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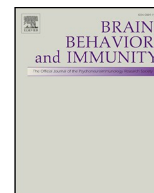
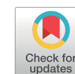


# Exploring cellular markers of metabolic syndrome in peripheral blood mononuclear cells across the neuropsychiatric spectrum



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ABSTRACT

Recent evidence suggests that comorbidities between neuropsychiatric conditions and metabolic syndrome may precede and even exacerbate long-term side-effects of psychiatric medication, such as a higher risk of type 2 diabetes and cardiovascular disease, which result in increased mortality. In the present study we compare the expression of key metabolic proteins, including the insulin receptor (CD220), glucose transporter 1 (GLUT1) and fatty acid translocase (CD36), on peripheral blood mononuclear cell subtypes from patients across the neuropsychiatric spectrum, including schizophrenia, bipolar disorder, major depression and autism spectrum conditions (n = 25/condition), relative to typical controls (n = 100). This revealed alterations in the expression of these proteins that were specific to schizophrenia. Further characterization of metabolic alterations in an extended cohort of first-onset antipsychotic drug-naïve schizophrenia patients (n = 58) and controls (n = 63) revealed that the relationship between insulin receptor expression in monocytes and physiological insulin sensitivity was disrupted in schizophrenia and that altered expression of the insulin receptor was associated with whole genome polygenic risk scores for schizophrenia. Finally, longitudinal follow-up of the schizophrenia patients over the course of antipsychotic drug treatment revealed that peripheral metabolic markers predicted changes in psychopathology and the principal side effect of weight gain at clinically relevant time points. These findings suggest that peripheral blood cells can provide an accessible surrogate model for metabolic alterations in schizophrenia and have the potential to stratify subgroups of patients with different clinical outcomes or a greater risk of developing metabolic complications following antipsychotic therapy.

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## 1. Introduction

Major neuropsychiatric conditions represent a significant burden on worldwide health, accounting for 31% of years lived with disability (YLD) and a lifetime prevalence of over 20% of the global population (approximately 2.4% for bipolar disorder (BD), 1–2% for schizophrenia (SCZ) and autism spectrum conditions (ASC) and 17% for major depressive disorder (MDD), depending on geographic region (Akiskal et al., 2000; Grande et al., 2016; Hyman, 2008; Kahn et al., 2015; Kessler et al., 2005; McGrath et al., 2008; Merikangas, 2011). They are associated with substantial comorbidities including suicide, cardiovascular diseases, immune disorders, metabolic syndrome and substance abuse (Kahn et al., 2015; Malan-Müller et al., 2016; Mitchell et al., 2013). Current treatments are effective in only 40–60% of individuals (Lally and MacCabe, 2015; Huhn et al., 2014; Schooler, 2015; Leucht, 2013; Crespo-Facorro et al., 2013) with potential debilitating side effects, such as extrapyramidal symptoms, weight gain and over-sedation, in addition to treatment refractory symptoms, such as negative and cognitive symptoms, being persistent problems (Berton and Nestler, 2006; Wong and Licinio, 2004). A fundamental lack of understanding about neuropsychiatric pathophysiology and the interaction between genetic (Zhang, 2019) and environmental risk factors, limited characterization of the mechanisms of existing drugs and a paucity of relevant preclinical models have significantly impeded progress towards improved therapies. Moreover, neuropsychiatric heterogeneity and diagnostic instability have emphasized the need to identify readily accessible biomarkers which are capable of stratifying patient populations and predicting clinical response to treatment (Bromet, 2011; Chan, 2011).

