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Immunomodulatory effects of antipsychotic treatment on gene expression in first-episode psychosis

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ABSTRACT

Previous studies suggest immunological alterations in patients with first-episode psychosis (FEP). Some studies show that antipsychotic compounds may cause immunomodulatory effects. To evaluate the immunological changes and the possible immunomodulatory effects in FEP, we recruited patients with FEP (n = 67) and matched controls (n = 38), aged 18–40 years, from the catchment area of the Helsinki University Hospital and the City of Helsinki, Finland. Fasting peripheral blood samples were collected between 8 and 10 a.m. in 10 ml PAXgene tubes. We applied the NanoString nCounter in-solution hybridization technology to determine gene expression levels of 147 candidate genes reflecting activation of the immune system. Cases had higher gene expression levels of *BDKRB1* and *SPP1/osteopontin* compared with controls. Of the individual medications used as monotherapy, risperidone was associated with a statistically significant upregulation of 11 immune system genes, including cytokines and cytokine receptors (*SPP1*, *IL1R1*, *IL1R2*), pattern recognition molecules (*TLR1*, *TLR2* and *TLR6*, *dectin-1/CLEC7A*), molecules involved in apoptosis (*FAS*), and some other molecules with functions in immune activation (*BDKRB1*, *IGF1R*, *CR1*). In conclusion, risperidone possessed strong immunomodulatory properties affecting mainly innate immune response in FEP patients, whereas the observed effects of quetiapine and olanzapine were only marginal. Our results further emphasize the importance of understanding the immunomodulatory mechanisms of antipsychotic treatment, especially in terms of specific compounds, doses and duration of medication in patients with severe mental illness. Future studies should evaluate the response pre- and post-treatment, and the possible role of this inflammatory activation for the progression of psychiatric and metabolic symptoms.

1. Introduction

Patients with first-episode psychosis (FEP) show a systemic low-grade inflammation (Fernandes et al., 2016; Miller et al., 2011), which is a predictor of poorer treatment outcome (Martinez-Cengotitabengoa et al., 2016; Mondelli et al., 2015), cognitive decline (Bulzacka et al., 2016; Martinez-Cengotitabengoa et al., 2014), and increased risk for metabolic changes and cardiovascular morbidity (Mori et al., 2015; Russell et al., 2015). While both external (e.g. smoking) and endogenous (e.g. obesity, stress) factors contribute to dysregulation of the immune system in early psychosis (Leza et al., 2015), there is accumulating evidence suggesting that antipsychotics may have immunomodulatory effects (Baumeister et al., 2016; Borovcanin et al., 2013; Cotel et al., 2015; Crespo-Facorro et al., 2008, 2014; Holmes

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et al., 2016; MacDowell et al., 2013; Miller et al., 2011; Mondelli et al., 2015; Obuchowicz et al., 2017). Based on *in vitro* and animal models, individual antipsychotics differ in their immunomodulatory effect, but the results have not provided a consistent profile (Baumeister et al., 2016). Additionally, one human study proposed that between-compound differences in the response at treatment onset are possible (Meyer et al., 2009). It has been presented that the immunomodulatory effects of medications can be expected to reflect differences in the prevalence of metabolic side effects between antipsychotics, i.e. weight-increasing effects correlate with the ability to decrease proinflammatory cytokine production (Fonseka et al., 2016).

The effect of antipsychotic treatment on dynamic inflammatory processes is likely to be both direct and indirect (Kriisa et al., 2016; Manu et al., 2014). Animal studies suggest that antipsychotics affect the inflammatory cells in a dose-dependent manner (da Cruz Jung et al., 2016; Obuchowicz et al., 2017; Sarvari et al., 2014). This direct effect could be exerted, for instance, on the immune cells, where receptors for neurotransmitters are known to mediate a wide variety of crucial immune functions (Arreola et al., 2016; Herr et al., 2017; Levite, 2016). The activation of the immune system could also be secondary to the metabolic disturbance (Nousen et al., 2013): antipsychotic medications have an indirect effect through critical regulatory genes of adipogenesis and lipid metabolism (Sarvari et al., 2014). Antipsychotics could potentially exacerbate the inflammatory processes in peripheral tissues (blood, fat, liver) (da Cruz Jung et al., 2016). Moreover, in addition to central effects on neuroinflammation (MacDowell et al., 2013; Mondelli et al., 2017; Monji et al., 2013; Obuchowicz et al., 2017), antipsychotics have central effects on endocrine disturbances (Emsley et al., 2015). A better understanding of the factors causing activation of the immune system at antipsychotic treatment onset is of interest for personalized treatment, not only to reach an improved psychiatric outcome, but especially to prevent metabolic changes and increased mortality (Dieset et al., 2016).

We hypothesized that immunological activation is present in patients with FEP and specific antipsychotic treatments may be associated with these changes. Thus, we studied the expression levels of 147 immunological genes in blood samples from 67 FEP patients after onset of treatment and from 38 matched healthy controls.

2. Methods

2.1. Data sampling and clinical evaluation

FEP patients (age 18–40 years) attending their first treatment for psychosis were recruited from the catchment area of the University Hospital District of Helsinki and Uusimaa and the City of Helsinki. The inclusion criteria for the study was receiving a score of at least 4 on items assessing delusions (unusual thought content) or hallucinations in the Brief Psychiatric Rating Scale-Extended (BPRS-E) (Ventura et al., 1993), and being fluent in the Finnish language. Substance-induced psychotic disorders and psychotic disorders due to a general medical condition were excluded. Baseline assessment was conducted as soon as the patient had entered treatment and was able to give an informed consent according to the treating personnel.

