MDD in SMD than in HC. This is in line with the previously shown clinical and genetic correlation between these disorders (109). Immune mechanisms are suggested as part of the aetiology in both ADHD, PTSD and MDD (83, 255, 297, 298), and similarly to SCZ and BD there seem to be abnormalities in cytokine levels for these disorders (192). However, there is still great uncertainty about the specifics of the function and underlying pathways of the shared genetic regions, as well as great heterogeneity in studies of immune markers. Hence, part of the explanation behind the current lack of findings might be that the overlapping genetic variants are too weakly linked with immune pathways or to the current chosen immune markers. Additionally, factors related to the explained variance of the different PRSs, and the sample size of the current study might limit the power to detect the hypothesised associations.

**PRS for SMD-related mental phenotypes and immune abnormalities**

We found lower PRS-EA in SMD than HC, in line with the cognitive difficulties seen in patients with SMD (299). Further, the main analyses showed significant negative associations between PRS-EA and both IL-1Ra and IL-18, surviving correction for multiple testing and confirmed in subgroup analyses in SCZ and BD separately. Educational attainment is considered a reliable proxy for cognitive abilities, although also influenced by other factors, such as personality traits (124, 300). Both cognitive abilities and educational attainment are genetically associated with SCZ and BD, however, the relationship is complex as the shared loci show different direction of effects both between and within the disorders (117, 124, 301). Educational attainment is linked with immune system activity and lower educational attainment seem to be associated with increased levels of inflammatory markers, particularly CRP and fibrinogen (302). In line with the current findings, previous studies have detected higher levels of IL-1Ra associated with poorer cognition in patients with BD and associations between IL-18 and cognition in patients with chronic schizophrenia (303, 304). IL-1Ra and IL-18 are both part of the IL-1 family and processed in the intracellular multiprotein complex called Nod-like receptor (NLR) family, pyrin domain-containing 3 (NLRP3) inflammasome (204, 305, 306). The NLRP3-inflammasome is involved in innate immune response to infections and in autoimmune diseases and associated with CNS pathologies including major depression and neurodegenerative disorders (306-308). Furthermore, the inflammasome has been suggested as a mediator between stress and depression (307). The current association
with PRS-EA might similarly indicate a link between cognition related traits and low-grade inflammation through inflammasome activity in subgroups of patients with SMD.

Interaction analysis suggested disease specific associations between personalityPRS and immune markers in the combined SMD sample and SCZ subsample. Although nominally, increased IL-1Ra was associated with decreased PRS-OPEN and PRS-EXTRA, and increased sTNFR-1 was associated with decreased PRS-OPEN. Personality might be linked with immune system activity through common genetic liability or personality mediated impact of stressors (309). Research investigating immune markers and personality have focused on CRP and IL-6 and a meta-analysis show negative associations with conscientiousness and openness (252). This is in line with the current negative associations between PRS-OPEN and IL-1Ra and sTNFR-1. SCZ and BD are both positively genetically correlated with openness (Lo et al., 2017), reflected in our findings of higher PRS-OPEN in SMD than HC. Moreover, SCZ and BD are characterised clinically by lower extraversion (129, 310), supporting the current negative associations with IL-1Ra. Thus, the current associations might suggest a disease specific role for immune activity in subgroups of SMD patients with lower genetic liability of openness and extraversion.

10.2 Autoimmune disease and immune abnormalities in severe mental disorders

We found a positive association between presence of autoimmune disease and sIL-2R, indicating that autoimmune mechanisms might be involved with the elevated plasma levels of sIL-2R seen in SCZ patients. The association was highly significant (p<0.001) but explained only minor amounts of the plasma level difference between the SCZ and HC. The functional repertoire of sIL-2R is not entirely mapped, but evidence suggest that elevated levels of sIL-2R reflect T lymphocytes activity and that levels of sIL-2R is increased during infections, general inflammation and with autoimmune diseases (311). sIL-2R is elevated in many autoimmune diseases of which some are genetically associated with SCZ, such as MS, RA and T1D (116, 311). Further, sIL-2R seem to correlate with autoimmune disease activity in the general population (312). Notably, the cytokine is included in the diagnostic criteria of hemophagocytic lymphohistiocytosis, a disease characterized by pro-inflammatory cytokine storm (311). In SCZ, sIL-2R is increased in both acutely ill and chronic patients, as well as after treatment, and hence it seems to be a trait, rather than a state, marker (194). However, it is not disease specific, as it is elevated also in patients with BD and MDD (194). sIL-2R is also implicated in the macrophage T lymphocyte theory of SCZ, where density of sIL2R in locus coeruleus is found to be associated with psychotic symptoms (151). Furthermore, sIL-
2R is a part of the compensatory immune-regulatory reflex system (CIRS) which is implicated in SCZ pathophysiology (225). Altogether, the current association between presence of autoimmune disease and increased plasma levels of sIL-2R in SCZ fit well with previous literature and suggest a role for autoimmune mechanisms linking SCZ and low-grade inflammation, particularly related to adaptive immune response involving T lymphocytes.

10.3 Infections and immune abnormalities in severe mental disorders

We found that the number of hospital-treated infections was higher in both patient groups compared to HC, which is in line with previous literature (42, 78). However, in combination with general practice treated infections, there were less total number of previous infective episodes in SCZ than both BD and HC. We know that there is a difference in help-seeking behaviour between patients with SCZ and the general population in that they seek help at a much later stage of disease and are thus less frequent users of primary care and more frequently admitted to the emergency department (13, 313). Therefore, an explanation of the lower-than-expected number of general-practice treated infections in SCZ in the current thesis could be that infections in people with SCZ either recover before help-seeking is initiated or get so serious that hospital admittance is needed at once. However, a previous study found an association between number of anti-infective treated infections outside of hospital and increased risk of subsequent SCZ (314). Although their methods differed slightly from ours in that they defined infective episodes as redeemed prescriptions for an anti-infective agent in primary care, these findings are conflicting with the current results.

We found negative correlations between number of previous infections and sgp130 in SCZ and BD, and IL-18 in BD, indicating a possible link between infection episodes and peripheral levels of immune markers. However, in the fully adjusted models there were no associations. This could mean that the effect from prenatal, childhood and adult infection events, that is suggested to be involved with SMD development (43, 315, 316), is not reflected by immune marker levels measured in patients with developed disorder. Moreover, it might be that these mechanisms are not reflected by the specific immune markers chosen for the current study. Interestingly, a recent study found that PRS for infections could predict a small amount of SMD risk, but only a modest part of this was mediated by actual infections (317). Although their outcome was development of SMD and not immune marker aberrations, their finding is partly in line with the lack of association seen in the current study. Furthermore, lack of findings could also be explained by power issues, in that we would need larger sample size with subsequent larger number of participants with previous infections to
capture the rather modest differences in immune marker levels between SMD and HC. Also, if associations between infections and immune abnormalities are relevant only for a subgroup of patients, this could be camouflaged in the full sample.

10.4 Treatment resistance in schizophrenia spectrum disorders

We found an association between PRS-SCZ and TR to antipsychotics in SCZ patients. The association was limited to the PRS at threshold \( p = 0.01 \) for inclusion of SNPs. Our finding complements previous related findings of higher PRS-SCZ in patients treated with clozapine (247) and increased chance of treatment response with lower PRS-SCZ in first-episode psychosis (248), altogether suggesting a subgroup of SCZ with genetically based less effect from regular antipsychotic treatment. This is in line with previous studies indicating that patients with TR constitute a subgroup of the disorder which is different from non-TR (235) or that represent a more “genuine schizophrenia” (235, 318-320). Biological differences, such as a lower level of striatal dopamine synthesis capacity and higher level of glutamate in the anterior cingulate cortex (39, 237), might contribute to the lack of effect from antipsychotics in these patients. However, there are previous studies not finding associations between PRS-SCZ and TR (239, 249, 250). This inconsistency between studies might have various explanations. First, it could be due to heterogeneity of criteria used to define TR, ranging from a history of clozapine and hospitalisation (247, 249) to less strict criteria including lack of reduction in psychotic symptoms to neuroleptics (239). Using the consensus criteria and guidelines developed by TRRIP, we found a TR prevalence in our sample of 33.6%, corresponding well with the literature (235, 240). Secondly, using different thresholds for inclusion of SNPs might have impacted the results (239, 248-250). Finally, differences in sample characteristics, ranging from first-episode psychosis to more certain SCZ with longer duration of disease, could affect the findings (248, 249). Interestingly, after publication of paper III, a GWAS on TR in schizophrenia found significant associations between PRS for TR and TR status in independent samples, suggesting a single-nucleotide variation-based heritability in TR (321).

In addition to PRS-SCZ, we investigated non-genetic factors previously associated with TR. We found that winter births were less common in TR than in non-TR and that season of birth was significantly associated with TR, also after adjusting for PRS-SCZ. A recent study found that severe SCZ requiring hospital admission was not related with birth seasonality (322). However, excess winter births in SCZ is repeatedly reported (323), an observation thought to be related with the immune hypothesis in SMD. Interestingly, other risk factors of SCZ also
seem to be associated with TR, such as living in less urban areas, as opposed to the finding that SCZ in general is associated with urbanicity (239, 324). Despite lower prevalence of winter births, immune mechanisms are suggested in TR and a recent study found indications for a unique immune signature in this patient group (325). Altogether, these findings support the previous suggestion that TR patients with SCZ might be a subgroup of SCZ, with potential genetic liability to TR that should be further investigated.

10.5 Discussion of methods

When studying the occurrence of diseases in a population and factors related to this, there is always a chance of random and systemic error. Random error happens by chance and can be limited by increasing the sample size. Systemic error (bias) is related to the way data is collected, analysed, interpreted and published and cannot be minimised by increasing sample size. If bias occur, this can influence the result of a study by violating internal validity, i.e. the characteristics of a study to produce valid results (326). Thus, it is central to be aware of biases when planning, designing and interpreting a scientific study. Confounding is a type of bias that occur when there exist one or more factors not accounted for that is associated both with the primary exposure of interest as well as the outcome, leading to a distortion in the estimated measure of association. Thus, it is important to map potential confounders and adjust for these.

In addition to internal validity, there is external validity, which can be described as the generalisability of the findings and applicability of the hypothesis that is generated from a study to more general circumstances than the study setting (326). In other words, internal validity is important for the accuracy of the study and the external validity is important for the relevance (327).

In the following sections I will discuss various aspects concerning internal and external validity of relevance for the current thesis.

10.5.1 Data collection and classification

Data collection is vulnerable to different types of biases that should be taken into consideration in the planning and interpretation of a study. Information bias occur when there is a systemic distortion in the information that is collected (326). A type of information bias is misclassification bias, when there is a distortion in the detection of exposure and/or outcome (326), for example when an individual is assigned to a category different from the one to
which they should be assigned. In the TOP study the participants go through a comprehensive diagnostic interview performed by clinical psychologists and physicians that have been trained by the UCLA training program (258) and cases of doubt are discussed with senior professor in psychiatry. This have resulted in good diagnostic inter-rater reliability (259) and thus probability of misclassification bias related to SMD diagnosis in the current thesis can be considered as low. Misclassification bias is relevant to consider also in paper I, where we used diagnostic information from national health registries to classify participants as either having or not having an autoimmune disease and to determine number of previous infections. To our knowledge, data entered in these registries are not quality checked and thus entirely dependent on the capabilities of the clinician. Further, the diagnostic codes used in these registries are not designed for infection- or autoimmune-related events in specific and therefore some of these codes can cover more broadly, for example ‘thyroid disease’ which can have both autoimmune and non-autoimmune causes. Cases of such doubt were not included in the current study to increase the specificity of these variables. Consequently, some participants could have been misclassified as not having had an infection or autoimmune disease when in fact they did. However, since we had access to two different registries using different diagnose classification systems, it was often possible to use one of the registries to confirm uncertainty or fill in where information was missing to account for potential misclassifications. Also, we used the same procedure for patients and controls.

Misclassification bias is also relevant for paper III, where participants often were defined as TR or non-TR based on the participants own reporting. Attempting to minimize the risk of this being an issue we carefully adapted the consensus criteria from TRRIP for application on our retrospective data (240). Although this approach cannot be considered as good as with a prospective study design, the resulting prevalence of TR in our study was similar to that in the literature (245), indicating that the current classification procedure was adequate. Finally, misclassification resulting to responses or diagnosis used in a GWAS can potentially reduce the predictive ability of the selected SNPs, with negative impact for the predictive power of the calculated PRS (328).

Another type of information bias is recall bias. This is a concern when a participant is requested to report previous events and do not report correctly what happened due to failure to remember. The chances of recall bias are typically greater in conditions with cognitive difficulties and/or longer time since the event of interest. People with severe mental illness more often have cognitive problems (329) and therefore information collected from cases in
the TOP sample could be particularly vulnerable to recall biases. This is a potential issue in paper III, where we collected information about type of antipsychotic drug, duration of treatment, dosage and side effects the last five years prior to inclusion in order to classify participants as TR or non-TR. Also, diagnostical interviews could be affected by recall bias, with consequences for the respective diagnosis that were set. The chances of recall bias were however reduced by accessing medical journals and information from the referring party, although this was not always possible.

A third type of bias to consider is selection bias. This is when the participants included in a study might not be representative of the source or target population, potentially causing an distorted exposure-outcome relationship (326). Selection bias might affect internal validity if for example the cases and controls are not drawn from the same population or the external validity if the study sample is not representable of the study population. In the TOP study, patients are in general referred from in- or outpatient clinics in the hospital, and sometimes from the general practitioner or by self-assignment. The participation is voluntary and requires participants who are motivated to contribute to research and able to complete a quite comprehensive study protocol. This potentiates the risk for volunteer bias if the people who volunteer for participation in a respective study differ in relevant characteristics from the target population, potentially leading to a weakened generalizability of results. For example, it is likely that the sickest and worst functioning patients do not participate as often, as they might be unable to complete a full research assessment, due to for example frequent hospital admittance. On the other side, the best functioning patients might as well not participate, since they do not want to prioritize research over work or family. Hence, volunteer bias might be a relevant issue in the current study. Attempting to minimize the risk of this we work hard to reach out to and have regular contact with different types of clinics, both in- and outpatient wards. We spread information about the study and how to refer participants, we hold lectures and present results. Also, we have a consultation phone available for clinicians and offer assessment where the patient is located or taxi for participants with difficulties of travelling.

10.5.2 Statistical model

In paper I and paper II we applied ANCOVA to investigate associations between immune marker levels and previous infections, autoimmune disease and PRS for SMD, autoimmune disease and SMD-related mental phenotypes. ANCOVA is a parametric technique for investigating a relationship between a dependent and independent variable when you need to control for one or more other continuous variables. Before performing ANCOVA, we
assessed the distribution of the variables and to meet the normality assumption we needed to
to logarithmically transform all immune marker measurements as well as remove outliers
outside 1.5 or 3 x the IQR of first and third quartile. Additionally, we ran preliminary
correlation analyses with the independent variables to ensure that there was no strong
correlation between these (collinearity). Further, we excluded all participants with CRP level
above 10 to avoid acute infections as underlying cause of aberrant immune marker levels.

Inclusion of participants for the TOP study has been ongoing since 2003 and blood has
similarly been sampled at varying time points with subsequent different durations of freezer
storage time. In the first operating years TOP mainly included cases, thus the distribution of
freezer storage time differs between SMD and HC. As freezer storage time might affect
immune marker levels (330) we adjusted for this in all analyses including immune markers.
Additionally, we adjusted for age and sex in all three papers, a standard procedure when
analysing biological markers.

In paper III we applied logistic regression to investigate the relationship between PRS and
TR. Logistic regression is a parametric technique for prediction of categorical outcomes with
two or more categories and requires similar assumptions as ANCOVA. For this study, we
carefully selected the most relevant variables to include based on previous literature. Further,
we made sure that there were no correlations above 0.8 between independent variables and
potential issues with outliers were ruled out by assessing Q-Q plots.

Some additional variables were included in the statistical model when we investigated PRS.
As the participants in the TOP study are included at different time points, so are the lab
analyses performed at different time points, which makes it important to adjust for potential
differences related to the respective genetic analysis batches. Further, the performance of PRS
can be affected by genetic differences between populations and therefore it is important to
account for ethnicity (331). To achieve a uniform structure throughout the first study we used
the same sample for both genetic and non-genetic analyses in paper I and did not exclude
based on ethnicity, but instead adjusted for ethnicity in the analyses including PRS-SCZ and
PRS-BD. In paper II and III we restricted the sample to only including European participants.
Also, we adjusted for genetic principal components in all analyses including PRS to account
for ancestry.

When investigating cytokines, it might be relevant to adjust for BMI, smoking status and
alcohol use (332). Smoking is suggested to have immunosuppressive and immunomodulatory
effects (333), BMI is associated with abnormal cytokine levels (334) and alcohol consumption is associated with pro-inflammatory cytokines (332). In paper I we evaluated whether to adjust for these factors by performing separate bivariate correlations between BMI, smoking status (smoking regularly, yes/no) and alcohol use (number of units of alcohol last two weeks) with significant variables from the main ANCOVA analyses (autoimmune disease, PRS-SCZ); if significantly correlating, these variables were also included in fully adjusted ANCOVAs. This method was chosen to avoid unnecessary reduction of the sample size due to incomplete data of these variables (40-80% of total sample size). As BMI correlated significantly with autoimmune disease and PRS-SCZ, it was included in the main ANCOVA, although with no significant effect on sIL-2R levels.