In recent years a considerable body of evidence has indicated the association of metabolic disturbances with neuropsychiatric conditions. For example, metabolic syndrome, which comprises visceral adiposity, insulin resistance, hyperglycemia, increased triglycerides, reduced high density lipoprotein (HDL) cholesterol and elevated blood pressure, is prevalent in people with SCZ and is thought to contribute to an increased risk of premature mortality due to cardiovascular disease and type 2 diabetes (Mitchell et al., 2013). Relative to the general population, people with SCZ die on average 15–30 years earlier, largely as a result of cardiovascular complications, and show a 2–3 fold increased prevalence of type 2 diabetes (Pillinger, 2017). While metabolic abnormalities have received most attention in SCZ, a recent metanalysis of 198 studies including 52,678 participants with a severe mental illness suggested that the risk of metabolic syndrome may be similarly elevated in individuals with other major neuropsychiatric conditions such as BD and MDD (Vancampfort, 2015). In the past, these risks have been largely attributed to side effects of specific neuropsychiatric medications, such as the atypical antipsychotics clozapine and olanzapine (Vancampfort, 2015), and the high prevalence of smoking among patients (Meyer and Stahl, 2009; Correll et al., 2014). However, recent data suggests that there might be a predisposition to metabolic abnormalities in subgroups of patients which precedes the onset of pharmacological treatment or extended illness duration (van Beveren, 2014; Schwarz, 2014; Herberth, 2011; Guest, 2010). In the case of SCZ, evidence includes reports of elevated circulating insulin and insulin-related peptides (Guest, 2010, 2011), impaired glucose tolerance (Meyer and Stahl, 2009), hepatic insulin resistance (Meyer and Stahl, 2009), hyperlipidaemia (Correll et al., 2014) and abnormal glucose metabolism in the brain and peripheral tissues (Herberth, 2011; Holden and Mooney, 1994). Importantly, subsets of these metabolic abnormalities have also been reported in unaffected siblings of SCZ patients relative to controls (van Beveren, 2014), identified through largescale *meta*-analyses of glucose homeostasis in drug-naïve patients at disease onset (Pillinger, 2017) and associated with polygenic risk of SCZ (Tomasik et al., 2019), suggesting that they might represent a pre-existing risk factor which is linked to the biological aetiology of the disease. While investigations into putative metabolic disturbances in other neuropsychiatric conditions are more limited (Barone et al., 2018; Cheng et al., 2017; Coello et al., 2019; Hajek et al., 2015; Vancampfort, 2014), shared heritability and significant overlap of genetic risk loci and biological pathways (Lee, 2013) between major neuropsychiatric conditions suggests that common metabolic alterations are plausible. This is highlighted by the enrichment of genetic risk loci in gene sets related to insulin secretion in the largest genome-wide association study of BD to date (Stahl et al., 2019), evidence of mitochondrial dysfunction in ASC (Cheng et al., 2017) and reports of hyperglycemia and hypertriglyceridemia in MDD (Vancampfort, 2014).

At the same time alterations in the immune system have been reported across several neuropsychiatric conditions. These include proinflammatory cytokine alterations in first-episode drug-naïve MDD and SCZ patients (Juncal-Ruiz, 2018; Kakeda et al., 2018; Miller et al., 2011), differences in the relative proportions and activation profile of circulating peripheral blood mononuclear cells (PBMCs) in SCZ and ASC (Suzuki et al., 2013; Beumer et al., 2012; Herberth, 2010; Jyonouchi and Geng, 2019); activation of brain microglia suggested by *post-mortem* immunohistochemistry and *in vivo* positron emission tomography (PET) studies in ASC and SCZ (Bloomfield et al., 2016; Trépanier et al., 2016; van Kesteren et al., 2017) and enrichment of genetic risk loci in genomic regions which are key to immunological function (e.g. major histocompatibility complex (MHC) region and B cell subtype-specific gene expression enhancers in SCZ (Ripke, 2014)). Moreover subsets of these immune alterations appear to be linked to active symptomatology (e.g. interleukin (IL)-6 and tumour necrosis factor-α in MDD (Dowlati, 2010) and IL-6, IL-1β and transforming growth factor-β in SCZ (Miller et al., 2011), disease severity (e.g. GSK-3β phosphorylation in PBMCs in BD (Polter et al., 2010)) and putative aetiological stages in disease progression (e.g. synaptic pruning during postnatal development proposed for SCZ (Sekar, 2016) and ASC (Thomas et al., 2016)). These changes might have implications also for the central nervous system (CNS), as expression of subsets of genes in whole blood correlates with gene expression in multiple brain regions (Sullivan et al., 2006).

Although the direction of causality between these immune and metabolic alterations and psychiatric symptomatology in the CNS is widely debated (Dantzer et al., 2008), it is likely that such alterations characterize subgroups of patients with distinct prognostic features (Schwarz, 2014). For example, in SCZ elevated peripheral cytokines (IL-1β, IL-18, IL-8 and IL-2) have been suggested to characterize a subgroup of patients with treatment refractory symptoms, such as cognitive deficits (Fillman et al., 2016), while oxidized low density lipoprotein receptor expression and peripheral insulin resistance have been shown to predict clinical response to antipsychotic treatment in drug-naïve patients (Tomasik et al., 2016; Tomasik et al., 2019). Likewise, in MDD baseline peripheral IL-8 levels (Liu et al., 2020) and plasma concentrations of high-sensitivity C-reactive protein (Raison et al., 2013) have been associated with antidepressant or adjunctive therapy responses, respectively, while in BD features of metabolic syndrome have been associated with adverse clinical outcomes such as treatment resistance, rapid cycling and progression to chronic illness (Coello et al., 2019). Finally, in addition to associations with treatment response, metabolic and immune markers have shown the potential to predict side-effects of treatment, notably weight gain and metabolic syndrome following second-generation antipsychotic administration in SCZ (Crespo-Facorro et al., 2019; Kim, 2014; MacNeil and Müller, 2016; Schwarz et al., 2015).