Controls, matched by age, sex and region of residence, were identified from the Finnish Population Register Center and assessed with the same protocol as the patients. The exclusion criteria for the controls were lifetime history of psychotic disorder, and as for the patients, chronic neurological, endocrinological or cardiovascular diseases, and any condition that prevents Magnetic Resonance Imaging.

The Axis I diagnostics were assessed for both cases and controls using the Structured Clinical Interview for DSM-IV-TR Axis I disorders, Research Version, Patient Edition (First et al., 2002 (Revision January 2007)). The study interviews were conducted by trained research nurses or psychologists, and all interviews were reviewed together with a senior psychiatrist (JS) before assigning the DSM-IV diagnosis. We also reviewed medical records from all psychiatric treatment contacts for the diagnostic assessment.

The interviewer measured weight and height and we calculated body mass index (BMI) (kg/m^2), as described in detail previously (Keinanen et al., 2015). The information on type and duration of medication was collected from medical records. To analyze the effect of antipsychotics, we identified patients who were on only one antipsychotic medication (referred hereafter as monotherapy). Duration of antipsychotic treatment was calculated from the onset of any antipsychotic treatment to the date of the interview. In addition, data were gathered on sociodemographic factors, functioning, somatic illness, substance use, and smoking as described earlier (Keinanen et al., 2015).

The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa and by the institutional review boards of the National Institute for Health and Welfare (THL), Helsinki, Finland, and the University of Helsinki. All participants gave a written informed consent.

2.2. Laboratory analytical methods

A fasting blood sample was collected at 8–10 a.m. Serum and plasma samples were immediately aliquoted and stored at −80 °C. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, high sensitivity C-reactive protein (hs-CRP), insulin and plasma glucose were measured by Abbott Architect ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA) in the laboratory of the Genomics and Biomarkers Unit at the National Institute for Health and Welfare. The laboratory has been accredited by Finnish Accreditation Service (FINAS) and it fulfills the requirements of the standards SFS-EN ISO/IEC 17025:2005. The scope of accreditation covers all analyses. The following methods were used: enzymatic assays for measuring total cholesterol, triglycerides and glucose, homogeneous method for direct measurement of HDL cholesterol, ultrasensitive immunoturbidimetric assays for hs-CRP and chemiluminescent microparticle immunoassay (CMIA) for insulin. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula. The mean inter-assay coefficients of variation (CVs) for total cholesterol, HDL cholesterol, triglycerides, hs-CRP, insulin and glucose were 1.0%, 1.1%, 0.8%, 2.8%, 1.9%, and 1.1%, respectively. We used homeostatic model assessment (HOMA) index to describe insulin resistance.

We extracted total RNA from blood samples collected from 67 patients and 38 controls. Blood was collected in PAXgene tubes (PreAnalytiX, Switzerland) and stored at −80 °C upon extraction with PAXgene Blood RNA kit (Qiagen). RNA yield and purity were assessed using NanoDrop and Qubit (Thermo Fisher Scientific). We applied the nCounter in-solution hybridization method using nCounter Sprint platform (NanoString Technologies, Inc, Seattle) to measure gene expression levels of candidate genes. The method utilizes color-barcoded probes to detect RNA molecules at high sensitivity (< 1 copy per cell). The method does not necessitate cDNA amplification prior to the analysis, which results in high quality since cDNA amplification can bias the original transcript counts (Prokopec et al., 2013). nCounter custom CodeSet probes for the assay were designed for 147 target genes of interest (see description and full names of the selected genes in Supplementary Table 1) and for five control genes (*PDCL, RBM48, SDR39U1, SPRYD7, TMEM87A*), selected based on their expression levels and stability in control samples of an RNA-sequencing study of systemic lupus erythematosus (GEO accession GSE72509), and not being, to our knowledge, associated with mental disorders nor activated by the immune system. For the full list of target probes and their isoform coverage see Supplementary Table 2. nCounter assays, each including 100ng total RNA, were conducted at the DNA Sequencing and Genomics Laboratory of the Institute of Biotechnology, University of Helsinki, Finland. For each assay cartridge, we randomized the samples

by disease status, sex, and blood sampling time point to avoid batch effects. Each assay resulted in on average 403,470 (151,992 – 887,787) molecule counts. Negative control probes to control for background hybridization showed on average 7.8 (1–24) counts and correlation of positive probes with the molar content of positive target molecules was on average 0.998, indicating good assay linearity. Data quality was further investigated for effect of different normalization parameters and possibility of batch effects was excluded with R package NanoStringNorm (Waggott et al., 2012). Finally, data were normalized using the control genes and positive control probes, and subtracting background counts that fell below mean + 2*SD of the negative control probes, using nSolver software v2.6 (NanoString Technologies). Based on these observations, the data quality was concluded to be excellent and we proceeded with statistical analyses. Expression heat maps for genes with expression fold change > 1.2 in medicated individuals relative to controls were drawn as described previously (Haarman et al., 2015).

2.3. Statistical analysis of clinical data

We used Pearson's χ^2 tests or *t*-test to compare differences in sociodemographic and clinical variables between cases and controls. Because several continuous variables were non-normally distributed, we used Spearman's rho for correlations and Mann-Whitney *U* test to test significance in between-group comparisons. For descriptive purposes, we present all p-values significant at the < 0.05 level. In the heat map analysis, the results were adjusted for multiple testing using the Benjamini-Hochberg method at the < 0.10 level. (Benjamini and Hochberg, 1995). We conducted power calculations for two samples (different sizes) using the t-tests of means. We used IBM SPSS Statistics v23.0 (SPSS Inc.) or R (R, 2013) for statistical analysis and graphical presentation.