In paper II, we investigated possible interaction effects between SMD/HC status and the different PRSs. Interactions reflect that the association between a dependent variable and an independent variable differ depending on another independent variable. In the current study, we found a significant interaction between SMD status and PRS-EXTRA in the analysis of IL-1Ra, revealing a SMD-specific negative association between PRS-EXTRA and IL-1Ra. This could implicate that there are genetic variants of extraversion interacting with SMD-related factors with impact on IL-1Ra aberration in SMD.

10.5.3 Multiple testing considerations

The probability of falsely rejecting the null hypothesis (type I error) and/or mistakenly not rejecting an actual false null hypothesis (type II error) should be minimized for increased credibility of the results. Although the analyses in paper I and II were somewhat hypothesis-based, the lack of other similar studies also makes these investigations explorative and potentially hypothesis generating. To limit the risk of missing out on hypotheses for future investigation we decided to apply a moderate correction of the significance threshold in these papers. In paper I we applied a corrected significance threshold of 0.025 (0.05/2) due to testing of genetic and non-genetic models and in paper II a corrected significance threshold of 0.0125 (0.05/4) due to testing PRS for the four following groups: autoimmune diseases, mental disorders, cognitive traits and personality traits. Additionally, we chose the current immune markers specifically for their known association with SMD and the PRSs based on indicated genetic association with SMD and association with immune markers. In paper III, due to testing of specific TR hypotheses with PRS analyses including five different p-value thresholds for inclusion of SNPs, we applied a stricter Bonferroni correction of the significance threshold of 0.01 (0.05/5).
10.6 Strengths and limitations

A strength applying to all three papers is the inclusion of a large, well-characterised sample from the TOP study, where we use a comprehensive research protocol and have standardised routines and procedures for clinical assessment and collecting blood samples. Moreover, the close cooperation with the referring clinics and the possibility to confer with medical records additionally strengthens the quality of the participant data. Specific strengths related to *paper I* was the access to national health registries which gave a unique overview of infection and autoimmune disease history for each participant. The comprehensive assessment of biochemical parameters and immune markers enabled us to choose relevant markers for the research goals in both *paper I* and *paper II*. A strength applying to *paper III* is the broad collection of data which gave us the opportunity to apply consensus-based criteria for defining TR and to perform analyses with relevant adjustments.

There are several limitations to address. One general limitation for all three papers is the cross-sectional, observational design, which makes us unable to indicate causality in the respective associations. However, the inclusion of genetic components invites us to cautiously interpret direction of effect, since genetic risk is inherited and in principle precedes the other variables. Limitations in *paper I* include lack of registry data before 2006, leaving out years of information about infections and autoimmune diseases, particularly for the older participants. The lack of early-life information about infections and autoimmunity involves an uncertainty about the representativeness of the rate of infection and autoimmune disease variables. This is particularly a concern regarding infections, as infective episodes often are delimited and single occurring, while autoimmune diseases are more often chronic or intermittent and therefore probably registered also after 2006 even though they were discovered before. Further questioning the representativeness of the current sample is the finding that patients with SCZ had less general practice treated infections than HC, which is at odds with the literature (314). However, there are indications of genetic components influencing the susceptibility to acquire infections, which is in favour of a more stable lifetime occurrence of infection episodes that would strengthen the representativeness of the rate of infections in *paper I* (335). Bias from potential blood handling issues cannot be fully excluded, e.g., since immediate freezing was not feasible, plasma was isolated and frozen within the next working day. Nevertheless, although somewhat conflicting results (336), studies suggest reasonable stability of immune markers under various conditions (337).
In both *paper I* and *paper II* there is a chance that the included immune markers, although carefully chosen based on previous association with SMD, were not the most relevant for the current tested associations. Also, while the immune link is evident for autoimmune diseases, this is less clear for mental phenotypes. However, immune system involvement is indicated for the specific mental phenotypes selected for PRS analyses in the current thesis. Another limitation in both papers is repeated testing. As previously discussed, this was accounted for by a moderate correction of the significance threshold, as a stricter correction might be at the expenses of indicating relevant hypotheses for further testing. Other limitations include the varying sample sizes and explained variance of GWASs used for PRS calculation for the different phenotypes, complicating comparison of the results.

In *paper III*, limitations include the use of a cross-sectional design, which made us unable to assess actual symptom reduction during different medication regimes. This made us unable to implement the ‘lack of reduction of symptoms’ criterion for classification of TR (240). However, due to our research protocol we were still able to adapt the prospective based criteria to match TR rates shown previously by others (234, 235). Further, due to lack of serum level data of previous medication we could not assess actual adherence in previous trials. Finally, excluding participants with a first-time episode of psychosis could mean that patients with fast recovery were underrepresented in the study sample. Although we cannot fully exclude this as a source of bias, the lack of significant correlations between PRS-SCZ and PANSS-total or GAF-F suggest that variation in symptoms would not have substantially affected the results.

### 10.7 Clinical implications

Increased understanding of SMD aetiology and disease mechanisms have potential clinical implications including more precise classification of clinically relevant subgroups and development of more effective treatment. The cross-sectional design, somewhat explorative approach and small effects in *paper I* and *II* make it difficult to derive direct clinical implications from the current findings. However, our attempt to test the potential contribution of relevant genetics and non-genetic factors in low-grade inflammation in SMD can add to the current understanding of immune mechanisms that might ultimately make way for future tailored treatment in acute and prophylactic settings, including immune-targeted alternatives. Also, mapping biological mechanisms in SMD could result in an increased understanding and acceptance of these disorders for patients and relatives. Moreover, although increased
identification of biological mechanisms might induce a feeling of hopelessness in some patients, such knowledge will hopefully in general lead to a reduction in mental health stigma.

In paper III we found indications for predictive abilities of PRS-SCZ on treatment response in SCZ. The positive and negative predictive values were not sufficient for clinical use and the explained variance suggests that PRS-SCZ is currently not suited for individual prediction of TR. However, it is argued that future PRSs can be utilized clinically for risk assessment, outcome prediction and clinical decision-making in clinical psychiatry, at least in combination with other risk factor measures (89, 338). Thus, genetic risk might be a valuable contributing factor in future multifactorial prediction models of treatment response (321), particularly if upcoming, larger GWASs enable calculation of PRSs that capture more of the genetic variance. These prediction tools can benefit patients by offering a more tailored treatment with limited degree of trial- and error testing of medication, subsequently reducing the burden of side effects.

The complex nature of SMD calls for a multifaceted approach in clinical assessment, monitoring and treatment in the years to come, potentially including data on polygenic risk as well as immune markers. Tailored treatment approaches building on new knowledge about biological mechanisms might make way for a more personalized balance between pharmacological and conversational therapy optimised for each patient.
The current thesis investigated the role of central immune activating conditions and genetics in immune marker abnormalities in SMD and TR to antipsychotics in SCZ, to explore potential underlying mechanisms. Overall, there were sparse evidence to support involvement of infections, autoimmunity and genetics in low-grade inflammation in SMD, albeit with some exceptions that should be followed up in future studies. These include suggested associations between cytokine levels (sIL-2R, IL-1Ra and IL-18) and PRS-SCZ, PRS-EA, PRS-ANX, PRS-PSOR and presence of autoimmune disease. Further, there were indications for a potential role for PRS-SCZ in predicting treatment response to antipsychotics.

Several recommendations for future research can be drawn from the current work. It would be interesting to test associations between infections and autoimmune disease with immune marker aberrations with registry data covering longer time periods. Further, the findings should be tested in studies with prospective designs, as this will give insight to intrapersonal fluctuations in immune marker levels and possible assessment of more ‘real time’ association between infections and immune activity, which might indicate effect directions. Future studies looking into polygenic risk and TR should include larger upcoming GWASs with increased number of identified common genetic risk loci and improved PRSs. Further, exploring the role for PRS together with other relevant variables in multifactorial prediction model could be an interesting approach.

The current thesis provided new insight to the role of polygenic susceptibilities, infections and autoimmunity in immune marker abnormalities in SMD, suggesting that the low-grade inflammation of these disorders is mainly explained by other factors. The few indicated associations of autoimmune disease and different PRSs with abnormal immune marker levels might generate hypotheses for further investigation. The association between PRS-SCZ and TR adds to the current knowledge and could turn out to be an important link for future tailoring of treatment when more of the genetic composition of SMD and treatment response is uncovered.
12 REFERENCES


100. Dennison CA, Legge SE, Pardiñas AF, Walters JTR. Genome-wide association studies in schizophrenia: Recent advances, challenges and future perspective. Schizophr Res. 2020;217:4-12.


296. Almulla AF, Al-Rawi KF, Maes M, Al-Hakeim HK. In schizophrenia, immune-inflammatory pathways are strongly associated with depressive and anxiety symptoms, which are part of a latent trait which comprises neurocognitive impairments and schizophrenia symptoms. J Affect Disord. 2021;287:316-26.


Paper I
Limited association between infections, autoimmune disease and genetic risk and immune activation in severe mental disorders

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A R T I C L E   I N F O

Keywords:
Immune system
Inflammation
Cytokines
Schizophrenia
Bipolar disorder
Polygenic risk score

A B S T R A C T

Background: Low-grade inflammation may be part of the underlying mechanism of schizophrenia and bipolar disorder. We investigated if genetic susceptibility, infections or autoimmunity could explain the immune activation.

Methods: Seven immune markers were selected based on indicated associations to severe mental disorders (IL-1Ra, sIL-2R, IL-18, sgp130, sTNFR1, APRIL, ICAM-1) and measured in plasma of patients with schizophrenia (SCZ, N = 752) and bipolar spectrum disorders (BD, N = 460) and healthy controls (HC, N = 938). Information on rate of infections and autoimmune diseases were obtained from Norwegian national health registries for a twelve-year period. Polygenic risk scores (PRS) of SCZ and BD were calculated from genome-wide association studies. Analysis of covariance were used to test effects of infection rate, autoimmune disease and PRS on differences in immune markers between patients and HC.

Results: Infection rate differed between all groups (BD > HC > SCZ, all p < 0.001) whereas autoimmune disease was more frequent in BD compared to SCZ (p = 0.004) and HC (p = 0.003). sIL-2R was positively associated with autoimmune disease (p = 0.001) and negatively associated with PRS of SCZ (p = 0.006) across SCZ and HC, however, associations represented only small changes in the difference of sIL-2R levels between SCZ and HC. Conclusion: There were few significant associations between rate of infections, autoimmune disease or PRS and altered immune markers in SCZ and BD, and the detected associations represented only small changes in the immune aberrations. The findings suggest that most of the low-grade inflammation in SCZ and BD is explained by other factors than the underlying PRS, autoimmunity and infection rates.

1. Introduction

Schizophrenia and bipolar disorder are severe mental disorders with shared genetic risk factors and clinical characteristics (Owen et al., 2016; Vieta et al., 2018). Parts of the genetic structure of these disorders was recently determined by large international genetic consortia (Mullins et al., 2021; The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020), enabling studies beyond effects of

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https://doi.org/10.1016/j.pnpb.2022.110511

Received 6 August 2021; Received in revised form 23 December 2021; Accepted 13 January 2022
Available online 19 January 2022

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single genetic variants. Moreover, environmental factors appear to impact risk throughout the entire early lifespan potentially involving epigenetic modifications (Aldinger and Schulze, 2017; McDonald and Murray, 2000), with large effects on disease occurrence (Marangoni et al., 2016; Stillo and Murray, 2019; van Os et al., 2010; van Os et al., 2005). However, despite recent scientific progress, the mechanisms at the interface of risk factors and pathophysiology are generally unknown, impeding progress in prevention and treatment.

The immune system has emerged as a pathophysiological candidate of severe mental disorders, however, with more heterogeneous findings in bipolar disorder than schizophrenia. Associations between psychosis and infectious disorders have long been suspected (Kapitska et al., 2020; Noll, 2007), including infections with toxoplasma gondii, influenza virus, herpes simplex virus type 2, cytomegalovirus and Borra disease virus (Arias et al., 2012; Benros and Mortensen, 2012; Brown and Derkits, 2010; de Barros et al., 2017). The increased risk of schizophrenia and bipolar disorder has been associated with infections both during (Barichello et al., 2016; Khandaker et al., 2013; Parboosingh et al., 2013) and after (Benros et al., 2011; Benros et al., 2013; Hickie et al., 2009; Oliveira et al., 2017) pregnancy as well as with paternal infections (Nielsen et al., 2013). A suggested mechanism in schizophrenia involves triggering of various cellular pathways within the central nervous system (CNS) secondary to activation of microglia by systemic inflammatory mediators (Monji et al., 2009; Smith, 1992). This “inflammatory hypothesis” is supported by evidence of low-grade systemic inflammatory mediators in both schizophrenia and bipolar disorder by findings of abnormal levels of several peripheral immune activation markers (Kroken et al., 2018; Rosenblat and McIntyre, 2017). Immune aberrations that have been repeatedly associated with these disorders include increased soluble interleukin 2 receptor (sIL-2R) levels, reflecting T cell activation, and activation of the proinflammatory interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF) signalling pathways as reflected by secreted levels of IL-1 receptor antagonist (IL-1Ra), IL-18, IL-6 and soluble TNF receptor 1 (sTNFR-1) (Hope et al., 2009; Hope et al., 2013; Kroken et al., 2018; Luo et al., 2019; Luo et al., 2016; March et al., 2016; Rosenblat and McIntyre, 2017; Wedervang-Resell et al., 2020a, 2020b). A meta-analysis indicated similar cytokine patterns in schizophrenia and bipolar disorder in both acute and chronic phases showing elevated sIL-2R, IL-1Ra, TNF-α and IL-6 in acutely ill patients and sIL-2R, IL-1p and IL-6 in chronic patients (Goldsmith et al., 2016; Kroken et al., 2018). Moreover, a proliferating inducing ligand (APRL), a cytokine belonging to the TNF-family, was recently found reduced in both schizophrenia and bipolar spectrum disorder compared with healthy controls (Engh et al., 2022). Alterations in cell adhesion molecules (CAMs) include elevated levels of the intercellular cell adhesion molecule 1 (ICAM-1) (Müller, 2019; Wedervang-Resell et al., 2020a, 2020b), which is expressed in microglial cells and astrocytes as well as on circulating lymphocyte subsets.

A range of autoimmune conditions are associated with schizophrenia and bipolar disorder (Chen et al., 2021; Eaton et al., 2006; Rosenblat and McIntyre, 2017) and seem to exert a small, but synergetic effect with hospital-treated infections on disease risk (Benros et al., 2011; Benros et al., 2013). Increased autoantibodies against neuronal cells (Ezeoke et al., 2013) and a genetic relationship between severe mental disorders and autoimmune diseases (Andreassen et al., 2015; Stringer et al., 2014; Wang et al., 2015) further support the immune system as a pathophysiological candidate.

Schizophrenia and bipolar disorder are highly heritable disorders (Smeland et al., 2020; Stahl et al., 2019) with immune candidate genes identified in genome-wide-association studies both within the Major Histocompatibility Complex (MHC) and outside MHC (Pouget, 2018). Of note, the MHC region which encodes a range of proteins critical for immune defence is the strongest signal in schizophreniaw GAS’ (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020). In particular there is a strong association with complement component 4 genes (Sekar et al., 2016). The MHC region was recently also linked with bipolar disorder (Mullins et al., 2021). Infection (Klebanov, 2018) and autoimmune (Hu and Daly, 2012; Skov et al., 2020) susceptibility similarly have strong genetic components; however, studies investigating the relationship between genetic risk of severe mental disorders and immune activation are few and inconclusive. One small report indicated associations between PRS of schizophrenia and bipolar disorder and increased C–C Motif Chemokine Ligand 4 (CCL4) in first-episode psychosis patients (Ma et al., 2019). Benros et al. (2016) found no effect of PRS of schizophrenia on the association between infections and the risk of schizophrenia.

Thus, we still do not know whether the low-grade inflammation of schizophrenia and bipolar disorder originate from associated infections, autoimmunity or overlapping genetic risk factors. The aim of the current study is to investigate the relationship between altered immune markers and the rate of infections, autoimmune disease and PRS. Immune markers were chosen based on robust links with schizophrenia or bipolar disorder. The study sample includes patients with schizophrenia and bipolar spectrum disorders and healthy controls, and national registry data are applied to obtain a comprehensive estimate of differences in occurrence of infections and autoimmune diseases.