In the present study we integrate biological markers of immunity and metabolism by measuring the cell-surface expression of key mediators of metabolic homeostasis, glucose transporter 1 (GLUT1), insulin receptor (IR) and fatty acid translocase (CD36), on abundant PBMC subtypes ($CD4^+$ T cells, $CD8^+$ T cells, $CD4^-/CD8^-$ T cells, B cells and monocytes) from peripheral blood samples of neuropsychiatric patients and controls. The criteria for selection of these metabolic markers involved: 1) direct implication in metabolic syndrome (Boucher et al.,

2014; Guo, 2014; Pascual, 2004; Silverstein and Febbraio, 2009; Wang et al., 2006), 2) significant basal expression in subtypes of PBMCs and CNS cells, 3) previous associations to neuropsychiatric disease (De Silva, 2011; Herberth, 2011; Kahl, 2016; McDermott and de Silva, 2005; Melkersson and Persson, 2011; Tomasik, 2016; Xiuli et al., 2005; Zhao et al., 2006) and 4) availability of reproducible monoclonal antibodies validated for use in flow cytometry. First, we use this information to identify which major neuropsychiatric conditions, including ASC, BD, MDD and SCZ, are most likely to exhibit peripheral metabolic abnormalities in PBMCs. Subsequently, we characterize these abnormalities in relation to systemic indicators of metabolic syndrome, such as altered insulin sensitivity and glucose homeostasis, in addition to polygenic disease risk in drug-naïve patient samples. Finally, we assess the clinical relevance of PBMC metabolic markers for predicting early response to treatment or side effects, such as weight gain, in relevant conditions with a longitudinal clinical follow-up of up to 3 years.

## 2. Materials and methods

### 2.1. Clinical sample recruitment

Patients and matched typical control PBMC donors across the neuropsychiatric spectrum (NS study) were recruited as follows: autism spectrum condition (ASC; Cambridge Autism Research Centre, Cambridge University, Cambridge, UK; n = 25/group), bipolar disorder (BD; Foundation Biological Psychiatry, Sofia, Bulgaria; n = 13 patients and n = 15 controls; and Union House, Cambridgeshire and Peterborough Mental Health Foundation Trust, Cambridge, UK; n = 12 patients and 10 controls), major depressive disorder (MDD; Westfälische Wilhelms University Hospital, Münster, Germany; n = 25/group) and first-onset antipsychotic drug-naïve schizophrenia (SCZ; University Hospital Marqués de Valdecilla, Santander, Spain; n = 25/group; Tables S1 and S2). In the BD cohort, patients and respective controls from different clinical centers were matched within and between centers for age, sex and body mass index (BMI). For the extended SCZ (ES study) cohort, PBMCs from first-onset antipsychotic drug-naïve SCZ PBMC samples (n = 34) and matched controls (n = 39) were collected at the University Hospital Marqués de Valdecilla, Santander, Spain, and combined for analysis with the aforementioned SCZ cohort (total SCZ n = 58 and control n = 63 after excluding two samples with low cell viability (< 50%) from the analysis; Table S3). Control as well as SCZ groups were matched between the two SCZ cohorts for age, sex and BMI ($P > 0.05$). The medical faculty ethical committees responsible for the respective sample collection sites approved the study protocols. Informed consent was given in writing by all participants and clinical investigations were conducted according to the Declaration of Helsinki (World Medical Association, 2013) and Standards for Reporting of Diagnostic Accuracy (Bossuyt et al., 2003).

Diagnoses of neuropsychiatric pathology were conducted by experienced psychiatrists and were based on the Diagnostic and Statistical Manual of Mental Disorders-IV-Text Review (DSM-IV-TR) (Association, 2000). The severity of symptoms in SCZ was measured using the Brief Psychiatric Rating Scale (BPRS) (Leucht, 2005), the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1989). Measurements of serum biochemistry, including cholesterol, high-density lipoprotein, low-density lipoprotein, glucose, insulin and triglycerides, and body mass index (BMI) were conducted as part of the routine clinical follow-up. Follow-up data was collected at baseline, 3 weeks, 6 weeks, 3 months, 1 year and 3 years for BPRS, SAPS and SANS and at baseline, 3 months, 1 year and 3 years for BMI and serum biochemistry measurements. The exclusion criteria for patients and controls included: age below 18 years old, additional neuropsychiatric diagnoses, other neurological conditions including epilepsy, mental retardation, multiple sclerosis, immune/autoimmune conditions, infectious disease, metabolic conditions including diabetes, obesity, cardiovascular disease, hepatic and renal insufficiency, gastrointestinal conditions, endocrine conditions including hypo-/hyperthyroidism and hypo-/hypercortisolism, respiratory diseases, cancer, severe trauma, substance abuse including psychotropic drugs and alcohol, somatic medication for non-CNS indications with CNS side-effects and somatic medication affecting the immune system including glucocorticoids, anti-inflammatory/immuno-modulating drugs and antibiotics.