3. Results

3.1. Descriptors of cases and controls

Our dataset for the current analysis comprised 67 cases of FEP patients and 38 matched controls. The cases and controls did not differ in terms of gender, mean age, or BMI (Table 1). Cases had a lower HDL cholesterol level and a higher triglyceride level than the controls, reported more often lifetime asthma or allergy, and were more likely to have antidepressive treatment.

Most cases had a schizophrenia spectrum psychotic disorder diagnosis (Supplementary Table 3). Polypharmacy was relatively common, and thus, we present expression levels comparing cases on the three most common antipsychotic medications as monotherapy at baseline (risperidone n = 16, olanzapine n = 19, quetiapine n = 12) to the control group (n = 38). Four cases did not have any antipsychotic medication when the first blood sample was taken; for descriptive purposes, we present the expression levels despite the small number. Median duration of antipsychotic treatment for cases with a medication at baseline was 31.0 days (interquartile range (IQR) 13–67) in the total sample, 20.0 (IQR 12–75) for risperidone, 21 days (IQR 17–35) for olanzapine and 52 days (IQR 40–101) for quetiapine monotherapy (ns). Eleven cases (16.4%) and one control (2.6%) were using an antidepressant medication, and two (5.3%) had mood stabilizing medication.

Most patients (16/19, 84.2%) were hospitalized in the olanzapine monotherapy group, the proportion being half (8/16, 50%) in risperidone and one in four (3/12, 25.0%) in quetiapine monotherapy group. No difference in other confounders including diagnosis, BMI, triglycerids, glucose, insulin, hs-CRP, age, gender or symptom severity was detected when comparing cases with risperidone, quetiapine or olanzapine monotherapy at baseline (data not shown). The prevalence of antidepressants, smoking and allergy was small comparing the three monotherapy groups (antidepressants 3, 2 and 4, smoking 4, 2 and 4, and allergy 4, 1 and 5 in olanzapine, quetiapine and risperidone monotherapy groups).

3.2. Expression of inflammatory genes in cases vs controls

When comparing all cases to controls, irrespective of medication, cases had higher expression levels of *bradykinin receptor B1* (*BDKRB1*) (Fold change 1.46, p = 0.036) and *osteopontin/phosphoprotein 1 (SPP1)* (Fold change 1.50, p = 0.031) compared with controls, but this did not

Table 1
Sociodemographic, lifestyle and clinical characteristics of cases and controls.

Character	FEP patients (N = 67) n(%)/mean ± SD/median (IQR)	Controls (N = 38) n(%)/mean ± SD	p (chi-square/*t*-test/Mann-Whitney *U* test/ Kruskal-Wallis independent samples test)
Age, years	26.2 ± 5.7	28.3 ± 6.5	ns
Male	40 (59.7%)	22 (57.9%)	ns
No vocational or higher education	**36 (53.7%)**	**9 (23.7%)**	**0.003**
Employed, military or student	**51 (76.1%)**	**36 (94.7%)**	**0.015**
BMI (kg/m^2)	24.2 ± 4.5	23.9 ± 3.3	ns
Blood glucose (mmol/l)	4.3 ± 0.71	4.1. ± 0.43	ns
S-insulin mU/l) (3 outliers removed insulin < 30)	9.1 (5.9, 17.1)	7.8 (5.2, 11.0)	ns
HOMA index (3 outliers removed insulin < 30)	1.74 (1.1, 3.2)	1.41 (0.92, 1.94)	ns
HDL-C (mmol/l)	**1.28 ± 0.35**	**1.62 ± 0.045**	**< 0.001**
LDL-C (mmol/l)	2.93 ± 0.79	2.68 ± 0.80	ns
Triglycerides (mmol/l)	**1.16 (0.77, 1.60)**	**0.83 (0.64, 1.05)**	**0.005**
hs-CRP (mg/l)	0.92 (0.41, 2.66)	1.19 (0.60, 2.51)	ns
GAF	**36.1 ± 7.45**	**86.0 ± 7.6**	**< 0.001**
Autoimmunological disorders	2 (3.0%)	2 (5.3%)	ns
Allergy/asthma	**13 (19.4%)**	**1 (2.6%)**	**0.015**
Antidepressive medication, current	**11 (16.4%)**	**1 (2.6%)**	**0.033**
Antibiotic treatment during the past year	3 (4.5%)	0 (0%)	ns
Valproate	2 (3.0%)	0 (0%)	ns
Current smoking	14 (24.6%)	6 (16.2%)	ns
Never tried marijuana or cannabis	42 (77.8%)	33 (89.2%)	ns

FEP = First Episode Psychosis; BMI = Body Mass Index; kg = kilogram; HOMA index = homeostatic model assessment index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; hs-CRP = high sensitive C-reactive protein; mg = milligram; l = liter; GAF = Global Assessment of Functioning scale; n = number; SD = standard deviation; IQR = interquartile range; ns = not significant.