2 Methods

2.1. Study setting

The study is a part of the Thematically Organized Psychoysis study (TOP) at the Norwegian Centre for Mental Disorders Research (NORMENT). Patients between the ages of 18–65, recruited from the major hospitals in the Oslo region, with a diagnosis of schizophrenia spectrum disorders (SCZ: schizophrenia, schizoaffective disorder, schizo-affective disorder, delusional disorder, brief psychotic disorder and psychosis not otherwise specified (NOS)) or bipolar spectrum disorders (BD: bipolar 1 disorder, bipolar 2 disorder, bipolar disorder NOS and major depressive disorder with psychosis) were included. All participants must understand verbal and written information to the study and make a written informed consent. Patients were excluded if they had a history of severe somatic disease interfering with brain functioning including neurological disease, history of moderate or severe head trauma, or an IQ below 70. Healthy controls (HC) in the same age span as the patients were randomly selected from statistical records from the same catchment areas and invited to participate. HC inclusion was also based on lack of history of severe mental illness, neurological disorder, severe brain trauma, illicit drug abuse or dependency, or somatic conditions that interfere with brain function or close relatives with severe mental illness. Participants with CRP level above 10 mg/L were excluded from the analyses to remove a potential effect of acute infection on the immune markers. In the current study we included patients (N = 1484) and HCs (N = 1041) with registry data (Table 1) of which immune marker assessments during 2002–2018 and PRS data were available in 732 and 740 patients with SCZ, 460 and 475 patients with BD, and 938 and 915 HCs, respectively.

2.2. Clinical assessment

The Structured Clinical Interview (SCID-1) (First et al., 1995) for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (American Psychiatric Association, 2000) was used for diagnostic assessment. Diagnostic interviews were performed by clinical psychologists and physicians supervised by a senior professor of psychiatry. The research personnel were comprehensively trained for the interviews based on a UCLA training program (Ventura et al., 1998). The diagnostic inter-rater reliability of the TOP study has been found good with a diagnostic agreement of 82% and an overall kappa score of 0.77 (95% CI 0.66–0.84) (Ringen et al., 2008).
Table 1
Demographic and clinical data.

<table>
<thead>
<tr>
<th></th>
<th>SCZ, N = 926</th>
<th>BD, N = 558</th>
<th>HC, N = 1041</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex female, N (%)</td>
<td>369 (39.8)</td>
<td>325 (58.2)</td>
<td>482 (46.3)</td>
<td>&lt;0.001</td>
<td>BD &gt; HC &gt; SCZ</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>28 (14)</td>
<td>31 (18)</td>
<td>33 (15)</td>
<td>&lt;0.001</td>
<td>BD, HC &gt; SCZ</td>
</tr>
<tr>
<td>Ethnicity European&lt;sup&gt;b&lt;/sup&gt;, N (%)</td>
<td>748 (80.8)</td>
<td>494 (88.5)</td>
<td>1017 (98.3)</td>
<td>&lt;0.001</td>
<td>HC &gt; BD &gt; SCZ</td>
</tr>
<tr>
<td>Diagnosis, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>543 (58.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizoaffective</td>
<td>51 (5.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder 1</td>
<td></td>
<td>307 (55.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder 2</td>
<td></td>
<td>163 (29.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder NOS</td>
<td>29 (5.2)</td>
<td>163 (29.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td></td>
<td>59 (10.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychosis NOS</td>
<td>130 (14.0)</td>
<td>163 (29.2)</td>
<td>163 (29.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brief psychotic disorder</td>
<td>20 (2.2)</td>
<td>163 (29.2)</td>
<td>163 (29.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>53 (5.7)</td>
<td>163 (29.2)</td>
<td>163 (29.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotics&lt;sup&gt;c&lt;/sup&gt;</td>
<td>774 (83.7)</td>
<td>282 (50.7)</td>
<td></td>
<td>&lt;0.001</td>
<td>SCZ &gt; BD</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>110 (11.9)</td>
<td>199 (35.7)</td>
<td></td>
<td>&lt;0.001</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Lithium</td>
<td>18 (1.9)</td>
<td>19 (1.9)</td>
<td></td>
<td>&lt;0.001</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>267 (28.8)</td>
<td>206 (36.9)</td>
<td></td>
<td>&lt;0.001</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>CRe protein, median (IQR)</td>
<td>1.9 (3.4)</td>
<td>1.4 (3.0)</td>
<td>1.1 (2.1)</td>
<td>&lt;0.001</td>
<td>SCZ &gt; BD &gt; HC</td>
</tr>
<tr>
<td>Total infections, median (min-max)</td>
<td>2.0 (0-35)</td>
<td>5.0 (0-42)</td>
<td>3.0 (0-35)</td>
<td>&lt;0.001</td>
<td>BD &gt; HC &gt; SCZ</td>
</tr>
<tr>
<td>Infections, specialist health care, median (min-max)</td>
<td>0 (0-10)</td>
<td>0 (0-14)</td>
<td>0 (0-7)</td>
<td>&lt;0.001</td>
<td>BD &gt; SCZ &gt; HC</td>
</tr>
<tr>
<td>Autoimmune disease, N (%)</td>
<td>92 (9.9)</td>
<td>83 (14.9)</td>
<td>101 (9.7)</td>
<td>0.003</td>
<td>BD &gt; SCZ, HC</td>
</tr>
<tr>
<td>PRS-SCZ 0.05&lt;sup&gt;d&lt;/sup&gt;, median (IQR)</td>
<td>0.582 (1.4)</td>
<td>0.317 (1.2)</td>
<td>-0.223 (1.2)</td>
<td>&lt;0.001</td>
<td>SCZ &gt; BD &gt; HC</td>
</tr>
<tr>
<td>PRS-BD 0.05&lt;sup&gt;d&lt;/sup&gt;, median (IQR)</td>
<td>0.283 (1.4)</td>
<td>0.366 (1.31)</td>
<td>-0.007 (1.4)</td>
<td>&lt;0.001</td>
<td>SCZ &gt; BD &gt; HC</td>
</tr>
</tbody>
</table>


Abbreviations: BD = bipolar spectrum disorders, CRP = C-reactive protein, HC = healthy controls, IQR = interquartile range, NOS = not otherwise specified, N = number, PRS-BD = polygenic risk score of bipolar disorder, PRS-SCZ = polygenic risk score of schizophrenia, SCZ = schizophrenia spectrum disorders.

2.3. Registry data

Registry data of type and time of infections and autoimmune disease from 2006 to 2018 were available from the Norwegian Control and Payment of Health Reimbursement database (KUHR), including data from primary health care, and from 2008 to 2018 from the Norwegian Patient Registry (NPR), including data from the specialist health care. ICD-10 diagnostic codes (Quan et al., 2008) are used in NPR and ICP-2 diagnostic codes (Hofmans-Ookes and Lamberts, 1996) in KUHR.

Number of infections for the twelve-year period 2006–2018 (rate of infections) was retrieved from KUHR and NPR for all groups as an estimate of differences in rates prior to immune assessments; that is, number of infections was summed up for each participant and used as a continuous variable. Repeats of the same diagnosis of infection within and across NPR and KUHR in one person were individually evaluated as constituting the same or different illness episodes based on all available information including length of the interval between the diagnoses. If not possible to determine by the available information, diagnoses were assessed as unique episodes if consecutive equivalent diagnoses were registered more than one month a part; however, repeats of a diagnosis with similar time intervals were considered check-ups and constituting the same episode. All cases of doubt were determined by two physicians. Participants that were neither registered in NPR nor in KUHR were coded with no previously treated infection or autoimmune diseases (N = 78). Six participants were excluded from our analysis due to mismatching of IDs between the TOP sample and the registries.

Presence of autoimmune diseases was retrieved from KUHR and NPR for the 2006–2018 period for all groups as an estimate of differences prior to immune assessments; that is, autoimmune disease was coded as present or absent for each participant and used as a dichotomous variable. Details about infections and autoimmune diagnoses included are given in Supplementary Material.

2.4. Polygenic risk score (PRS)

DNA was extracted from blood and saliva samples collected at inclusion. Genotyping was performed on Human Omni Express 24 v.1.1 (Illumina Inc., San Diego, CA, USA) at deCODE Genetics (Reykjavik, Iceland). Pre-imputation quality control was performed using PLINK 1.9 (Chang et al., 2015). Briefly, variants were excluded if they had low genotyping rate (≤95%), deviated from Hardy-Weinberg equilibrium (p < 10<sup>-5</sup>), or occurred at significantly different frequencies in different genotyping batches (FDR < 0.5). Whole individual genotypes were excluded if they had low coverage (<80%) or high likelihood of contamination (heterozygosity above mean + 5 standard deviations). The quality-controlled genotypes were phased using Eagle (Loh et al., 2016), and missing variants were then imputed with MaCH (Dax et al., 2016; Li et al., 2010) using haplotype reference consortium (HRC) trans-ethnic reference panel (version 1.1) (McCarthy et al., 2016). Following the quality control and imputation procedure, variants with information score < 0.8 or minor allele frequency < 1% were removed. Additionally, individual genotypes were imputed with less than 75% confidence were set to missing, the remaining ones were converted to best guess hard allelic dosages. PRS were computed following the method described by Purcell et al. (2009) using PRSice-2 (Choi and O’Reilly, 2019) with default clamping parameters (250 kb clumping window, 0.10 LD r2 threshold using target sample for LD estimation). The high linkage disequilibrium (LD)-regions MHC region (chr6:25119106-33,854,733) and 8p23.1 (chr8:7200000-12,500,000) were excluded in all analyses. The PRS of schizophrenia, ‘PRS-SCZ’, was based on the latest meta-analysis from the Psychiatric Genomics Consortium Schizophrenia Working Group and included participants with European ancestry with schizophrenia and schizoaffective disorder (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020), after removing the TOP cohort. The PRS of bipolar disorder, ‘PRS-BD’, was based on a meta-analysis of 56 cohorts and included patients with European ancestry meeting international consensus criteria (DSM-IV, ICD-9, or ICD-10) for a lifetime diagnosis of bipolar disorder (Mullins et al., 2021), after
removing the TOP cohort. In accordance with previous literature (Wimberley et al., 2017; Wray et al., 2014), we used a p-value threshold of 0.05 for selection of single nucleotide polymorphisms (SNPs) in our PRS computation. The PRS-SCZ and PRS-BD were standardized before the analyses were performed.

2.5. Immune markers

Immune markers were selected based on previous evidence of associations to patient status, and constitute sIL-2R, a marker of T-cell activity, IL-1Ra, a marker of IL-1b activity, IL-18, a cytokine belonging to the IL-1 superfamily, soluble glycoprotein 130 (sgp130), a soluble form of the IL-6 co-receptor reflecting activity in the IL-6 system, sTNFR-1, one of the two main receptors of TNF-α reflecting TNF-activity, APRIL, a cytokine belonging to the TNF-family, and ICAM-1, a cell adhesion molecule (Akevold et al., 2014; Dinarello, 2018; Drexhage et al., 2010; George-Chandy et al., 2008; Girardin et al., 1992; Goldsmith et al., 2016; Kroken et al., 2018; Merch et al., 2016; Merch et al., 2019; Müller, 2019; Potvin et al., 2008). Following the same methods as described in Merch et al. (2019) plasma levels of the immune markers were measured in duplicate by enzyme immunoassays (EIA) by using commercially available antibodies (R&D Systems, Minneapolis, MN, USA) in a 384 mm format using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT, USA) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Bio-Rad, Hercules, CA, USA). Intra- and inter-assay coefficients of variation were <10% for all EIA. For immunoassays, blood was sampled using EDTA vials and the plasma was isolated within the next working day and stored at ~80 °C. Blood sampling was performed between 8:00 and 17:00.

2.6. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago II, version 26). For demographic and clinical data, we used Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. The main analyses were performed with analysis of covariance (ANCOVA). To comply with test assumptions, immune markers were logarithmically transformed with standardized removal of residuals more than 3 x inter-quartile range (IQR) or 1.5 x IQR below and above the first and third quartile, respectively, depending on the degree of deviation (3 IQR for sgp130, sTNFR-1, IL-18 and ICAM-1 and 1.5 IQR for IL-1Ra, sIL-2R and APRIL). Histograms, Q-Q-plots and Kolmogorov-Smirnov statistics were used to assess normal distribution. Potential issues with multicollinearity were ruled out. We first compared immune marker levels across groups (SCZ, BD, HC) using ANCOVA. We then ran bivariate correlations between rate of infections, presence of autoimmune disease and PRSs and immune markers in the patient groups, to select variables (p ≤ 0.1) for fully adjusted ANCOVAs. Lastly, we compared patient groups to HC with and without adjustments for of infections (twelve-year), presence of autoimmune disease and PRS, respectively, to address the impact of these factors on the effect of patient group. These main ANCOVA analyses were adjusted for age, sex and freezer storage time (Enroth et al., 2016) and additionally for ethnicity (European, Asian, African and others), batch and the two first genetic principal components in the PRS analyses. PRS-SCZ and PRS-BD were tested in analyses of SCZ and BD, respectively. To exclude major confounding effects of body mass index (BMI), smoking status (smoking regularly, yes/no) and alcohol use (number of units of alcohol last two weeks) of which we had incomplete data (40-80%), separate bivariate correlations were performed with significant factors of the main ANCOVA analyses (autoimmune disease, PRS-SCZ); if significant, these variables were also tested in fully adjusted ANCOVAs. Moreover, the main ANCOVAs were redone without participants with MDD (N = 59) to exclude potential bias due to heterogeneity within the BD group. Due to the established association of the selected immune markers with affective and psychotic conditions, we applied a corrected significance level of pF = 0.025 due to testing of non-genetic and genetic models (0.05/2).

3. Results

3.1. Demographic, clinical, genetic and immune data

Median age was significantly lower in SCZ compared to BD and HC (both p < 0.001). Frequency of European ethnicity differed between all groups (HC > BD > SCZ, all p < 0.001). Rate of infections differed significantly between groups (BD > HC > SCZ, all p < 0.001), as did rate of specialist health care treated infections (BD > SCZ, p = 0.043, SCZ > HC, p < 0.001 and BD > HC, p < 0.001). A diagnosis of one or more autoimmune diseases was significantly more frequent in BD than in SCZ and HC (p = 0.004 and p = 0.003, respectively). The PRS-SCZ differed significantly between all three groups (SCZ > BD > HC, all p < 0.001), and the PRS-BD was significantly higher in both patient groups compared to HCs (both p < 0.001). See Table 1 for details.

3.2. Associations of immune marker levels with diagnosis, rate of infections, autoimmune disease and PRS

Plasma levels of all immune markers differed significantly between patients and HC (Supplementary Table 1); however, case-control differences are more specifically reported in other papers from our group (Engh et al., 2022; Merch et al., 2017; Merch et al., 2019; Sheikh et al., 2021; Szabo et al., 2022). Infection rate correlated negatively with plasma levels of sgp130 (SCZ, r = −0.09, p = 0.04; BD, r = −0.16, p = 0.01 and IL-18 (BD, r = −0.10, p = 0.05). Presence of autoimmune disease correlated positively with IL-1Ra (SCZ, r = 0.11, p = 0.01), sIL-2R (SCZ, r = 0.16, p < 0.001), sgp130 (SCZ, r = 0.13, p = 0.002), sTNFR-1 (SCZ, r = 0.08, p = 0.05), ICAM-1 (SCZ, r = 0.07, p = 0.10) and negatively with APRIL (BD, r = −0.10, p = 0.06). PRS-SCZ correlated negatively with sIL-2R (SCZ, r = −0.11, p = 0.02). See Supplementary Table 2 for details of all correlation analyses.

3.3. Immune aberrations in patients explained by rate of infections, autoimmune disease and PRS

Comparisons of immune marker levels with and without adjustment of each of rate of infections, autoimmune disease and PRS of SCZ and BD versus HC were as follows:

Infection rate had no significant effect (p = 0.97 (sgp130, SCZ), p = 0.66 (sgp130, BD), p = 0.30 (IL-18, BD)) on the indicated immune markers in comparisons of patient groups with HC in fully adjusted models.

Presence of autoimmune disease was significantly positively associated with sIL-2R ([F(1,1021) = 10.305, p = 0.001, partial eta squared = 0.010]) in the adjusted model; the difference between SCZ and HC in plasma level of sIL-2R decreased from 11.2% to 10.7% when adding autoimmune disease to the model, reflecting a minor impact of presence of autoimmune disease on increased sIL-2R levels in SCZ. IL-1Ra, sgp130, sTNFR-1, APRIL and ICAM-1 were not significantly associated to autoimmune disease in the adjusted models (p = 0.12, p = 0.09, p = 0.40, p = 0.60 and p = 0.07, respectively).

PRS-SCZ was negatively associated with sIL-2R ([F(1,922) = 7.107, p = 0.01, partial eta squared = 0.008]) in the adjusted model; the difference between SCZ and HC in sIL-2R level increased from 15.1% to 17.2% when adding PRS-SCZ, reflecting minor negative impact of PRS-SCZ on sIL-2R levels in SCZ. See Table 2 for details of all ANCOVAs. In sub-sample analyses, BMI, but not smoking status or alcohol use, was significantly correlated with autoimmune disease and PRS-SCZ; however, no significant effect of BMI on sIL-2R levels were suggested in fully adjusted models (p = 0.99 and p = 0.69, respectively). Moreover, additional analyses of BD without participants with MDD did not change the main findings.

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Table 2
Main effects of rate of infections, autoimmune disease and PRS on immune markers in ANCOVA analyses. *

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Groups</th>
<th>Immune marker</th>
<th>df</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of infections</td>
<td>SCZ and HC</td>
<td>sgp130</td>
<td>11,091</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>Rate of infections</td>
<td>BD and HC</td>
<td>sgp130</td>
<td>1836</td>
<td>0.194</td>
<td>0.66</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>SCZ and HC</td>
<td>sIL-2R</td>
<td>11,188</td>
<td>1.056</td>
<td>0.30</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>HC</td>
<td>sIL-1Ra</td>
<td>11,067</td>
<td>2.435</td>
<td>0.12</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>BD and HC</td>
<td>APRIL</td>
<td>11,167</td>
<td>0.275</td>
<td>0.60</td>
</tr>
<tr>
<td>PRS-SCZ</td>
<td>SCZ and HC</td>
<td>sIL-2R</td>
<td>1922</td>
<td>7.107</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: APRIL = a proliferation inducing ligand, BD = bipolar spectrum disorder, df = degrees freedom, ICAM-1 = Intercellular Adhesion Molecule 1, IL-18 = interleukin-18, IL-1Ra = interleukin-1 receptor antagonist, PRS = polygenic risk score, SCZ = schizophrenia spectrum disorder, sIL-2R = soluble interleukin-2 receptor, sgp130 = soluble glycoprotein 130.

* Adjusted for: age, sex, freezer storage time, group, and additionally ethnicity, genetic principal component 1 and 2 and genetic batch in PRS analyses, dependent variables: immune markers.

4. Discussion

While we found significant differences in rate of infections and autoimmune diseases in patients with SCZ and BD compared to HC, only minor associations with abnormal immune marker levels were indicated. The highest rate of total infections was found in BD and the lowest in SCZ, while presence of autoimmune disease was higher in BD than in SCZ and HC. While some correlations with immune markers were observed, only sIL-2R was significantly associated with autoimmune disease and PRS-SCZ in fully adjusted models across SCZ and HC; however, these associations represented only minor changes in immune marker differences between the groups. Thus, although we found increased frequency of infections and autoimmunity in BD, the present results did not provide strong evidence that infectious or autoimmune burden or genetic risk contributes to the systemic inflammation observed in SCZ and BD.

The immune hypothesis of severe mental disorders states that infections and autoimmune diseases may be involved in the development of the disorders through activation of inflammatory and immune-mediated processes (Benros and Mortensen, 2020; Köhler et al., 2017). Indeed, numerous studies support a role of inflammatory factors in SCZ and BD pathophysiology as reflected by dysregulated levels of systemic inflammatory markers. Although the current findings cannot disprove the hypothesis, they provide little support. Rather, a pattern of minor or non-detectable influences of infections and autoimmune diseases on the inflammatory state was suggested. This adds novel information to our understanding of the inflammatory processes of these disorders. Notwithstanding, inflammatory and immune mediated mechanisms do not “depend on” classical autoimmune and infectious disorders. Several factors such as altered gut microbiota, molecular mimicry, danger associated molecular patterns (DAMPs) and enhanced oxidative stress could elicit local and systemic inflammation in patients with SCZ and BD. Importantly, metabolic disturbances including dyslipidaemia and insulin resistance secondary to antipsychotic treatment and decreased physical activity are frequent in severe mental disorders and promote subclinical inflammation (Henderson et al., 2015).

While the investigated markers were chosen based on previous findings of differences between patients and controls (Kroken et al., 2018; Merch et al., 2016), we cannot exclude that other markers of inflammation and immune activation than those selected in this study could be of importance in the pathogenesis of SCZ and BD and in addition related to infections, autoimmunity and PRS. However, current variation in the immune markers is in line with low-grade inflammation in SCZ and BD and supports their role in these disorders.

The current results provide some interesting insights into the role of specific immune marker levels and association with autoimmunity and PRS-SCZ. In analysis of SCZ and HC, associations to sIL-2R, a stable and reliable marker of T cell activation, survived adjustments for all confounders. The relationship between autoimmune disease and sIL-2R corresponds to previous studies indicating correlations of sIL-2R with disease activity in autoimmune diseases in the general population (Rubin and Nelson, 1990). sIL-2R might particularly be associated with immune diseases where enhanced T cell activation is thought to play an important pathogenic role (Adachi et al., 1999; Barak et al., 2009; Mavropoulou et al., 2020), some of which share genetic risk variants with SCZ (Pouget, 2018). IL-2R is implicated in the macrophage T-lymphocyte theory of SCZ, which involves failing of activated macrophages to properly control T-lymphocyte secretion of IL-2 and IL-2R (Smith, 1992). sIL-2R is also part of the compensatory immune-regulatory reflex system (CIRS) which has been implicated in the SCZ pathophysiology (Roozmans et al., 2020). While our findings indicate that sIL-2R is related to autoimmune diseases as well as to PRS-SCZ, plasma level increases in SCZ is not explained by the associations. Still, a common genetic susceptibility for SCZ and immune system activity is suggested.

A unique feature of the current study is the access to diagnostic data from primary care physicians in addition to the specialist health care. When analyzing hospital data separately, we found increased rates of infections in both SCZ and BD compared with HC; this is in line with previous reports (Jeppesen and Benros, 2019) of large registry-based studies showing increased risk of both disorders with more hospital-treated infections (Benros et al., 2011; Benros et al., 2013; Köhler-Forsberg et al., 2019; Pankiewicz-Dulacz et al., 2018). By adding data from primary health care, we obtained a more comprehensive characterization of the infection rate, which interestingly indicated increased rates in BD, and not in SCZ. However, the finding need replication and should be interpreted with caution, as the sample size and assessed period is limited and confounds are likely. Differences in help-seeking behaviour (Oud and Meyboom-de Jong, 2009) is a potential explanation, as people with SCZ and BD might be less eager to seek help from primary care while at the same time more frequent users of emergency departments (Niedzwiecki et al., 2018). Also, presence of autoimmune disease was more prevalent in BD, but not in SCZ, compared with HC. This is in contrast with previous findings of increased prevalence of autoimmune disease in both groups (Chen et al., 2021; Eaton et al., 2006) and may partly explain the minor impact of the association between autoimmune disease and sIL-2R in sIL-2R alterations in SCZ (Benros et al., 2011).

Associations of immune genetic variants with SCZ and BD and genetic pleiotropy with immune related phenotypes (Andreasen et al., 2015; Tylee et al., 2018; Wang et al., 2015), suggest that the low-grade inflammation of these disorders involve a shared genetic liability (Ripke et al., 2014; Stefansson et al., 2009). The current results indicate that alterations in immune markers display only a minimal degree of association with disease genetics. We found a significant negative association between PRS-SCZ and one immune marker level (sIL-2R); however, the effect was small, and the direction did not explain the increased level in SCZ. The lack of other significant associations with PRS in our study complement previous findings of no significant interaction between PRS-SCZ and hospital-treated infections on risk of SCZ (Benros et al., 2016). The findings also fit with the results from a twin study showing that variations in the human immune system is largely driven by non-heritable influences (Brodin et al., 2015). Still, it is possible that the PRS does not capture the selected immune markers, although a smaller study indicated an effect on the CCL4 chemokine (Maj et al., 2019).
Moreover, as immune activation might vary with clinical characteristics, such as negative symptoms (Goldsmith et al., 2018) or acute relapse (Miller et al., 2011), one might hypothesize associations in clinical subgroups. It is also possible that current PSS are not adequately pow-
ered, as they only capture up to 7.7% of the variance in the phenotype (The Schizophrenia Working Group of the Psychiatric Genomics Con-
sortium et al., 2020), and that the standard removal of the high LD MHC region to avoid spurious findings, might have limited the ability to indicate true associations (Mangalam et al., 2013; Sekar et al., 2016).

Strengths of the current study includes detailed characterized data and a large sample size. To our knowledge this is the first study to investigate the associations between immune markers and PSS, in-
fec tions and autoimmune in SCZ and BD simultaneously. This combi-
nation enabled us to probe the current immune-etiopathogenic model linking immunogenic conditions with inflammation and severe mental disorders. Limitations include lack of registry data before 2006, thus we were unable to record the complete life-time number of infections and autoimmune diagnoses prior to immune assessments. However, avail-
able twelve-year complete registry data, also including diagnoses after immune assessments, allowed us to compare diagnosis rates between groups and assess the impact of this variation on immune marker alter-
tations. In line with previous studies, we found higher rates of specialist health care treated infections in SCZ and BD compared to HC, indicating valid data. Still, lack of life-time data, including perinatal and early childhood infection rate (Barichello et al., 2016; Blomstrem et al., 2014), may be a reason for the negative findings, although supple-
menting the tests of twelve-year rate of infections with years at risk of infections prior to immune assessments (i.e. impact of age in adjusted models, data not shown), showed similar results. However, due to ge-
etic components in susceptibility to infections (Chapman and Hill, 2013), one could speculate that current rates of infections retrieved from registries, might also be an indicator of early infection rates. Other limitations are the cross-sectional design with measurement at one time point as well as assessing a limited range of immune markers and pathways; however, the immune marker selection was based on robust associations previous findings. Also, several factors suggested to in-
crease inflammation, such as social impairments, stress or trauma (Baumeister et al., 2016; Buske-Kirschbaum et al., 2007; Khandaker et al., 2017;Wieck et al., 2013) were not included. Due to the lack of consensus for choosing p-value threshold for PSS calculation, we cannot exclude the possibility of more appropriate thresholds than 0.05, with impact on the results. Lastly, the use of PSS’ computed from GWAS of participants of European ancestry in a sample of admixed ancestries, could affect PSS performance (Duncan et al., 2019), however, the target sample for PSS is mostly (90%) of European ancestry and all main an-
alyses of PSS were adjusted for ethnicity.

By investigating central immune activating conditions, genetics and inflammatory mediators in SCZ and BD concomitantly, we were able to test the immune etiopathogenic model of these severe mental disorders. Our findings of sparse association between markers of immune activa-
tion in SCZ and BD and rate of infections, autoimmune disease and ge-
etic susceptibility suggest that the inflammatory abnormalities of these disorders are mainly driven by other factors. Larger studies with lon-
titudinal data are needed to further clarify the mechanisms involved in inflammatory processes in SCZ and BD.

Role of funding sources

This work was supported by the Research Council of Norway (grant numbers 262656, 273291, 248778, 232373); and the South-Eastern Norway Regional Health Authority (grant number 2019-108, 2017-
112). The funding sources were not involved in collection, analyzing or inter-
pretation of data, writing of report or decision to submit this research paper.

Supplementary data to this article can be found online at https://doi.
Supplementary material for paper I:
Limited association between infections, autoimmune disease and genetic risk and immune activation in severe mental disorders

Content
Supplementary material
Supplementary table 1
Supplementary table 2
**Supplementary material.** Overview of included diagnoses of infections and autoimmune diseases retrieved from Norway Control and Payment of Health Reimbursement database (KUHR) and the Norwegian Patient Registry (NPR)

From NPR (specialist health care), all diagnoses from the general infection chapters A and B were included, except HIV and AIDS (B20-24), further, inflammatory diseases of the CNS (G00-G08), hordeolum and chalazion (H00), keratitis (H16), suppurative and unspecified otitis media, otitis media in diseases classified elsewhere (H66-67), cute rheumatic fever (I00-I02), acute pericarditis (I30), phlebitis and thrombophlebitis (I80), acute upper respiratory infections, influenza and pneumonia, other acute lower respiratory infections (J00-J22), chronic sinusitis (J32), peritonsillar abscess (J36), suppurative and necrotic conditions of lower respiratory tract (J85-86), acute appendicitis (K35), unspecified appendicitis (K37), abscess of anal and rectal regions (K61), peritonitis (K65), cholecystitis (K81), infections of the skin and subcutaneous tissues (L00-L08), infectious arthropathies (M00-M03), acute tubule-interstitial nephritis (N10), cystitis (N30), urethritis and urethral syndrome (N34), inflammatory diseases of prostate (N41), orchitis and epididymitis (N45), inflammatory disorders of the breast (N61), salpingitis and oophoritis, inflammatory disease of uterus, except cervix, inflammatory disease of cervix uteri, other female pelvic inflammatory disease (N70-73), infections of genitourinary tract in pregnancy (O23), puerperal sepsis, other puerperal infections (O85-86), infections of breast associated with childbirth (O91) and maternal infectious and parasitic diseases classifiable elsewhere but complicating pregnancy, childbirth and the puerperium (O98) were included as episodes of infection. From KUHR (primary health care), general infections (A70-78), lymphadenitis (B70), gastrointestinal infections (D70-73), appendicitis (D88), worms/other parasites (D96), infections conjunctivitis (F70), blepharitis/style/chalazion, eye infection (F72-73), trachoma (F86), ear infections (H70-74), infection of circulatory system (K70), infections of musculoskeletal system (L70), neurological infections (N70-73), respiratory infections (R71-78, R80-83), skin infections (S70-73, S76, S84), endocrine infections (T70), urological infections (U70-72), puerperal infections and infections complicating pregnancy (W70-71, W94), female genital infections (X70-74, X84, X90-92) and male genital infections (Y70-75) were included as episodes of infection.

From NPR, sarcoidosis (D86), thyrotoxicosis (E05), type 1 diabetes mellitus (E10), multiple sclerosis (G35), other demyelinating diseases of CNS (G37), iridocyclitis (H20), optic neuritis (H46), Crohn’s disease and ulcerative colitis (K50-51), intestinal malabsorption (K90),
psoriasis (L40), alopecia areata (L63), vitiligo (L80), lupus erythematos (L93), seropositive rheumatoid arthritis (M05), juvenile arthritis (M08), Sicca syndrome (M35) and ankylosing spondylitis (M45) were included as diagnoses of autoimmune disease. From KUHR, chronic enteritis/ulcerative colitis (D94), rheumatoid/seropositive arthritis (L88), multiple sclerosis (N86), psoriasis (S91) and insulin dependent diabetes (T89) were included as diagnoses of autoimmune disease.
**Supplementary table 1. Immune data across groups**

<table>
<thead>
<tr>
<th>Immune marker(^a), median (IQR)</th>
<th>SCZ</th>
<th>BD</th>
<th>HC</th>
<th>P-value(^d)</th>
<th>Group comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1Ra(^b)</td>
<td>224 (301)</td>
<td>191 (284)</td>
<td>185 (248)</td>
<td>&lt;0.001</td>
<td>SCZ&gt;BD, HC</td>
</tr>
<tr>
<td>sIL-2R(^c)</td>
<td>0.27 (0.16)</td>
<td>0.26 (0.15)</td>
<td>0.24 (0.12)</td>
<td>&lt;0.001</td>
<td>SCZ, BD&gt;HC</td>
</tr>
<tr>
<td>IL-18(^b)</td>
<td>982 (1555)</td>
<td>743 (1410)</td>
<td>692 (1164)</td>
<td>&lt;0.001</td>
<td>SCZ&gt;BD&gt;HC</td>
</tr>
<tr>
<td>sgp130(^c)</td>
<td>215 (59)</td>
<td>210 (55)</td>
<td>225 (58)</td>
<td>&lt;0.001</td>
<td>HC&gt;BD, SCZ</td>
</tr>
<tr>
<td>sTNFR-1(^c)</td>
<td>1.75 (0.69)</td>
<td>1.67 (0.52)</td>
<td>1.60 (0.77)</td>
<td>&lt;0.001</td>
<td>SCZ, BD&gt;HC</td>
</tr>
<tr>
<td>APRIL(^b)</td>
<td>250 (211)</td>
<td>244 (213)</td>
<td>326 (260)</td>
<td>&lt;0.001</td>
<td>HC&gt;SCZ, BD</td>
</tr>
<tr>
<td>ICAM-1(^c)</td>
<td>275 (115)</td>
<td>267 (105)</td>
<td>250 (95)</td>
<td>&lt;0.001</td>
<td>SCZ, BD&gt;HC</td>
</tr>
</tbody>
</table>

\(^a\)Reported in Engh et al., 2021; Mørch et al., 2017; Mørch et al., 2019; Sheikh et al., submitted; Szabo et al., 2022.

\(^b\)pg/ml; ‘ng/ml; \(^d\)ANCOVA for immune markers after log-transformation with adjustments for age, sex and freezer storage time.

Abbreviations: APRIL = A proliferation-inducing ligand, BD = bipolar spectrum disorders, HC = healthy controls, ICAM-1 = Intercellular Adhesion Molecule 1, IL-1Ra = Interleukin-1 Receptor antagonist, IL-18 = Interleukin-18, IQR = interquartile range, SCZ = schizophrenia spectrum disorders, SMD = severe mental disorders (SCZ and BD), sgp130 = soluble glycoprotein 130, sIL-2R = soluble Interleukin-2 Receptor, sTNFR-1 = soluble Tumour Necrosis Factor 1.
**Supplementary table 2.** Bivariate correlations\(^a\) between immune markers and rate of infections, autoimmune disease and PRS in SCZ and BD separately.