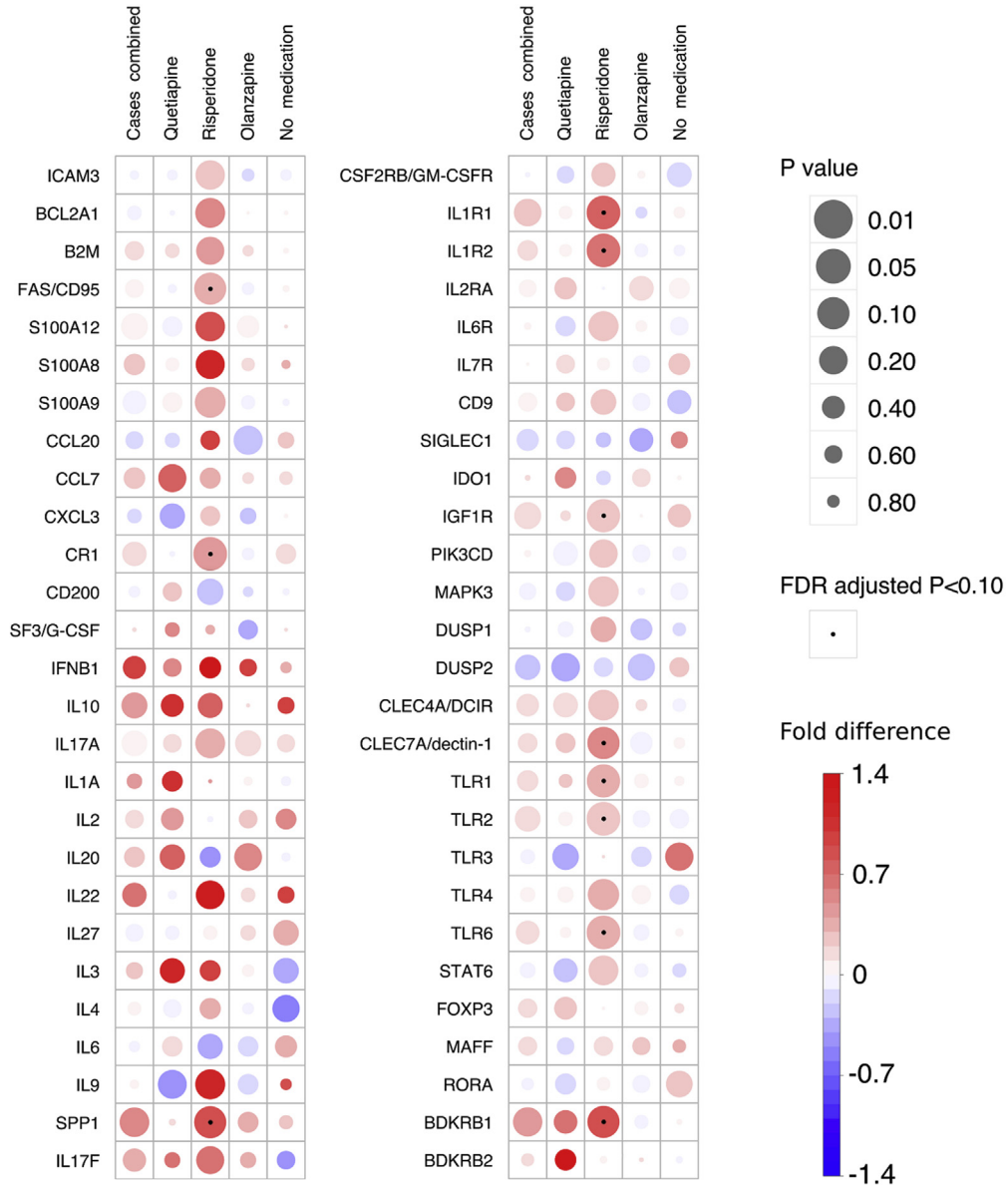

Fig. 1. A heatmap of the genes with a significant ($p < 0.05$) difference in the expression level between cases and controls. We compared controls to the total sample and to the monotherapy groups on quetiapine, risperidone, or olanzapine.

remain significant after correction for multiple testing (Fig. 1, with correction for multiple testing, Supplementary Table 4, uncorrected values in groups, and Supplementary Table 5, individual levels).

3.3. Differences in gene expression according to medication

It was notable that the standard deviation for several genes was larger in the patient than in the control group, despite the larger size of the patient group. To examine whether the type of antipsychotic medication might explain the larger variance in the patient group, we compared four groups: patients on monotherapy with risperidone, olanzapine and quetiapine and the control group. In Fig. 1 and Supplementary Table 3 we describe the gene expression levels comparing cases with monotherapy to controls. After correcting for multiple testing, cases with risperidone monotherapy had upregulation of 11 genes compared with controls: *BDKRB1, CLEC7A, CR1, FAS, IGF1R, IL1R1, IL1R2, SPP1, TLR1, TLR2,* and *TLR6* (see a description of genes in Table 2 and Supplementary Table 1). Several other genes had a statistical difference with $p < 0.05$ before correction for multiple testing in the level of expression. These included (starting from the most significant genes): *CASP9, TLR4, S100A9, CLE4 (DCIR), PIK3CA, PIK3CG, IL6R, IL9, BCL2A1, IL17A, STAT6, PIK3R5, IL17*RA, *S10012, STAT3, ICAM3, IFNGR2, MAPK1,* and *S100A8*. For quetiapine and olanzapine, none of the genes showed a statistically significant effect after correcting for multiple testing.