<table>
<thead>
<tr>
<th></th>
<th>SCZ</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-1Ra</td>
<td>sIL-2R</td>
<td>sgp130</td>
<td>sTNFR-1</td>
<td>IL-18</td>
<td>APRIL</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>Rate of infections</td>
<td>0.02</td>
<td>0.03</td>
<td><strong>-0.09</strong></td>
<td>-0.05</td>
<td>-0.05</td>
<td>0.02</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>(0.58)</td>
<td>(0.55)</td>
<td>(0.04)</td>
<td>(0.28)</td>
<td>(0.21)</td>
<td>(0.67)</td>
<td>(0.46)</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td><strong>0.11</strong></td>
<td><strong>0.16</strong></td>
<td>0.13</td>
<td><strong>0.08</strong></td>
<td>0.04</td>
<td>0.01</td>
<td><strong>0.07</strong></td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(&lt;0.001)</td>
<td>(0.002)</td>
<td>(0.05)</td>
<td>(0.28)</td>
<td>(0.83)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>PRS-SCZ</td>
<td>-0.04</td>
<td><strong>-0.11</strong></td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.06</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>(0.40)</td>
<td>(0.02)</td>
<td>(0.73)</td>
<td>(0.60)</td>
<td>(0.19)</td>
<td>(0.42)</td>
<td>(0.43)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-1Ra</td>
<td>sIL-2R</td>
<td>sgp130</td>
<td>sTNFR-1</td>
<td>IL-18</td>
<td>APRIL</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>Rate of infections</td>
<td>n/a</td>
<td>0.03</td>
<td><strong>-0.16</strong></td>
<td>-0.08</td>
<td><strong>-0.10</strong></td>
<td>0.07</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.66)</td>
<td>(0.01)</td>
<td>(0.19)</td>
<td>(0.05)</td>
<td>(0.21)</td>
<td>(0.31)</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>n/a</td>
<td>0.05</td>
<td>-0.01</td>
<td>-0.07</td>
<td>-0.05</td>
<td><strong>-0.10</strong></td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.44)</td>
<td>(0.87)</td>
<td>(0.22)</td>
<td>(0.30)</td>
<td>(0.06)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>PRS-BD</td>
<td>n/a</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.04</td>
<td>-0.03</td>
<td>0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.91)</td>
<td>(0.26)</td>
<td>(0.55)</td>
<td>(0.60)</td>
<td>(0.53)</td>
<td>(0.30)</td>
</tr>
</tbody>
</table>

\(^a\)Pearson’s r for autoimmune (dichotomous) and PRS, Spearman’s rho for rate of infections. P-value given in parenthesis, ≤0.1 in bold.

Abbreviations: APRIL = A proliferation-inducing ligand, BD = bipolar spectrum disorders, ICAM-1 = intercellular adhesion molecule 1, IL-1Ra = interleukin 1 receptor antagonist, IL-18 = interleukin 18, n/a = not applicable, PRS = polygenic risk score, sgp130 = soluble glycoprotein 130, sIL-2R = soluble interleukin 2 receptor, sTNFR-1 = soluble tumor necrosis factor receptor 1, SCZ = schizophrenia spectrum disorders.
Paper II
Immune marker levels in severe mental disorders: associations with polygenic risk scores of related mental phenotypes and psoriasis

Maren Caroline Frogner Werner, Katrine Verena Wirgenes, Alexey Shadrin, Synve Hoffart Lundvang, Linn Rødevand, Gabriela Hjell, Monica Bettina Elkjaer Greenwood Ormerod, Marit Haram, Ingrid Agartz, Srdjan Djurovic, Ingrid Melle, Pål Aukrust, Thor Ueland, Ole Andreas Andreassen and Nils Eiel Steen

Several lines of evidence implicate immune abnormalities in the pathophysiology of severe mental disorders (SMD) and comorbid mental disorders. Here, we use the data from genome-wide association studies (GWAS) of autoimmune diseases and mental phenotypes associated with SMD to disentangle genetic susceptibilities of immune abnormalities in SMD. We included 1004 patients with SMD and 947 healthy controls (HC) and measured plasma levels of IL-1Ra, sIL-2R, gp130, sTNFR-1, IL-18, APRIL, and ICAM-1. Polygenic risk scores (PRS) of six autoimmune disorders, CRP, and 10 SMD-related mental phenotypes were calculated from GWAS. General linear models were applied to assess the association of PRS with immune marker abnormalities. We found negative associations between PRS of educational attainment and IL-1Ra ($P = 0.01$) and IL-18 ($P = 0.01$). There were nominal positive associations between PRS of psoriasis and sgp130 ($P = 0.02$) and PRS of anxiety and IL-18 ($P = 0.03$), and nominal negative associations between PRS of anxiety and sIL-2R ($P = 0.02$) and PRS of educational attainment and sIL-2R ($P = 0.03$). Associations explained minor amounts of the immune marker plasma-level difference between SMD and HC. Different PRS and immune marker associations in the SMD group compared to HC were shown for PRS of extraversion and IL-1Ra (interaction effect (IE), $P = 0.002$), and nominally for PRS of openness and IL-1Ra (IE, $P = 0.02$) and sTNFR-1 (IE, $P = 0.04$). Our findings indicate polygenic susceptibilities to immune abnormalities in SMD involving genetic overlap with SMD-related mental phenotypes and psoriasis. Associations might suggest immune genetic factors of SMD subgroups characterized by autoimmunity or specific mental features.

INTRODUCTION

Bipolar disorder (BD) and schizophrenia (SCZ) are among the leading causes of disability worldwide. The pathophysiology of these severe mental disorders (SMD) is not fully understood. Their estimated heritability is 60–80% and recent genome-wide association studies (GWAS) have revealed a large number of common genetic risk variants [1, 2] with extensive overlap between the disorders [3]. Moreover, evidence suggests extensive genetic overlap with several associated traits [4] and comorbid disorders [1, 5]. The growing body of GWAS can be used to generate polygenic risk scores (PRS) of SMD-related phenotypes to study core features of SMD [6].

Involvement of the immune system in SMD is supported by several lines of evidence [7, 8]. Genetic loci in immune-related regions are associated with SCZ and BD [9] and infections during pregnancy, potentially inducing immune activation, increase the child’s risk of developing SCZ and BD decades later [10]. Similarly, large registry studies have identified an increased risk associated with prior infections [11, 12]. Inflammation seems to modulate brain mechanisms underlying clinical characteristics of SMD [13]. Moreover, similar signs of systemic low-grade immune activation and inflammation are found in SCZ and BD [14] and associations with symptoms [15] and pharmacological treatment response [16] are reported. However, the causative mechanisms of immune abnormalities, as well as the pathogenic importance of the different pathways that are upregulated, are unknown.

Autoimmune disease is characterized by immune-mediated attacks by autoantibodies and self-reactive lymphocytes in particular T cells and is a known comorbidity of SMD [17]. There is evidence of shared genetic risk with SMD [9] and neuropsychiatric symptoms in autoimmune diseases [17]. While the immune signaling system is extensive and autoimmune diseases are associated with different circulating immune markers [18], some of these markers are also linked to SMD, suggesting not only...
shared genetic risk factors, but also shared immune-related mechanisms. Such markers include interleukin-18 (IL-18) and interleukin-1 receptor antagonist (IL-1Ra) implicated in among others rheumatoid arthritis (RA), a proliferating-inducing ligand (APRIL) involved in RA and systemic lupus erythematosus (SLE), interleukin-6 (IL-6) in RA and inflammatory bowel disease (IBD) and soluble interleukin-2 receptor (sIL-2R) as a marker of T-cell activation involved in a wide range of autoimmune disorders like IBD and SLE [19–22].

Several mental traits and disorders are genetically associated with SMD [23]. Such examples of mental disorders include major depressive disorder (MDD), anxiety disorders, and autism spectrum disorders (ASD) [24–27]. Further, the genetic association between SMD and different mental traits include cognitive abilities [28], a core impairment of SMD and personality traits of openness, extraversion, and neuroticism [29, 30]. The immune system is also suggested to be involved in these SMD-related disorders and traits. Comorbid mental disorders are associated with immune marker abnormalities [31–34] and immune-related genetic factors [9, 35, 36]. Cognitive abilities correlate with immune factors [37] and immune mechanisms in personality traits are indicated [38].

Physiological levels of immune markers are in addition to a range of non-genetic factors, influenced by complex genetics [39]. Thus, mapping the genetic architecture of immune abnormalities in SMD may provide novel pathophysiological knowledge. Applying multiple PRS of SMD-related phenotypes for explaining characteristics within SMD was recently suggested [4]. Here, we tested the hypothesis that PRS of autoimmune or SMD-related mental phenotypes with immune associations can explain detectable, but small amounts [40] of immune marker abnormalities in SMD. We applied PRS of autoimmune diseases and mental phenotypes, including mental disorders [31–33, 41], cognitive phenotypes [37, 42], and personality traits [38] in a naturalistic sample of SMD to investigate associations with immune markers. SCZ and BD spectrum-specific analyses were further explored.

METHODS

Study setting
The study is a part of the Thematically Organized Psychosis Study (TOP) at the Norwegian Centre for Mental Disorders Research (NORMENT). The TOP study includes patients from the major hospitals in the Oslo region with a diagnosis of a SCZ spectrum (schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, and psychosis not otherwise specified (NOS), from here termed “SCZ”) or BD spectrum (bipolar 1 disorder, bipolar 2 disorder, bipolar disorder NOS, and major depressive disorder with psychotic features, from here termed “BD”) disorder as the main inclusion criteria. Participants must be able to provide written informed consent and be between the ages of 18–65. Healthy controls (HC) are randomly selected from statistical records from the same catchment areas as the patients. HC are included based on no history of severe mental illness, illicit drug abuse or dependency, or close relatives with severe mental illness. Both patients and HC are excluded based on somatic conditions interfering with brain function, neurological disorder, IQ < 70, or history of severe head trauma. In this study, we additionally applied the following exclusion criteria: data of participants other than European ethnicity were not included in the analyses to avoid issues with population stratification, and participants with CRP level of 10 or above (N = 114) were excluded to remove potential effects of acute infection on the immune markers. The resulting sample with immune measures included N = 1004 patients with SMD and N = 947 HCs (Table 1), out of which PRS data was available for N = 1802 individuals (Table 2). Immune measures from the current sample have been reported in overlapping samples [43–47].

Clinical assessment
Diagnoses are made by use of the Structured Clinical Interview (SCID-1) for the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).

Table 1. Demographics and clinical data.

<table>
<thead>
<tr>
<th>SCZ, N = 595</th>
<th>BD, N = 409</th>
<th>HC, N = 947</th>
<th>SMD vs HC*</th>
<th>Subgroup comparisonsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex female, N (%)</td>
<td>253 (42.5)</td>
<td>240 (58.7)</td>
<td>436 (46.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>31.6 (10.1)</td>
<td>35.0 (12.4)</td>
<td>34.0 (9.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>GAF-symptoms, mean (SD)</td>
<td>45 (12)</td>
<td>58 (12)</td>
<td>n/a</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>GAF-Function, mean (SD)</td>
<td>46 (13)</td>
<td>56 (13)</td>
<td>n/a</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Medication, N (%)</td>
<td>494 (83.0)</td>
<td>200 (48.9)</td>
<td>n/a</td>
<td>SCZ &gt; BD</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>82 (13.8)</td>
<td>147 (35.9)</td>
<td>n/a</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Lithium</td>
<td>15 (2.5)</td>
<td>69 (16.9)</td>
<td>n/a</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>170 (28.6)</td>
<td>147 (35.9)</td>
<td>n/a</td>
<td>BD &gt; SCZ</td>
</tr>
<tr>
<td>Diagnose, N (%)</td>
<td>339 (57.0)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>36 (6.1)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Schizophreniform</td>
<td>85 (14.3)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder 1</td>
<td>229 (56.0)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder 2</td>
<td>123 (30.1)</td>
<td>n/a</td>
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<tr>
<td>Bipolar disorder NOS</td>
<td>19 (4.6)</td>
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<tr>
<td>Major depressive disorder</td>
<td>38 (9.3)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Psychosis NOS</td>
<td>82 (13.8)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Brief psychotic disorder</td>
<td>14 (2.4)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>39 (6.6)</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

BD bipolar spectrum disorders, GAF general assessment of functioning scale, ns not significant, n/a not applicable, SCZ schizophrenia spectrum disorders, SMD severe mental disorders.
\*p value of SMD versus HC: chi square for categorical variables, t test for continuous variables.

**p value of SMD versus HC: chi square for categorical variables, ANOVA for continuous variables.
Table 2. Polygenic risk scores.

<table>
<thead>
<tr>
<th>Polygenic risk scores#</th>
<th>SCZ</th>
<th>BD</th>
<th>HC</th>
<th>P valueb</th>
<th>SMD vs HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune diseases and CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>0.04 (1.0)</td>
<td>0.03 (0.9)</td>
<td>−0.07 (1.0)</td>
<td>0.18</td>
<td>n/a</td>
</tr>
<tr>
<td>IBD</td>
<td>0.01 (1.0)</td>
<td>−0.04 (1.0)</td>
<td>0.05 (1.0)</td>
<td>0.13</td>
<td>n/a</td>
</tr>
<tr>
<td>PSOR</td>
<td>−0.03 (0.9)</td>
<td>−0.04 (0.9)</td>
<td>0.08 (0.9)</td>
<td>0.06</td>
<td>HC &gt; SMD</td>
</tr>
<tr>
<td>RA</td>
<td>−0.002 (1.0)</td>
<td>−0.04 (1.0)</td>
<td>−0.02 (1.0)</td>
<td>1.0</td>
<td>n/a</td>
</tr>
<tr>
<td>SLE</td>
<td>−0.03 (0.8)</td>
<td>−0.05 (0.7)</td>
<td>−0.12 (0.7)</td>
<td>0.74</td>
<td>n/a</td>
</tr>
<tr>
<td>TID</td>
<td>0.05 (0.8)</td>
<td>0.10 (0.8)</td>
<td>0.06 (0.8)</td>
<td>0.12</td>
<td>n/a</td>
</tr>
<tr>
<td>CRP</td>
<td>−0.02 (0.9)</td>
<td>0.00 (0.9)</td>
<td>−0.04 (0.9)</td>
<td>0.84</td>
<td>n/a</td>
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<tr>
<td>Mental disorders</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>0.05 (1.0)</td>
<td>0.11 (1.0)</td>
<td>−0.09 (0.9)</td>
<td>&lt;0.001</td>
<td>SMD &gt; HC</td>
</tr>
<tr>
<td>ANX</td>
<td>0.17 (1.0)</td>
<td>0.12 (0.9)</td>
<td>−0.09 (1.0)</td>
<td>&lt;0.001</td>
<td>SMD &gt; HC</td>
</tr>
<tr>
<td>ASD</td>
<td>0.03 (1.1)</td>
<td>0.08 (1.1)</td>
<td>−0.01 (1.0)</td>
<td>0.15</td>
<td>n/a</td>
</tr>
<tr>
<td>MDD</td>
<td>0.06 (1.0)</td>
<td>0.01 (1.0)</td>
<td>−0.08 (1.0)</td>
<td>0.04</td>
<td>SMD &gt; HC</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.05 (1.0)</td>
<td>−0.02 (0.9)</td>
<td>−0.11 (0.9)</td>
<td>0.002</td>
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<td>Cognitive traits</td>
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<td>COG</td>
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<td>0.07 (1.0)</td>
<td>0.02</td>
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<td>−0.01 (1.0)</td>
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<td>SMD &gt; HC</td>
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</table>

*ADHD attention-deficit hyperactivity disorder, ANX anxiety, ASD autism spectrum disorder, BD bipolar spectrum disorders, CD Crohn’s disease, COG cognition, CRP C-reactive protein, EA educational attainment, EXTRA extraversion, HC healthy controls, IBD inflammatory bowel disease, MDD major depressive disorder, n/a not applicable, NEURO neuroticism, OPEN openness, PC principal component, PSOR psoriasis, PTSD post-traumatic stress disorder, RA rheumatoid arthritis, SCZ schizophrenia spectrum disorders, SD standard deviation, SMD severe mental disorders (SCZ and BD), SLE systemic lupus erythematosus, TID type 1 diabetes.

#Standardized values.

bP value of SMD vs HC: ANCOVA with adjustments for PC1, PC2, and genetic batch.
Kolmogorov–Smirnov statistics were used to assess distributions of data. For group comparison of demographic and clinical data, we used t test and ANOVA for continuous variables and Chi-Square test for categorical variables. Pearson’s r or Spearman’s rho were used for correlation analyses. The main analyses were performed with Analysis of Covariance (ANCOVA), with immune markers as dependent variables. Immune marker distributions were carefully assessed, and all markers were logarithmically transformed with standardized removal of residuals more than 3× interquartile range (IQR) or 1.5× IQR below and above the first and third quartile, respectively, depending on the degree of deviation (3 IQR for sgp130, sTNFR-1, IL-18, and ICAM-1 and 1.5 IQR for IL-1Ra, sIL-2R, and APRIL). First, we identified PRS and immune marker variables with indicated (P < 0.1) differences between SMD and HC (Table 2 and Supplementary Table 2) and bivariate correlations (in SMD, Supplementary Table 3). We then performed the main analyses with these variables comparing immune marker differences between SMD and HC (“diagnosis variable”) with and without adjustments for PRS to assess the effect of PRS on immune marker differences. Moreover, all PRS correlating with immune markers in SMD were tested for interaction effects with the diagnosis variable. Main analyses, including interactions, were repeated in subsamples by substituting SMD with SCZ (“subsample SCZ-HC”) and BD (“subsample BD-HC”), respectively. Main analyses were adjusted for age at blood sampling, sex, freezer storage time, genetic principal components 1 and 2 and genetic batch by including these as independent variables in the ANCOVA. Based on established genetic overlap with SMD and immune links of SMD-related phenotypes, we applied a moderate correction of the significance level of P = 0.0125 (0.05/4) to correct for testing of PRS of the four groups of autoimmune disorders, mental disorders, cognitive traits, and personality traits.

RESULTS
Demographic data and immune markers
The mean age was lower in SMD than in HC (P = 0.04) with younger participants in SCZ than in the BD and HC (both P < 0.001). The proportion of females was lowest in SCZ and highest in BD (all P < 0.001), see Table 1. Plasma levels of all immune markers differed significantly between SMD and HC (Supplementary Table 2); however, case-control differences are more specifically reported in other papers from our group [43–47] [submitted]. Bivariate correlation analyses of PRS and immune marker levels in SMD indicated three correlations with PRS of autoimmune diseases (“PRS-Autoimm”) and seventeen for PRS of mental disorders, traits, or cognitive abilities (“PRS-Ment”). Of these, there were one PRS-Autoimm (i.e., PRS-PSOR) and seven PRS-Ment (i.e., PRS-ANX, PRS-MDD, PRS-ADHD, PRS-PTSD, PRS-OPEN, PRS-COG, and PRS-EA) with the indicated difference between SMD and HC. Details are found in Table 2 and Supplementary Table 3.

Immune marker levels: main effects of PRS
PRS-Autoimm: PRS-PSOR was nominally positively associated with plasma levels of sgp130 (P = 0.02); the plasma-level difference between SMD and HC decreased by 0.2 percentage points when adding PRS-PSOR to the model.
PRS-Ment: PRS-ANX was nominally negatively associated with sIL-2R (P = 0.02) and positively with IL-18 (P = 0.03); the plasma-level difference between SMD and HC of sIL-2R and IL-18 increased by 0.6 percentage points and decreased by 2.0 percentage points, respectively, when adding PRS-ANX to the models. PRS-EA was negatively associated with IL-1Ra (P = 0.01) and IL-18 (P = 0.01), and at nominal level with sIL-2R (P = 0.03); the plasma-level difference of IL-1Ra (18.6%) and sIL-2R (13.2%) were unchanged when adding PRS-EA to the models, whereas the plasma-level difference of IL-18 decreased by 0.7 percentage points. See Table 3 for statistical details of PRS main effects. There were no other significant main effects of PRS in the main analyses.

Immune marker levels: interaction effects of PRS
PRS-Autoimm: There were no significant interaction effects between PRS and the diagnosis variable.
PRS-Ment: Nominal SMD specific negative associations was found for PRS-EA and IL-1Ra (P = 0.02 [interaction effect, P = 0.002]) and for PRS-OPEN and IL-1Ra (P = 0.02 [interaction effect, P = 0.002]).

Table 3. Main effects of polygenic risk scores on immune markersa.

<table>
<thead>
<tr>
<th>Immune marker</th>
<th>df</th>
<th>F-ratio</th>
<th>P value</th>
<th>ηp²</th>
<th>PP change</th>
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<tr>
<td>Total sample</td>
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<td></td>
<td></td>
<td></td>
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<td>PRS-PSOR</td>
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<td>1,118</td>
<td>5.86</td>
<td>0.02</td>
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<td>sIL-2R</td>
<td>1,1050</td>
<td>5.19</td>
<td>0.02</td>
<td>0.005</td>
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<tr>
<td></td>
<td>IL-18</td>
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<td>4.77</td>
<td>0.03</td>
<td>0.003</td>
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<td>IL-1Ra</td>
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<td>6.46</td>
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<td>0.006</td>
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<td>sIL-2R</td>
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<td>4.79</td>
<td>0.03</td>
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<td></td>
<td>IL-18</td>
<td>1,1520</td>
<td>6.93</td>
<td>0.01</td>
<td>0.005</td>
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<td>Subsample SCZ-HC</td>
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<td>0.006</td>
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<td>1,1196</td>
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</table>

PRS-ANX: anxiety, BD bipolar spectrum disorders, df degrees freedom, EA educational attainment, IL-18 interleukin-18, IL-1Ra interleukin-1 receptor antagonist, ηp² partial eta squared, PP percentage points, PRS polygenic risk score, PSOR psoriasis, SCZ schizophrenia spectrum disorders, sIL-2R soluble interleukin-2 receptor, sgp130 soluble glycoprotein 130.

a Independent variables: PRS-age, sex, freezer storage time, patients vs healthy controls, genetic principal components 1 and 2 and genetic batch; dependent variables: immune markers.

bSubsample SCZ-HC: participants with schizophrenia spectrum disorders and healthy controls; subsample BD-HC: participants with bipolar spectrum disorders and healthy controls.

cPercentage point changes in immune marker plasma-level difference between diagnostic subgroups and healthy controls by adding PRS to the statistical model.
Supplementary Fig. 1. There were no other significant interaction effects between PRS and the diagnosis variable.

Subsample analysis

For separate analyses of SCZ-HC and BD–HC subsamples, see Table 3 and Supplementary Results. In general, subsample findings corresponded with the main findings. The association between PRS-EA and IL-1Ra in the BD–HC subsample survived correction for multiple testing (P = 0.01) and in addition there was a SCZ-specific association for PRS-EA and ICAM-1 (P = 0.003, interaction effect, P = 0.04). Levene’s test was significant in ANCOVA analysis of sTNFR-1 and in several analyses of IL-18.

DISCUSSION

The main findings of the study were significant associations of PRS-EA, and nominal associations for PRS-PSOR and PRS-ANX, with immune marker levels across patients with SMD and HC. Nominal associations specific for patients of immune levels and PRS-EXTRA and PRS-OPEN were detected by interaction analysis. Despite the limited explanatory power of PRS, the large sample allowed well-adjusted analyses indicating associations of genetic risk for autoimmune diseases and in particular for mental phenotypes with components of low-grade inflammation and immune activation in SMD; however, the correlations constituted small changes in immune marker differences between patients and HC. The involvement of several PRS-Ment might indicate effects on immune activation related to specific phenotypic features within SMD.

PRS-EA was negatively associated with IL-1Ra and IL-18 across SMD and HC, and both associations were confirmed in the SCZ-HC and BD-HC subsamples. We used educational attainment as a proxy for cognitive ability based on known associations [72], including genetic correlation [73]. Importantly, the clinical relevance of our findings is substantiated by reports of cognitive correlations in patient groups. In particular, elevated IL-1Ra is found to be associated with poorer cognition in BD [74] and IL-18 is associated with cognition although with mixed directions in Alzheimer’s disease and SCZ [75, 76]. Moreover, as IL-1Ra is regarded as a marker of IL-1b activity, the combined effects of IL-1Ra and IL-18 could implicate the involvement of Nod-like receptor (NLR) family, pyrin domain-containing 3 (NLRP3) inflammasomes, the major source of both IL-1 and IL-18. Interestingly, NLRP3 inflammasomes have recently been suggested to link psychological stress, depression, and systemic illness [77] and have been linked to severe mental illness [78]. An interaction effect in the subsample analysis suggested a SCZ-specific negative relationship of PRS-EA with ICAM-1 (Supplementary Results). Increased ICAM-1 with decreased general cognitive abilities is found in older people [79]. Similarly, a previous PRS study support of a role for cell adhesion molecules (CAMs) in cognitive function in SCZ [80]. Thus, our findings indicate a polygenic interplay regulating cytokines involved in the cognitive symptom dimension of SMD, potentially explaining immune abnormalities in subgroups with cognitive impairments.

In geno-phenotypic associations with PRS-Autoimm and the examined inflammatory markers, however, our findings suggest an association between PRS-PSOR and sgp130 abnormalities in SMD. Psoriasis is an autoimmune disease with modest systemic aberrations of IL-6, CRP, TNF-α, E-selection, and ICAM [81] and comorbidity with SCZ [82]. Gp130 is the common signal receptor in the IL-6 cytokine family and reflect the activation of several of these cytokines. The positive correlation is also supported by elevated expression of gp130 in patients with psoriasis [83]. Although a minor impact on the difference between SMD and HC, the current association of sgp130 might reflect the genetic risk of sgp130 abnormalities in autoimmune-related subgroups of SMD, in line with the broad comorbidity of autoimmune diseases with SMD [17]. The trend-level lower PRS-PSOR in SMD versus HC is at odds with a recently indicated small positive genetic correlation between SCZ and psoriasis [84]. The difference might be related to the current use of another and more recent GWAS of psoriasis. However, the finding suggests a potential of leveraging genetics of autoimmune diseases in elucidating immune mechanisms in SMD with the increasing power of GWAS.

Associations of PRS-ANX with IL-18 and sIL-2R were also indicated. Anxiety is genetically correlated to [3] and a common comorbidity of SCZ. The comorbidity exists also in prodromal stages [85], suggesting associations beyond overt psychotic symptoms. PRS-ANX was positively associated with IL-18 across SMD and HC, as well as in SCZ. The positive link with IL-18 is further supported by elevated levels of IL-18 in anxiety including increased levels with severity, indicating clinical implications [86]. While PRS-ANX was negatively associated with sIL-2R across SMD and HC, subgroup analyses also suggested a BD-specific negative association not detected in the SMD samples. Others have reported decreased levels of sIL-2R in anxiety [87], thus supporting the current negative correlation. However, sIL-2R may vary with clinical stage in BD [88]. Thus, the interaction between PRS-ANX and BD suggest an interplay with other susceptibilities in sIL-2R regulation related to clinical characteristics. Moreover, the positive association of PRS-ANX with IL-18 and a negative association with sIL-2R, as robust marker of T-cell activation could implicate that innate and adaptive immunity may differently affect anxiety.

In addition to the main effects of PRS of autoimmune and SMD-related mental phenotypes, PRS of personality traits were related to immune markers, particularly IL-1Ra, in interactions with SMD. The findings are in line with the documented association of personality traits with immune aberrations [38]. Decreased PRS-OPEN and PRS-EXTRA were both associated with increased IL-1Ra in SMD and SCZ, similar to the correlation between PRS-OPEN and sTNFR-1 in SMD. Interestingly, both openness and SCZ may be linked to dopamine activity in substantia nigra [89] and creativity [90] and a negative association with psychotic experiences was recently shown in a non-clinical sample [91]. The effects correspond with evidence suggesting widespread interactions in immune mechanisms [92]. Although speculative, one might hypothesize a mechanism involving the hypothalamic–pituitary–adrenal (HPA) axis given its association with immune responses and dysregulation in SMD [93, 94]. The interactions support complex associations underlying immune mechanisms in psychiatric disorders, in line with previous studies [95]. Moreover, common immune pathways linking personality traits and SMD symptomatology may be hypothesized.

The strengths of this study are large, well-characterized sample of participants and a selection of immune markers robustly associated with SMD. The inclusion of SMD-related mental PRS in combination with the immune markers is to our knowledge the first of its kind. Limitations include a cross-sectional format that makes us unable to assess the stability of the associations. Also, the repeated testing increases risk of type 1 error; however, the analyses were based on known immune differences between SMD and HC and all PRS phenotypes were selected based on genetic associations to SMD in association samples. Otherwise, given the lack of studies of underlying mechanisms of systemic inflammation in SMD, a stricter correction for multiple testing may lead to ignoring hypotheses that merit further investigation. Still, the moderate correction for multiple testing and significant Levene’s test of some of the main ANCOVAs, warrants cautious interpretation of the results. Moreover, plasma was isolated and frozen within the next working day, as immediate freezing was not feasible. Although conflicting evidence [96] studies suggest reasonable stability of immune markers under various conditions [97]. Thus, while bias from potential blood handling issues or other non-specific effects...
cannot be fully excluded, a significant impact on the results seems less likely in the current setting of well-adjusted analyses and a large sample size. Moreover, major influence by unidentified factors is further prevented by the careful removal of outliers from the immune markers. The main associations explained small amounts of the difference in immune marker plasma level between SMD and HC. This is in line with findings in comparable studies [40]. The limited explanatory power is not unexpected given the complexity of SMD mechanisms, the impact of non-genetic influences on the immune system including stress responses related to clinical state [98], high level of polygenicity with potential interaction effects and missing heritability of the phenotypes [66, 99] and uncertain heritability of immune components [100].

In conclusion, we found associations of PRS of immune-linked SMD-related mental phenotypes and nominally of psoriasis with immune markers in SMD and HC. This suggests that genetic susceptibility of these phenotypes might be involved in mechanisms underlying systemic immune abnormalities often seen in SMD, which seem to be partly disease-specific. Although representing small changes, our findings indicate genetic susceptibilities in SMD immune dysregulation for further investigation, potentially of specific interest in subgroups based on autoimmune or mental characteristics, which could be of future relevance in personalized intervention studies. Moreover, the involvement of IL-18a and IL-18 in the detected association with various PRS may underscore a particular role for innate immunity in SMD.

REFERENCES

54. Choi SW, O
53. Purcell SM, Wray NR, Stone JL, Visscher PM, O
ACKNOWLEDGEMENTS
The authors thank the patients for participating in the study and contributing colleagues at NORMENT, especially the bio-psychiatry, genetic, biostatistical, clinical and database group. We would like to thank the research participants and employees of 23andMe, Inc. for making this work possible.

AUTHOR CONTRIBUTIONS
MCFW and NES wrote the first draft of the paper. MCFW, KVW, SHL, LR, GH, MBEGO, MH, IA, SD, IM, PA, TU, OAA, and NES recruited participants and gathered clinical data and blood samples or analyzed immune markers. NES, OAA, and KWW planned, supervised, and coordinated the work. MCFW, NES, OAA, KWW, and AS analyzed the data and interpreted the results. All authors contributed to and revised the article for important intellectual content and approved the final article. All authors are accountable for all aspects of the work.

FUNDING
This work was supported by the Research Council of Norway (grant numbers 262656, 273291, 248778, and 223273); and the South-East Norway Regional Health Authority (grant numbers 2019-108 and 2017-112).

COMPETING INTERESTS
Author OAA has received Speaker’s honorarium from Lundbeck, Sunovion, and is a consultant to HealthLytix. The remaining authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-022-01811-6.

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Supplementary material for paper II:

Immune marker levels in severe mental disorders: associations with polygenic risk scores of related mental phenotypes and psoriasis

**Content**

Supplementary results
Supplementary table 1
Supplementary table 2
Supplementary table 3
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**Supplementary results.** Interaction effects of PRS in diagnostic subsamples

*Subsample SCZ-HC*: SCZ specific negative associations were found for PRS-EA and ICAM-1 (p = 0.003 [interaction effect, p = 0.01]) and PRS-EXTRA and IL-1Ra (p = 0.03 [interaction effect, p = 0.001]).

*Subsample BD-HC*: A BD specific negative association was found for PRS-ANX and sIL-2R (p = 0.01 [interaction effect, p = 0.04]).
### Supplementary table 1. Summary of the GWASs used for calculation of PRS

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<th>Phenotype abbreviation</th>
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<td>Min-Tzu Lo</td>
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**Supplementary table 2. Immune data**

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<tr>
<td>sIL-2R&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 (0.2)</td>
<td>0.26 (0.2)</td>
<td>0.24 (0.1)</td>
<td>&lt;0.001</td>
<td>SMD&gt;HC</td>
</tr>
<tr>
<td>sgp130&lt;sup&gt;c&lt;/sup&gt;</td>
<td>215 (51)</td>
<td>208 (59)</td>
<td>224 (60)</td>
<td>&lt;0.001</td>
<td>HC&gt;SMD</td>
</tr>
<tr>
<td>sTNFR-1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.75 (0.7)</td>
<td>1.68 (0.5)</td>
<td>1.60 (0.8)</td>
<td>0.001</td>
<td>SMD&gt;HC</td>
</tr>
<tr>
<td>IL-18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>978 (1613)</td>
<td>769 (1447)</td>
<td>731 (1226)</td>
<td>&lt;0.001</td>
<td>SMD&gt;HC</td>
</tr>
<tr>
<td>APRIL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>250 (217)</td>
<td>253 (242)</td>
<td>334 (272)</td>
<td>&lt;0.001</td>
<td>HC&gt;SMD</td>
</tr>
<tr>
<td>ICAM-1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>271 (116)</td>
<td>273 (107)</td>
<td>251 (97)</td>
<td>&lt;0.001</td>
<td>SMD&gt;HC</td>
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</tbody>
</table>

<sup>a</sup>Reported in Engh et al., 2021; Mørch et al., 2017; Mørch et al., 2019; Szabo et al., 2022; Sheikh et al., submitted.