We conducted secondary analyses to explore the associations between the differentially expressed genes (DEG) with confounders (Table 3). Duration of antipsychotic treatment in the total sample did not correlate with any of the differentially expressed genes in the total patient sample nor in monotherapy groups. Among the patients, *FAS* correlated with age (rho = 0.30, p = 0.014), *SPP1* with positive BPRS-E symptom score during the past week (rho = −0.25, p = 0.040), and *IGF1R, CLEC7, TLR1* and *TLR2* with triglycerids (rho = 0.30, p = 0.017; rho = 0.26, p = 0.039, rho = 0.26, p = 0.040, and rho = 0.28, p = 0.023, respectively).

We present the power calculation of the needed size for future studies to replicate the findings, given the observed effect (Cohen's d) (Supplementary Figures 1 to 5). In the risperidone group, the effect size

Table 2
Names and description of the genes with a significant ($p < 0.05$) difference in the expression level between cases and controls. We compared controls to the total sample and to the monotherapy groups on quetiapine, risperidone, or olanzapine.

Gene	Symbol	Function (NCBI gene database)
Intercellular adhesion molecule 3	*ICAM3*	Expressed by all leucocytes, may be the most important ligand for leukocyte adhesion protein LFA-1 in the initiation of the immune response
BCL2 related protein A1	*BCL2A1*	A member of the BCL-2 protein family; apoptosis regulation
Beta-2-microglobulin	*B2M*	Antigen presentation, HLA class I accessory molecule
Fas cell surface death receptor	*FAS/CD95*	A member of the TNF-receptor superfamily. Interaction of this receptor with its ligand allows the formation of a death-inducing signaling complex, but is also involved in transducing proliferative signal to T cells
S100 calcium binding protein A12	*S100A12*	S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. Involved in specific calcium-dependent signal transduction pathways; its regulatory effect on cytoskeletal components may modulate various neutrophil activities
S100 calcium binding protein A8	*S100A8*	May function in the inhibition of casein kinase and as a cytokine
S100 calcium binding protein A9	*S100A9*	An antimicrobial protein, antifungal and antibacterial activity
C-C motif chemokine ligand 7	*CCL7*	Attracts macrophages, an in vivo substrate of matrix metalloproteinase 2
C-C motif chemokine ligand 20	*CCL20*	Has chemotactic activity for lymphocytes, can repress proliferation of myeloid progenitors. A Th17 chemoattractant
C-X-C motif chemokine ligand 3	*CXCL3*	A chemokine, chemoattractant for neutrophils
Complement component 3b/4b receptor 1	*CR1/CD200*	Mediates cellular binding to particles and immune complexes that have activated complement
Colony stimulating factor 3	*CSF3/G-CSF*	A chemokine secreted by macrophages and monocytes
Interferon beta 1	*IFNB1*	Belongs to the type I class of interferons, which are important for defense against viral infections
Interleukin 10	*IL10*	A cytokine that downregulates the expression of Th1 cytokines and costimulatory molecules on macrophages; enhances B cell survival, proliferation, and antibody production. Downregulates antigen presentation of dendritic cells
Interleukin 17A	*IL17A*	A proinflammatory cytokine produced by activated T cells
interleukin 1 alpha	*IL1A*	A member of the interleukin 1 cytokine family, produced by monocytes and macrophages
Interleukin 2	*IL2*	A cytokine that is important for the proliferation of T and B lymphocytes
Interleukin 20	*IL20*	A cytokine that transduces its signal through signal transducer and activator of transcription 3 (STAT3) in keratinocytes. A specific receptor for this cytokine is expressed in skin
Interleukin 22	*IL22*	A cytokine that is produced by T helper (Th) 17 cells, γδ T cells, NKT cells, and innate lymphoid cells (ILCs). Supports mucosal immunity against bacteria and fungi
Interleukin 27	*IL27*	This protein is related to interleukin 12A (IL12A). It interacts with Epstein-Barr virus induced gene 3 (EBI3), a protein similar to interleukin 12B (IL12B), and forms a complex that has been shown to drive rapid expansion of naïve but not memory CD4(+) T cells. The complex is also found to synergize strongly with interleukin 12 to trigger interferon gamma (IFNG) production of naïve CD4(+) T cells
Interleukin 3	*IL3*	This cytokine is capable of supporting the proliferation of a broad range of hematopoietic cell types and is also neurotrophic
Interleukin 4	*IL4*	A cytokine produced by activated T cells. Th2-type cytokine
Interleukin 6	*IL6*	A major proinflammatory cytokine, pyrogen, maturation of B cells
Interleukin 9	*IL9*	A regulator of a variety of hematopoietic cells, implicated particularly in asthma
Secreted phosphoprotein 1	*SPP1/osteopontin*	A cytokine that upregulates expression of interferon gamma and interleukin 12
Interleukin 17F	*IL17F*	A cytokine expressed by activated T cells that shares sequence similarity with IL17
Colony stimulating factor 2 receptor beta common subunit	*CSF2RB*	A beta chain of the high affinity receptor for IL-3, IL-5 and CSF
Interleukin 1 receptor type 1	*IL1R1*	A receptor for interleukin 1 alpha, interleukin 1 beta, and interleukin 1 receptor antagonist
Interleukin 1 receptor type 2	*IL1R2*	Binds IL1A, IL1B, and IL1R1, and acts as a decoy receptor that inhibits the activity of its ligands
Interleukin 2 receptor subunit alpha	*IL2RA*	The interleukin 2 (IL2) receptor alpha (IL2RA) and beta (IL2RB) chains, together with the common gamma chain (IL2RG), constitute the high-affinity IL2 receptor
Interleukin 6 receptor	*IL6R*	The receptor of IL6
Interleukin 7 receptor	*IL7R*	A receptor for IL7. IL7 is a cytokine important for B and T cell development
CD9 molecule	*CD9*	A member of the transmembrane 4 superfamily, also known as the tetraspanin family
Sialic acid binding Ig like lectin 1	*SIGLEC1*	A transmembrane protein expressed only by a subpopulation of macrophages and is involved in mediating cell-cell interactions
Indoleamine 2,3-dioxygenase 1	*IDO1*	A rate-limiting step in tryptophan catabolism to N-formyl-kynurenine; plays a role in antimicrobial and antitumor defense, neuropathology, immunoregulation, and antioxidant activity
Insulin like growth factor 1 receptor	*IGF1R*	Binds insulin-like growth factor
Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	*PIK3CD*	A class I PI3K found primarily in leukocytes
Mitogen-activated protein kinase 3	*MAPK3*	A member of the MAP kinase family which act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development
Dual specificity phosphatase 1	*DUSP1*	Inactivates mitogen-activated protein (MAP) kinases *in vitro*, plays a role in the human cellular response to environmental stress
Dual specificity phosphatase 2	*DUSP2*	Inactivates mitogen-activated protein kinases
C-type lectin domain family 4 member A	*CLEC4A/DCIR*	A pattern recognition receptor, plays a role in inflammatory and immune response
C-type lectin domain family 7 member A	*CLEC7A/dectin-1*	A pattern recognition receptor that recognizes a variety of beta-1,3-linked and beta-1,6-linked glucans from fungi and plants, plays a role in innate immune response
Toll-like receptor 1	*TLR1*	Toll-like receptors play a fundamental role in pathogen recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity

(*continued on next page*)

Table 2 (*continued*)

Gene	Symbol	Function (NCBI gene database)
Toll-like receptor 2	*TLR2*	This protein is a cell-surface protein that can form heterodimers with other TLR family members to recognize conserved molecules derived from microorganisms known as pathogen-associated molecular patterns (PAMPs). Activation of TLRs by PAMPs leads to an up-regulation of signaling pathways to modulate the host's inflammatory response. TLR2 is also thought to promote apoptosis in response to bacterial lipoproteins
Toll-like receptor 3	*TLR3*	This receptor is most abundantly expressed in placenta and pancreas, and is restricted to the dendritic subpopulation of the leukocytes. It recognizes dsRNA associated with viral infection, and induces the activation of NF-kappaB and the production of type I interferons. It may thus play a role in host defense against viruses
Toll-like receptor 4	*TLR4*	This receptor has been implicated in signal transduction events induced by lipopolysaccharide (LPS) found in most gram-negative bacteria. Mutations in this gene have been associated with differences in LPS responsiveness
Toll-like receptor 6	*TLR6*	This receptor functionally interacts with toll-like receptor 2 to mediate cellular response to bacterial lipoproteins. A Ser249Pro polymorphism in the extracellular domain of the encoded protein may be associated with an increased of asthma is some populations
Signal transducer and activator of transcription 6	*STAT6*	A transcription activator, plays a central role in exerting IL4 mediated biological responses (Th2 differentiation)
Forkhead box P3	*FOXP3*	A transcriptional regulator. A master regulator of Tregs
MAF bZIP transcription factor F	*MAFF*	A transcription factor, involved in e.g. the cellular stress response
RAR related orphan receptor A	*RORA*	A member of the NR1 subfamily of nuclear hormone receptors; among its roles is to aid in the transcriptional regulation of some genes involved in circadian rhythm
Bradykinin receptor B1	*BDKRB1*	Bradykinin is generated in pathophysiologic conditions such as inflammation, trauma, burns, shock, and allergy; the gene encodes one of its receptors
Bradykinin receptor B2	*BDKRB2*	A receptor for bradykinin

was big and even small samples are sufficient to replicate the findings.

4. Discussion

We compared the expression levels of 147 genes between 67 FEP patients and 38 controls matched for age, gender and area of residence. A difference was detected only for two genes, a higher level of *BDKRB1* and *osteopontin/SPP1* expression was found in cases (all cases irrespective of medication) compared with controls. We also compared the expression levels between controls and patients on risperidone, quetiapine or olanzapine monotherapy. Use of risperidone associated with an unexpectedly strongly increased expression level of 11 pro-inflammatory genes after correction for multiple testing. These include genes that are known to be involved in immune activation, including cytokines and cytokine receptors (*ILR1, ILR2*), osteopontin (*SPP1),* pattern recognition molecules (*TLR1, TLR2, TLR6, dectin-1/CLEC7A*), molecules involved in apoptosis (*FAS*), and some other molecules with functions in immune activation (*BDKRB1, IGF1R, CR1*). The importance of these findings was further supported by a difference in the expression levels of several other genes belonging to the same groups, such as several interleukins. Thus, risperidone seemed to have pro-inflammatory immunomodulatory effects, since the response included factors involved in activating pro-inflammatory processes like *TLR*s, mainly linked to the activation of innate immune cells. In addition, genes controlling apoptosis were likely activated as a consequence of the pro-inflammatory pathway activation.