<sup>b</sup>pg/ml; <sup>c</sup>ng/ml; <sup>d</sup>p-value of SMD vs HC: ANCOVA for immune markers (log-transformed) with adjustments for age, sex and freezer storage time.

Abbreviations: APRIL = A proliferation-inducing ligand, BD = bipolar spectrum disorders, HC = healthy controls, ICAM-1 = Intercellular Adhesion Molecule 1, IL-1Ra = Interleukin-1 Receptor antagonist, IL-18 = Interleukin-18, IQR = interquartile range, SCZ = schizophrenia spectrum disorders, SMD = severe mental disorders (SCZ and BD), sgp130 = soluble glycoprotein 130, sIL-2R = soluble Interleukin-2 Receptor, sTNFR-1 = soluble Tumour Necrosis Factor 1.
Supplementary table 3. Bivariate correlations\textsuperscript{a} between PRS and immune markers in all patients

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<tr>
<th></th>
<th>IL-1Ra</th>
<th>sIL-2R</th>
<th>sgp130</th>
<th>sTNFR-1</th>
<th>IL-18</th>
<th>APRIL</th>
<th>ICAM-1</th>
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<td></td>
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<td>-0.02</td>
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<td>(0.41)</td>
<td>(0.69)</td>
<td>(0.35)</td>
<td>(0.93)</td>
<td>(0.39)</td>
<td>(0.11)</td>
<td>(0.78)</td>
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<td>-0.01</td>
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<tr>
<td></td>
<td>(0.90)</td>
<td>(0.96)</td>
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<td>(0.79)</td>
<td>(0.22)</td>
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<td>0.04</td>
<td>-0.01</td>
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<tr>
<td></td>
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<td>(0.43)</td>
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<td>(0.83)</td>
<td>(0.21)</td>
<td>(0.12)</td>
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<td>(0.38)</td>
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<td>(0.85)</td>
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<td>(0.63)</td>
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<tr>
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<td>(0.57)</td>
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<tr>
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<td>(0.73)</td>
<td>(0.60)</td>
<td>(0.18)</td>
<td>(0.25)</td>
<td>(0.49)</td>
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<tr>
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<tr>
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<td>(0.51)</td>
<td>(0.46)</td>
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<tr>
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<td>PRS-COG</td>
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</tr>
<tr>
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<td>(0.48)</td>
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<td>(0.43)</td>
<td>(0.18)</td>
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<td>PRS-EA</td>
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<td>-0.08</td>
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<td>-0.08</td>
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<tr>
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<td>(0.08)</td>
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<td>(0.04)</td>
<td>(0.01)</td>
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<tr>
<td><strong>Personality traits</strong></td>
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<td>-0.04</td>
</tr>
<tr>
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<td>(0.44)</td>
<td>(0.82)</td>
<td>(0.75)</td>
<td>(0.69)</td>
<td>(0.39)</td>
<td>(0.25)</td>
</tr>
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<td>-0.01</td>
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<tr>
<td></td>
<td>(0.68)</td>
<td>(0.31)</td>
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<td>(0.42)</td>
<td>(0.87)</td>
<td>(0.98)</td>
<td>(0.60)</td>
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<tr>
<td>PRS-OPEN</td>
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<td>-0.02</td>
</tr>
<tr>
<td></td>
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<td>(0.20)</td>
<td>(0.40)</td>
<td>(0.03)</td>
<td>(0.22)</td>
<td>(0.55)</td>
<td>(0.56)</td>
</tr>
</tbody>
</table>
The correlation coefficient (Pearson’s r except Spearman’s rho for PRS-SLE) and p-value in parenthesis is given, p ≤0.1 in bold

Abbreviations: ADHD = attention deficit hyperactivity disorder, AGREE = agreeableness, ANX = anxiety, APRIL = A proliferation-inducing ligand, ASD = autism spectrum disorder, CD = Crohn’s disease, COG = cognition, Cogn traits = cognitive traits, CRP = C-reactive protein, EA = educational attainment, EXTRA = extraversion, IBD = inflammatory bowel disease, IL-1Ra = Interleukin-1 Receptor antagonist, IL-18 = Interleukin-18, ICAM-1 = Intercellular Adhesion Molecule-1, MDD = major depressive disorder, NEURO = neuroticism, OPEN = openness, PSOR = psoriasis, PTSD = post-traumatic stress disorder, RA = rheumatoid arthritis, sIL-2R = soluble Interleukin-2 Receptor, sgp130 = soluble glycoprotein 130, sTNFR-1 = soluble Tumour Necrosis Factor-1, SLE = systemic lupus erythematosus, T1D = type 1 diabetes
Supplementary figure 1. Scatterplots of immune markers and polygenic risk scores based on interaction effects between PRS and diagnosis group (HC and SMD).

Abbreviations: EXTRA = extraversion, HC = healthy controls, IL-1Ra = Interleukin-1 Receptor antagonist, OPEN = openness, PRS = polygenic risk score, SMD = severe mental disorders, sTNFR-1 = soluble Tumour Necrosis Factor-1.
Paper III
Indicated association between polygenic risk score and treatment-resistance in a naturalistic sample of patients with schizophrenia spectrum disorders

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Abstract

Article history:
Received 4 December 2019
Received in revised form 2 March 2020
Accepted 5 March 2020
Available online 11 March 2020

Keywords:
Antipsychotic agents
Psychotic disorders
Precision medicine
Genetic research
Pharmacogenomics
Biological psychiatry

Article info

1. Introduction

Schizophrenia is a severe mental disorder that has a profound impact on affected individuals and imposes large economic costs on the society (Chong et al., 2016; Knapp et al., 2004). Schizophrenia is ranked among the most disabling disorders globally (Charlson et al., 2018), with recovery rates as low as 13.5% (Jaa kslaen et al., 2013). The disorder is associated with a significant loss of productivity (Knapp et al., 2004) and a 10–20 years shorter life expectancy (Chesney et al., 2014). Antipsychotic drugs have become a cornerstone in the treatment of schizophrenia (Leucht et al., 2012). Although varying in targeted receptors (Kusumi et al., 2015), all antipsychotics share the property of regulating dopamine signalling (Amato et al., 2018). The efficacy in reducing symptoms differs only but little between the drugs (Leucht et al., 2013).

Among individuals diagnosed with schizophrenia, about one third display treatment-resistance (TR) (Elkis, 2007), with persisting disabling symptoms after adequate trials of antipsychotic drugs. Recently, the Treatment Response and Resistance in Psychosis (TRRIP) working group was established to solve difficulties in comparing studies, interpreting meta-analyses and replicating research on TR, by standardizing the definition of TR (Howes et al., 2017). Clozapine is a well-
documented antipsychotic drug against treatment-refractory symptoms (Siskind et al., 2016), but due to its severe adverse effects, clinicians often refrain from prescribing this drug (Warne et al., 2014). Thus, many patients are being subjected to a trial-and-error testing of drugs with various troublesome side effects (Iversen et al., 2018), which adds to the burden of the disorder itself (Charlson et al., 2018). Moreover, patients with TR tend to have more impaired functioning, poorer psychosocial adjustment, higher rates of hospitalization and represent a higher cost to society relative to antipsychotic-responsive patients (Gillespie et al., 2017; Iasevoli et al., 2016; Kennedy et al., 2014). Mapping predictors of antipsychotics response may take us closer to personalized medicine in schizophrenia (Lally et al., 2016; Lally and MacCabe, 2015).

It has been suggested that TR might be a categorically distinct subgroup (Gillespie et al., 2017) or represent the most severe cases on a continuum (Molen et al., 2019). MRI studies of the brain show that TR patients have lower grey matter volumes compared with both treatment responders and healthy volunteers (Anderson et al., 2015). Moreover, patients with TR seem to have lower levels of striatal dopamine synthesis capacity (Demjaha et al., 2012), as well as alterations in glutamate concentration (Demjaha et al., 2014) compared to treatment responders. In addition, patients with TR seem to have specific neurocognitive deficits (de Bartolomeis et al., 2013; Joob et al., 2002), and treatment response to their first antipsychotic trial, including lack of remission during the first three months, seems to predict long-term outcome of the disorder (Agid et al., 2011; Friis et al., 2016; Kolakowska et al., 1985). It has also been hypothesized that patients with TR may share genetic underpinnings (Nucifora et al., 2019). This is further supported by findings showing that family members of patients with TR are more likely to have a diagnosis of schizophrenia in comparison with family members of responsive patients with schizophrenia (Hajj et al., 2019; Silverman et al., 1987), indicating a common genetic component (Joob et al., 2005). Taken together, these findings indicate that TR might be at least partly determined by the burden of genetic risk for schizophrenia.

Recent GWAS in schizophrenia have established a large number of single nucleotide polymorphisms (SNPs) associated with the disorder (Pardinas et al., 2018). This has enabled the generation of a schizophrenia polygenic risk score (PRS-SZ) (Tesli et al., 2014) representing the weighted genetic predisposition of an individual to the disorder. PRS is a new and promising genetic measure in psychiatry (Pardinas et al., 2018; Purcell et al., 2009) as well as in medicine in general (Torkamani et al., 2018). There are some recent studies of PRS-SZ and TR in schizophrenia, however with conflicting results. Associations between PRS-SZ and a history of clozapine treatment (Frank et al., 2015) and lack of response to antipsychotics (Zhang et al., 2019) have been reported, suggesting that the genetics of schizophrenia is also involved in TR. Three other studies found no association between PRS-SZ and TR (Legge et al., 2018; Martin and Mowry, 2016; Wimberley et al., 2017). There are several possible explanations for the different results. There is a variation of strategies for selecting SNP thresholds and number of thresholds tested (Wimberley et al., 2017; Zhang et al., 2019; Legge et al., 2019). Moreover, various definitions of TR are applied (Wimberley et al., 2017; Frank et al., 2015). In the study by Zhang et al. (2019), antipsychotic efficacy was assessed based on symptom scores as opposed to studies specifically investigating TR (e.g. Frank et al., 2015). Also, samples vary between first episode patients and more chronic conditions as well as in methods of determining diagnoses (Zhang et al., 2019; Wimberley et al., 2017). Hence, the genetic architecture of TR is mainly unresolved.

Efforts have been made to understand clinical correlates underlying biological mechanisms and predictive factors for TR (Nucifora et al., 2019; Wimberley et al., 2016). Clinical and demographic factors associated with TR include earlier age at onset (Legge et al., 2019; Wimberley et al., 2016), lifetime drug abuse (Wimberley et al., 2016), poorer premorbid social adjustment (Legge et al., 2019) and living in less urban area (Legge et al., 2019). Decreased plasma level of antipsychotic drugs have also been associated with TR (Mccutcheon et al., 2018), suggesting that adherence and pharmacokinetic factors need to be addressed when investigating TR.

In the current study, we aimed to determine the potential of PRS-SZ to explain the heterogeneity in treatment response in a large, naturalistic sample of patients with schizophrenia spectrum disorders using the most recent consensus criteria for defining treatment resistance (Howes et al., 2017). We hypothesized that there is an increased risk of TR associated with increasing PRS-SZ and thus overlapping mechanisms for schizophrenia and TR. Additionally we investigated previous suggested non-genetic predictors of TR such as earlier age at onset, lifetime drug abuse and family history of psychosis (Frank et al., 2015; Legge et al., 2019; Meltzer et al., 1997; Wimberley et al., 2016). We also investigated dose serum ratio of antipsychotic medication in relation to TR, serving to account for non-adherence as a possible confounder and drug turnover as a possible contributor in TR mechanism.

2. Methods

2.1. Participants

As part of the Thematically Organized Psychosis (TOP) study, participants (N = 321) were included in the current study if they fulfilled diagnostic criteria for a schizophrenia spectrum disorder, defined as schizophrenia (N = 195), schizophreniform disorder (N = 5), schizoaffective disorder (N = 66), psychosis not otherwise specified (N = 39), brief psychotic disorder (N = 2) or delusional disorder (N = 14). Patients were recruited to the TOP study from the mental health clinics of the major hospitals in Oslo, currently covering a catchment area of close to 700,000 inhabitants. All patients were between 18 and 65 years of age and able to provide consent of participation. Patients were excluded if they had a history of severe somatic disease interfering with brain functioning including neurological disease, history of moderate or severe head trauma, or an IQ below 70. Participants recruited during their first psychotic episode were excluded from the current study as they were in the initial phase of antipsychotic drug trials. Demographic data, information about ancestry and information about current and past five years drug treatment were collected by interview and from medical records, as was information on duration, adherence and adverse effects of the treatment. See Table 1 for details.

2.2. Clinical characteristics

2.2.1. Clinical assessment

Diagnoses were made by using the Structured Clinical Interview (SCID-1 (First et al., 1995) for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (American Psychiatric Association, 2000). Symptoms of psychosis were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and function was assessed using the Global Assessment of Functioning (GAF—F) scale (Endicott et al., 1976). Previous psychiatric history was recorded based on interviews and medical records. Diagnostic interviews were performed by psychologists and physicians supervised by a senior professor in psychiatry. The research personnel are all comprehensively trained for the interviews based on a UCLA training program (Ventura et al., 1998).

2.2.2. Treatment resistant schizophrenia (TR) classification

Patients were classified as being TR or non-TR; the classification was adapted for retrospective data based on the Treatment Response and Resistance in Psychosis (TRRIP) working group’s consensus criteria (Howes et al., 2017). A patient was defined as being TR based on (during the five years prior to inclusion) either 1) history of treatment with clozapine or 2) two or more failed trials of antipsychotic treatment, each of at least six weeks duration and with therapeutic dosage. At least one of the antipsychotics had to be a second generation antipsychotic. Previous
Table 1
Demographics, clinical characteristics and medication in TR and non-TR.

<table>
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<tr>
<th></th>
<th>TR, N</th>
<th>Non-TR, N</th>
</tr>
</thead>
<tbody>
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<td>Sex female, N (%)</td>
<td>(33.6)</td>
<td>(66.4)</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>41 (72)</td>
<td>103 (48.4)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>25 (6.4)</td>
<td>24.5 (4.7)</td>
</tr>
<tr>
<td>Age at onset, median (IQR)</td>
<td>22.5 (9)</td>
<td>23 (13)</td>
</tr>
<tr>
<td>Season of birth (Dec-March), N (%)</td>
<td>26 (24.1)</td>
<td>76 (35.7)</td>
</tr>
<tr>
<td>Family history of psychosis, N (%)</td>
<td>30 (21.4)</td>
<td>44 (30.3)</td>
</tr>
<tr>
<td>PANSS-total, median (IQR)</td>
<td>60 (19)**</td>
<td>56 (23)</td>
</tr>
<tr>
<td>PANSS-positive, median (IQR)</td>
<td>15 (7)**</td>
<td>12 (7)</td>
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<td>PANSS-negative, median (IQR)</td>
<td>14 (7)**</td>
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</tr>
<tr>
<td>GAF-F, median (IQR)</td>
<td>41 (13)**</td>
<td>45 (13)</td>
</tr>
</tbody>
</table>

Missing data for %b,%16,%21,%65 patients.

Abbreviations: BMI = Body Mass Index; GAF-F = Global Assessment of Functioning, functioning scale; IQR = Intergroup range; N = Number; NOS = Not otherwise specified; PANSS: Positive and Negative Syndrome Scale; SD = Standard deviation; TR = Treatment-resistance.

 différence* ≤ 0.05.

* p ≤ 0.05.

** p ≤ 0.01.

*** p ≤ 0.001.

trials were classified as failed if there were change in antipsychotic agents. The current trial was classified as failed if the patient had significant symptoms (a few patients had frequent psychotic episodes [five or more during the five years] and were thus classified as TR regardless of current symptoms). Current significant symptoms were defined as at least one score of at least moderate severity on the PANSS positive subscale together with a score of 60 or less on the GAF functioning scale (GAF—F), indicating at least moderately impaired functioning (Howes et al., 2017). The antipsychotic treatment was not counted as a failed trial if the medication was stopped due to adverse effects. Antipsychotic drugs used for indications other than psychosis, typically as needed for sleep or anxiety, did not count as a trial of antipsychotic treatment (see Supplementary table). Patients having used a third, or more, antipsychotic drugs during the last five years after the two initial trials, were classified as having treatment resistance regardless of ongoing symptoms. We did not apply any limitation on the maximum duration of an antipsychotic trial. Based on the criteria, both patients currently in remission and patients with psychotic symptoms could be classified as TR. Patients not fulfilling the criteria for TR were classified as non-TR. According to this definition, 108 patients (33.6%) were classified as having treatment resistance in our sample. See Table 1 for details.