We found increased expression of *BDKRB1* and osteopontin/*SPP1* in cases compared with controls, and this was not dependent only on risperidone treatment, although patients on risperidone had higher expression levels than all patients combined. *BDKRB1* is a bradykinin receptor that plays a role in initiating innate immune response (Alper et al., 2016; Dutra, 2017). Kinins are pro-inflammatory peptides that are released and act at the site of injury and inflammation, such as brain, and work through bradykinin 1 receptors. While we found no reports in psychosis, a previous Finnish study associated copy number variations of BDKRB receptors with autism (Kanduri et al., 2016). *SPP1* gene codes for osteopontin, also known as early T cell activating factor 1 (ETA-1), which is a pro-inflammatory cytokine-like glycoprotein released by macrophages and other innate immune cells including microglia (Kahles et al., 2014; Yu et al., 2017). Osteopontin and adhesion molecules are involved in lymphocyte adhesion and trafficking into the brain. Osteopontin is released in ischemic brain injury, and may have a role in neurodegenerative disease or in neuroprotection (Chan et al., 2014; Schroeter et al., 2006; Yu et al., 2017). Thus, the possible role of osteopontin in FEP warrants further study.

Increased expression of several *TLR*s was clearly associated with risperidone in our study. Our results suggest that risperidone induces upregulation of *TLR*s and has thus immune-stimulatory effect. There are

Table 3
Correlations of potential confounders with differentially expressed genes in all patients, n = 67.

Gene	Age	BMI	Insulin	Triglycerids	Hs-CRP	BPRS-E positive symptom score during the past week	BPRS-E negative symptom score during the past week	Duration of medication
FAS/CD95	**0.30***	0.048	0.13	−0.15	0.12	0.004	0.048	0.011
CR1	0.21	0.064	0.14	−0.21	0.081	−0.091	0.089	0.066
SPP1	−0.058	0.049	0.19	0.056	0.081	**−0.25***	−0.045	0.17
IL1R1	0.12	0.053	0.13	−0.22	0.070	−0.033	−0.007	0.049
IL1R2	0.066	−0.013	0.18	−0.23	0.13	−0.020	0.046	0.019
IGF1R	0.059	0.12	0.11	**−0.30***	0.014	−0.12	0.12	0.076
CLEC7A/dectin-1	0.056	0.049	0.065	**−0.26***	0.069	0.010	0.069	0.10
TLR1	0.16	0.12	0.15	**−0.26***	0.12	0.060	0.087	0.077
TLR2	0.13	−0.005	0.071	**−0.28***	0.027	0.023	0.098	0.075
TLR6	0.15	0.14	0.22	−0.23	0.11	0.046	0.11	0.054
BDKRB1	0.14	0.062	0.13	0.038	0.084	0.22	0.096	−0.21

*P < 0.05, **P < 0.01, BMI = Body Mass Index; hs-CRP = high sensitivity C-reactive protein; BPRS-E = the Brief Psychiatric Rating Scale-Extended.

also studies suggesting involvement of *TLR*s in the pathogenesis of schizophrenia and bipolar disorder (Venkatasubramanian and Debnath, 2013). Genetic studies suggest association with schizophrenia and bipolar disorder for *TLR2* and *TLR4* polymorphisms (Garcia-Bueno et al., 2016a,b; Kang et al., 2013; Oliveira et al., 2014a, 2014b). Also, in peripheral cells of patients with schizophrenia and bipolar disorder, it was demonstrated that *TLR2* and *TLR4* agonist-mediated cytokine release was different from controls (McKernan et al., 2011). Another study detected significant reductions in *TLR3* and *TLR5* mRNA in the monocytes of patients with an acute phase of schizophrenia (Chang et al., 2011). One study with drug-naïve schizophrenia patients compared *TLR* expression in monocytes and T cells before and after treatment with risperidone (Keri et al., 2017). They found an increased percentage of TLR4+ and TLR5+ monocytes and TLR5+ T_{reg}/T_{act} cells before treatment. After the treatment period, they observed normalized TLR4+ monocytes and an upregulation of TLR2+ monocytes and T_{reg}/T_{act} cells. Thus, several studies have supported the idea that TLR molecules might contribute to an altered immune activation in psychosis and other neuropsychiatric disorders (Garcia Bueno et al., 2016a,b; Venkatasubramanian and Debnath, 2013). In the risperidone group, we further observed marginal differences in gene expression of TLR adaptor molecules *TIRAP* and *MYD88*, and downstream kinases *MAPK1* and *MAPK2*. It is not excluded that the patients with FEP in our study may have responded to risperidone in an altered way and aberrant TLR-mediated inflammatory signaling may play a role in immunological alterations observed in patients with psychiatric disorders.

In addition to the upregulation of TLRs, we found increased expression of *IL1R*s and *FAS* suggesting the activation of inflammasome pathway and apoptotic processes, respectively, in patients treated with risperidone. Thus, the effect of risperidone was clearly immune-stimulatory in our study, which is in contrast to some earlier findings to be reviewed below.

Previously, some studies have compared controls with FEP patients before and after a specific antipsychotic medication. These studies have shown that risperidone decreased the levels of proinflammatory cytokines in the patients. In a study with 69 drug-naïve first-episode schizophrenia patients and 60 healthy controls, higher proportions of Th17 cells and higher plasma levels of IFNγ and IL6 were found before treatment onset in the patients compared with controls, while during the treatment the proportions of Th17 cells decreased (Ding et al., 2014). In 55 drug-naïve FEP patients and 57 controls (Noto et al., 2014), increased IL6, IL10 and TNFα were reported in FEP before treatment. Four weeks after the onset of risperidone treatment, levels of IL6, IL10 and IL4 decreased and IL17 was increased (Noto et al., 2014). In a larger dataset combining several clinical cohorts, including in total 180 antipsychotic-naïve first-episode schizophrenia patients and 350 matched controls (de Witte et al., 2014), the serum levels of IL1RA, IL10 and IL15 were significantly increased in drug-naïve patients; for 32 patients, a 6-week follow-up on quetiapine, risperidone or olanzapine was reported, showing similar decrease in IL10 and IL1R levels for all groups. In 62 drug-naïve FEP patients on risperidone, there was an initial decrease in the levels of IL-1β and IL6 but not TNFα at 2 and 4 weeks, but the levels returned to original pro-inflammatory level by 2 or 6 months (Song et al., 2014). However, in previous works on dendritic cells of healthy adults (Chen et al., 2012) and murine macrophages (da Cruz Jung et al., 2016), risperidone increased the production of proinflammatory cytokines such as IL6, IL8 and TNFα, but decreased anti-inflammatory cytokines like IL10.

We evaluated whether the medication first caused a difference in glucose or lipid metabolism or anthropometric measures, which then would have resulted in the differences in inflammatory response. Notably, while this is a cross-sectional study during the first weeks of treatment, there was no difference in BMI between cases and controls, nor in distinct monotherapy groups. Also, there was no difference in plasma glucose and LDL measurements in the total sample, and in glucose or lipid levels between the three monotherapy groups. We detected higher triglyceride levels and lower HDL in cases, in line with a recent meta-analysis (Pillinger et al., 2017). Antipsychotics differ in the immediate effect on fatty acid and cholesterol biosynthesis (Cai et al., 2015) and inflammatory response in adipocytes (Sarvari et al., 2014). While the most significant genes did not correlate with insulin or hs-CRP, a small correlation was detected with triglycerides for four genes. This suggests that the difference in triglyceride levels and proinflammatory profile between cases and controls most likely reflects a rapid, direct effect of medication on regulatory cells of adipogenesis and lipid metabolism (Nousen et al., 2013).

Previous studies have found increased percentage of circulating blood cells with early apoptosis in bipolar patients compared with controls, but the effect of medication was not addressed in these studies (Fries et al., 2014; Scaini et al., 2017). Also, in drug-naïve FEP patients, susceptibility to apoptosis was increased based on an analysis of cell growth and cell viability in fibroblasts (Batalla et al., 2015; Gasso et al., 2014).

We detected a difference between cases and controls only in the expression levels of two genes, which previously had not been linked with psychosis, and did not remain significant after controlling for multiple testing. Furthermore, we could not replicate some of the previous findings in cohorts which should be comparable to our study in terms of duration of medication and in having similar BMI in cases and controls (Di Nicola et al., 2013; Noto et al., 2014). Di Nicola et al. present several explanations for detecting so few differences between cases and controls in gene expression compared with studies measuring cytokine levels. It seems that gene expression in blood is more specific to changes in blood immune cells (Di Nicola et al., 2013) as opposed to changes in circulating cytokines, which reflect the inflammation in adipose tissue or liver (Chen et al., 2017). Also, gene expression in blood cells may change more rapidly than cytokine levels and thus, gene expression reflects more recent than chronic activation of the immune system (Di Nicola et al., 2013). As our results also suggest, for some genes the lack of significant results as a total group could be due to the opposing effects of the specific compounds.

A limitation of the current study is that since clinical treatment was prioritized and was most often needed before the patients were able to give informed consent, we could not compare the same patients before and after antipsychotic treatment. In this naturalistic setting, confounder by indication and lack of control for compliance, pharmacogenetics or blood levels limit our conclusions about the role of dosing; these are important to control for in future studies. The size of subsamples was small for monotherapy groups, and we could not control for the effect of some other factors with immunomodulatory effect such as antidepressants. While we cannot exclude a smaller immunomodulatory effect for quetiapine and olanzapine, in the risperidone group the differences should be detectable already with small sample sizes. It is also possible that the patients actually had an intrinsically disturbed immune response before medication, and the immunomodulatory response to that is weaker in the risperidone monotherapy group than in other groups. The true long-term dynamics of the immune response as well as long-term differences in its effect on outcome remain open. Given the strong evidence for differences in the metabolic side effect profiles of specific compounds, it is not surprising to see differences also in inflammatory responses, and our results might fit an initial anorexigenic effect for risperidone in line with the hypothesis of Fonseka et al. (2016). Whole blood sampling is a feasible method, provides a composite estimate of inflammatory response in the blood cells, and some evidence supports compatibility to measurement of expression from peripherial blood mononuclear cells (Bondar et al., 2014); other methods are needed to evaluate the role of specific cell types. The strengths include high-quality laboratory analysis of samples with a novel medium-throughput technology for measuring mRNA abundances (Prokopec et al., 2013), epidemiologically representative data and a comprehensive set of inflammatory markers.

In conclusion, we detected that cytokines, their receptors, and other

inflammatory markers, which all are related to innate immune response, were robustly activated in the patients using risperidone monotherapy. The immunomodulatory effect of antipsychotics is likely to be a dynamic response, where immunological state before medication, individual characteristics driving immune response, and the type of cells responding affect the specific characteristics of the response at each moment. Our results further emphasize the importance of understanding the immunomodulatory mechanisms of antipsychotic treatment, especially in terms of specific compounds, doses and duration of medication in patients with severe mental illness. Further studies are needed to understand the possible role of this inflammatory activation for the progression of the disease and metabolic comorbidities.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2018.11.008.

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