2.3. Polygenic risk score for schizophrenia

DNA was extracted from blood and saliva samples collected in the clinic. Genotyping was performed on Human Omni Express-24 v.1.1 (Illumina Inc., San Diego, CA, USA) at deCODE Genetics (Reykjavik, Iceland). Quality control was performed using PLINK 1.9 (Purcell et al., 2007). Briefly, variants were excluded if they had low coverage (<95%), had low minor allele frequency (MAF) (<0.01), deviated from Hardy-Weinberg equilibrium (p < 10^-8), or occurred at significantly different frequencies in different genotyping batches (FDR < 0.5). Whole individual genotypes were excluded if they had low coverage (<95%) or high likelihood of contamination (heterozygosity above mean + 5 standard deviations). MaCH software (Das et al., 2016; Li et al., 2010) was used to impute the genotypes of all participants onto reference haplotypes derived from samples of European ancestry in the 1000 Genome Project (genomic build GRCh37). The PRS-SZs were based on a meta-analysis of all Psychiatric Genomics Consortium Schizophrenia Working Group’s genome-wide association sub-studies except TOP (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), which included patients with schizophrenia and schizoaffective disorder. The summary statistics were quality controlled by removing variants that met any of the following conditions: MAF < 0.05; imputation quality (ratio between observed and expected allelic variance) <0.8; not present in more than half of the sub-studies. Variants from the MHC region were also excluded. The remaining variants were clumped into independent regions on the basis of the linkage disequilibrium structure of the 1000 Genomes Phase III European population. PLINK v1.9 was used with the following parameters: –clump-p1 1.0 –clump-p2 1.0 –clump-r2 0.2 –clump-kb 500. The allelic dosage coefficients (or logarithms of the odds ratios) of the variants with minimum p-values from all independent regions were used in constructing the PRS-SZs. These were calculated for all individuals following Purcell et al.'s (2009) recipe of multiplying the number of effect alleles they carried by the allelic dosage coefficients calculated in the meta-analysis. Only European subjects were included in our sample to avoid confounding from population stratification.

2.4. Medication and serum levels

Blood was withdrawn from antecubital vein in the morning for assessments of antipsychotic drug serum level by methods previously described (Steen et al., 2017). Standardised relationships between dose and serum level of antipsychotics were calculated to enable the comparison of several antipsychotic drugs: First, the dose of each participant’s primary antipsychotic drug was divided by the Defined Daily Dose (DDD) (Leucht et al., 2016) of the drug to obtain a standardised dose for each participant. Second, the measured serum level of this antipsychotic drug was divided by the median of the antipsychotic drug’s reference range (Hienke et al., 2018). Finally, the standardised dose was divided by the standardised serum level, to obtain a relationship between dose and serum level comparable across antipsychotics indicating the antipsychotic drug’s turnover.

2.5. Ethics

All participants gave written consent of participation after a written and oral description of the study. The study was approved by The Regional Ethics Committee, The Norwegian Data Inspectorate, and the Norwegian Directorate of Health approved the biobank.

2.6. Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago II, version 24). To investigate differences in demographic and clinical variables we used chi-square tests for the categorical variables, independent sample t-test for normally distributed continuous variables and Mann Whitney U test for non-parametric distributions. Normality was assessed with Q-Q-plots, histograms and Kolmogorov-Smirnov statistics. Potential issues with multicollinearity and outliers in the regression analyses were ruled out. To assess the association of PRS-SZ with TR we used binary logistic regression. Patients were coded as either TR or non-TR and this binary variable was set as the dependent variable. The standardised PRS for
schizophrenia (PRS-SZ) was set as the predictor variable with adjustments for ancestry (using the twelve first genetic principal components giving the highest classification correctness of TR and non-TR in the statistical model), and for genotyping batch (7 batches) in addition to age at inclusion and sex (main model). Adjustments for time since first episode of the disorder and for time since first drug treatment were also tested. Regression analyses were performed with PRS-SZs based on different SNP significance thresholds (Wimberley et al., 2017; Zhang et al., 2019), due to testing of five inclusion thresholds, we applied a Bonferroni-corrected significance level of \( p < 0.01 \) for the effect of PRS-SZ. Secondry logistic regression analyses with the significantly associated PRS-SZ were performed by additionally adjusting for all the included diagnoses first, and then including season of birth (summer versus winter) and dose serum ratio, separately. Spearman’s rho was applied to quantify the correlation between PRS-SZ and PANSS-total to assess the effect of PRS-SZ on current symptom severity, as well as the correlation between PRS-SZ and GAF—F. Spearman’s rho was also calculated to confirm the correlation between PRS-SZ at different thresholds. We also performed an additional logistic regression analysis after excluding all clozapine users to rule out a specific association with clozapine use, as well as a regression analysis without PRS-SZ to demonstrate the contribution of PRS-SZ to the model. Finally, we ran the same analysis including only the subgroup of patients with a schizophrenia diagnosis.

3. Results

3.1. Study sample, demographic and clinical variables

A diagnosis of schizophrenia was more frequent in the TR group than in the non-TR group \( (p < 0.05) \) and the prevalence of delusional disorder was lower in the TR group than the non-TR group \( (p < 0.01) \). Current use of anticonvulsants and lithium was significantly higher in the TR group \( (p < 0.05) \). The positive \( (p < 0.001) \), negative \( (p < 0.05) \) general \( (p < 0.05) \) and total \( (p < 0.01) \) PANSS scores were higher and the GAF-functioning score was lower \( (p < 0.001) \) in the TR group compared to the non-TR group. Winter birth was less common in TR \( (p < 0.05) \), and the dose serum ratio was higher in TR \( (p < 0.05) \). Drug abuse and family history of psychosis only showed a trend level difference \( (p = 0.07 \) and \( p = 0.09 \) respectively). See Table 1 for details.

3.2. PRS-SZ and the relationship to TR

Higher PRS-SZ was significantly associated with TR-status \( (p = 0.003, \text{odds ratio} 1.595 \text{CI: 1.148–1.973}, \text{Omnibus test} = (21, N = 321, 35.77, p = 0.023)) \). There was a strong and significant correlation between PRS-SZ and GAF—F. The association between PRS-SZ and PANSS was still significant \( (p = 0.004) \), dose serum ratio did not have significant effect when added to the regression model \( (p = 0.653) \). PRS-SZ based on other GWAS p-value thresholds were not significantly associated with TR \( (\text{GWAS p-value thresholds of} p = 5 \times 10^{-8}, p = 0.05, p = 0.1 \text{ and } p = 0.5 \text{ yielded p-values of} 0.649, 0.074, 0.963 \text{and} 0.458, \text{respectively}) \).

Table 2 Classification table of the logistic regression model.a

<table>
<thead>
<tr>
<th></th>
<th>Observed TR (N)</th>
<th>Observed Non-TR (N)</th>
<th>Predicted TR (N)</th>
<th>Predicted Non-TR (N)</th>
<th>Percentage correct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>20</td>
<td>42</td>
<td>54</td>
<td>29.6%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>76</td>
<td>193</td>
<td>76</td>
<td>193</td>
<td>90.6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>90.6%</td>
<td>70.1%</td>
<td>70.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>61.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative predictive value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The model included polygenic risk score, sex, age, principal components 1–12, batch number (independent variables) and Treatment-resistance and non-treatment resistance (dependent variable).

In the current study, we found PRS-SZ at \( p = 0.01 \) to be significantly associated with antipsychotic drug TR in patients with a schizophrenia spectrum disorder. This indicates PRS-SZ as a potential biological marker of TR and supports earlier assumptions of genetic factors involved in TR. The findings indicate genetic mechanisms in common for schizophrenia and TR. Season of birth was significantly associated with TR, also when adjusting for PRS-SZ and diagnosis. Associations to PRS-SZs based on other GWAS significance thresholds were non-

Fig. 1. Standardised polygenic risk score \( (p = 0.01) \) for schizophrenia (y-axis) in patients with treatment-resistance (TR) and non-treatment resistance (non-TR), grouped by schizophrenia only and all patients (unadjusted mean). Error bars represents one standard error.
significant, suggesting the current threshold as the most suitable for TR prediction (Zhang et al., 2019).

The current main finding is in line with previous findings of a higher likelihood of responding to treatment among first-episode psychosis patients with a low PRS-SZ (Zhang et al., 2019), indicating PRS-SZ as a biological marker regardless of duration of illness. An important strength of our sample is the inclusion of patients with a well-established diagnosis and with a higher likelihood to have received several trials of antipsychotics beyond the initial antipsychotic treatment (Haddad and Correll, 2018), assuring the validity of the effect of PRS-SZ on TR in schizophrenia spectrum disorders. Our finding is also in line with the increased PRS-SZ observed in patients with a history of clozapine treatment compared to patients with no such history (Frank et al., 2015).

TR status in our sample was based on the most recent consensus definition criteria (Howes et al., 2017), yielding a TR rate of 33.6%. Contrary to the current study, several other studies have restricted the definition of TR to involving clozapine use, which may limit the representativeness of the TR sample (Howes et al., 2017; Kelly et al., 2010). The three studies that did not find significant association between PRS-SZ and TR (Legge et al., 2019; Martin and Mowry, 2016; Wimberley et al., 2017) also used definitions of treatment-resistance somewhat different from those following the recent consensus-based criteria (Howes et al., 2017). Wimberley et al. (2017) defined TR as first occurrence of either clozapine initiation or hospitalization during antipsychotic treatment within 18 months after at least two periods of different antipsychotic monotherapy, thus introducing hospitalization as a criterion. According to their definition, 21% of the patients were defined as TR during follow-up, which is below the general estimate of 30–40% (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019). The definition used by Martin and Mowry (2016), based on clinical features and course, resulted in a TR prevalence of 37.09%, more like ours. In the study by Legge et al. (2019), TR was defined as either rating negatively on the Operational Criteria Checklist (OPCRIT) item 89 or receiving clozapine treatment, resulting in a TR prevalence of 52.4%. The authors explain the high frequency of TR as due to recruitment from clozapine clinics. They also used a more general criterion from the Operational Criteria Checklist for Psychotic Illness and Affective Illness to decide TR classification. Their sample might thus be less representative of TR (Howes et al., 2017; Kelly et al., 2010) than the present naturalistic sample. The lack of consistence in TR definition criteria could contribute to the varying results with regard to a PRS-TR association. The inconsistent findings could also be due to the use of GWAS of smaller samples (Frank et al., 2015).

The current study comprises a naturalistic sample with TR based on the most recent consensus criteria and a TR rate comparable with the genetically reported prevalence (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019). It is however possible that some of the current associations could be due to overlap between TR characteristics and illness severity. If the cohorts analysed in the GWAS included mostly chronic patients with more severe illness, one could speculate that the PRS-SZ association exists because individuals with TR tends to be more severely ill. Attempting to test this possibility, we performed a sub-analysis using the same model but excluding all clozapine users. In this analysis, we found effects similar to those found in the main analysis. Moreover, there were no significant correlations between PRS-SZ and symptom severity.

Season of birth was significantly associated with TR, in line with one previous study showing a lower prevalence of winter births in TR (Wimberley et al., 2016). The effect remained significant after adjustment for PRS-SZ, suggesting the existence of genuine effects unrelated to the genetics of the disorder, indicating the complexity of TR. Considering the increased prevalence of winter or spring births in schizophrenia (Davies et al., 2003), the finding supports the hypothesis of TR being a categorically distinct subgroup (Gillespie et al., 2017). It has been suggested that the TR group represents a more “genuine schizophrenia” with differences in response potentially representing genetic and sociocultural factors (Itil et al., 1966). Several subsequent studies have supported this hypothesis (Frank et al., 2015; Gillespie et al., 2017; Jobber et al., 1999; Wolkin et al., 1989). Studies exploring the mechanisms of TR have reported a lower level of striatal dopamine synthesis capacity (Demjaha et al., 2012) as well as higher levels of glutamate in the anterior cingulate cortex (Demjaha et al., 2014). Significant interactions between dopamine transporter variable number tandem repeats (DAT-VNTR) and the serotonin transporter (5HTT) polymorphism are also reported in TR (Blic et al., 2014). Further, a strong association was found between TR and the variants for brain-derived neurotrophic factor (BDNF), which is associated with schizophrenia (Di Carlo et al., 2019) and interacts with monoaminergic neurotransmitters (Zhang et al., 2013). Moreover, an increased number of rare copy number variants (CNV) are associated with TR (Martin and Mowry, 2016). This is in line with the current findings of TR linked to the genetics of schizophrenia (Owen et al., 2016). Furthermore, the current association between TR and PRS-SZ might indicate underlying factors in TR, as recent studies have revealed genetic pleiotropy between schizophrenia and phenotypes such as brain structure volumes (Terwisscha van Scheltinga et al., 2013; Smeland et al., 2018; Chen et al., 2019), immune related conditions (Andreasen et al., 2015) body mass index (Bahrami et al., 2020) and lipids (Andreasen et al., 2013), all of which potentially related to antipsychotic drug treatment response (Barry et al., 2019; Hutcherson et al., 2014; Noto et al., 2015; Pillinger et al., 2020).

The current study has several strengths. Our large, well-characterized sample enabled the application of criteria for TR well matching the most recent consensus-criteria, resulting in a TR rate in accordance with the literature (Elkis, 2007; Gillespie et al., 2017; Kane et al., 2019) and demonstrating associations to PRS-SZ and demographic data. We did not find any mediating effect of diagnosis. The naturalistic design assures relevance to clinical samples, with the current results being of special interest for treatment response in long-term and thus the long-term prognosis, as opposed to studies of first-episode patients with a different response patterns to antipsychotic medication (Haddad and Correll, 2018). The limitations include the cross-sectional design, which made us unable to assess actual reduction of symptoms during antipsychotic medication and therefore to implement the criterion of lack of 20% reduction of symptoms (Howes et al., 2017). This could lead to classification bias as some patients being classified as TR due to fulfilling symptom criteria might have experienced a 20% reduction of

### Table 3

<table>
<thead>
<tr>
<th>PRS-SZ</th>
<th>Unadjustedd</th>
<th>Adjusteda</th>
</tr>
</thead>
<tbody>
<tr>
<td>GWAS</td>
<td>0.541, OR = 0.9 (0.737–1.173)</td>
<td>0.548, OR = 0.9 (0.741–1.205)</td>
</tr>
<tr>
<td>0.01</td>
<td>0.007, OR = 1.4 (1.092–1.768)</td>
<td>0.003, OR = 1.5 (1.148–1.973)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.094, OR = 1.3 (0.962–1.645)</td>
<td>0.074, OR = 1.3 (0.974–1.788)</td>
</tr>
<tr>
<td>0.1</td>
<td>0.097, OR = 1.0 (0.780–1.291)</td>
<td>0.063, OR = 1.0 (0.748–1.320)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.566, OR = 1.1 (0.824–1.423)</td>
<td>0.458, OR = 1.1 (0.823–1.542)</td>
</tr>
</tbody>
</table>

**Abbreviations:** GWAS = Genome-Wide Association Study, PRS-SZ = Polygenic Risk Score for Schizophrenia, SNP = Single Nucleotide Polymorphism.

a Odds ratio (OR) given with 95% confidence interval.

b Explained variance (Nagelkerke pseudo R²) of the unadjusted/adjusted model.

c Explained variance (Nagelkerke pseudo R²) of the unadjusted/adjusted model.
symptoms during the present trial and hence should have been classified as non-TR. Moreover, although previous antipsychotic administrations were adequate in dosage and duration, we lack serum concentration data, and cannot exclude issues with adherence during these previous trials. However, thanks to our detailed protocol, we were still able to adapt the prospective based criteria to match the TR rates shown previously (Elkis, 2007; Gillespie et al., 2017; Lally et al., 2016), and confirm the PRS-SZ effect in the clozapine-free subsample. By excluding first-episode patients and missing out on individuals that did not survive long enough to be included in the study, we might have failed to include patients with a fast recovery or severe illness, decreasing the power and variability of the data. Moreover, we cannot fully exclude this as a source of bias, although there were no significant correlation between PRS-SZ and PANSS-total or GAF—F, suggesting that variation in symptoms would not have affected the results. Various strategies may be applied for choosing PRS-SZ threshold values (Wimmerly et al., 2017; Wray et al., 2014) and there exists no well-founded procedure; however, the thresholds selected in the current study have been previously used (Zhang et al., 2019) and results for all of them were reported. Moreover, the results were strictly adjusted for the number of levels analysed. The reason why only threshold p = 0.01 results in a significant association with PRS-SZ is matter for speculation. It might be that the PRS-SZ obtained with less stringent thresholds are too unspecific and therefore unable to detect the association, while more stringent thresholds exclude variants of importance.

In conclusion, we found an association between PRS-SZ at SNP significance threshold p = 0.01 and TR in patients with a schizophrenia spectrum disorder, as well as an association between season of birth and TR. This indicates an association between PRS-SZ and treatment response, although the failure to replicate the findings with other SNP significance thresholds makes the association less robust. The findings suggest that TR is related to genetic factors that also drive core physiological processes in schizophrenia spectrum disorders, as well as to factors independent of schizophrenia genetics. The positive and negative predictive values of the current model are not sufficient for clinical use. Still, the study confirms the utility of PRS-SZ as a predictive supplement based on forthcoming expansions of GWAS data.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2020.03.006.