### 2.2. PBMC isolation and culture

PBMCs were prepared within 4 h from blood collected into 7.5 ml sodium heparin tubes (BD Biosciences; ASC, BD and MDD) or acid citrate dextrose solution A tubes (BD Biosciences; SCZ). Whole blood was pelleted at 500 g for 10 min to remove platelet-rich plasma, diluted 1:1 with Dulbecco's phosphate-buffered saline solution (PBS; Sigma-Aldrich) and centrifuged over Ficoll-Paque PLUS (GE Healthcare) at 750 g for 20 min at 23 °C (15 min at 1000 g for BD samples from Sofia). PBMCs were extracted from the interphase, washed three times with PBS at 300 g for 10 min (once at 760 g for 10 min for BD samples from Sofia) and cryopreserved at $5*10^6$ cells/ml in heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich) (Roswell Park Memorial Institute (RPMI)-1640 medium with 25 mM Hepes, Ultraglutamin-1, 10% FBS and 1% penicillin–streptomycin for BD samples from Sofia) containing 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich). PBMCs were thawed at 37 °C and resuspended in sterile conditions in complete RPMI media with deoxyribonuclease (DNAse) (RPMI-1640 with sodium bicarbonate (Sigma-Aldrich), 10% FBS (Life Technologies), 50 U/ml penicillin and 50 µg/ml streptomycin (Life Technologies), 2 mM L-alanyl-L-glutamine dipeptide (Life Technologies) and 20 µg/ml DNAse (Sigma-Aldrich)). The cells were counted using a Coulter Counter (Beckman Coulter), pelleted, resuspended at $1*10^6$ cells/ml in FACS buffer (PBS with 0.5% bovine serum albumin (Sigma-Aldrich)), strained via a 40 µm cell strainer (Corning) and distributed into in 96-well polypropylene plates (Starlab). Samples from participants from different clinical groups (n = 200; NS and n = 73; ES), alongside quality control (QC) samples (n = 16; NS and n = 10; ES) from a single typical control donor, were randomized across different plate positions and experimental days to minimize measurement-related batch effects.

### 2.3. Staining for metabolic markers, immunophenotyping and cell viability

PBMCs were resuspended at $1*10^6$ cells/ml in FACS buffer with 20% human Fc receptor binding inhibitor (eBioscience) and incubated for 20 min at room temperature in 96-well polypropylene plates (Starlab). The PBMCs were stained in a total volume of 135 µl/tube with 0.5 µl of anti-human CD3 (UCHT1) PE-Cy7 (eBioscience), 0.5 µl of anti-human CD4 (SK3) PerCP-eFluor 710 (eBioscience), 0.5 µl of anti-human CD8 (SK1) APC-eFluor 780 (eBioscience) and 0.3 µl of anti-human CD14 (MφP9) V500 (BD Biosciences). Additionally, 10 µl of anti-human GLUT1 (202915) FITC, 20 µl of anti-human CD220 (IR) (3B6/IR) PE and 2.5 µl of anti-human CD36 (NL07) eFluor660 was added to wells stained with metabolic markers as per manufactures instructions, while an equivalent volume of FACS buffer was added to unstained control wells per sample. The cells were incubated in the dark for 30 min at room temperature. They were washed twice with 200 µl of FACS buffer, resuspended in 200 µl of FACS buffer with 1 µM DAPI (Sigma-Aldrich) and stored at 4°C until acquisition.

### 2.4. Data acquisition using flow cytometry

PBMC cell suspensions were acquired using an eight color FACSVerse flow cytometer (BD Biosciences) with 405, 488 and 640 nm laser excitation at an average flow rate of 2 µl/sec and an average threshold event rate of 1,000–2,000 events/sec. Multicolor Cytometer Setup and Tracking beads (BD Biosciences) were used for quality control and standardization of photomultiplier tube detector voltages

across multiple experimental runs. Fluorescence compensation was conducted using anti-mouse IgGκ antibody capture beads (Bangs Laboratories) labelled separately with anti-human CD3 (UCHT1) PE-Cy7, anti-human CD4 (SK3) PerCP-eFluor 710, anti-human CD8 (SK1) APC-eFluor 780, anti-human CD14 (MφP9) V500 (BD Biosciences), anti-human GLUT1 (202915) FITC, anti-human CD220 (IR) (3B6/IR) PE and anti-human CD36 (NL07) eFluor660, alongside single stain controls with DAPI.

### 2.5. Genotype analysis and calculation of polygenic risk scores

All patients were genotyped using the Illumina Infinium PsychArray Bead Chip and imputed using the standard SHAPEIT2/IMPUTE2 pipeline. PLINK 1.9 was used for the calculation of Polygenic Risk Scores (PRS) using methodologies described elsewhere (Wray, 2014). Briefly, based on the results of the SCZ-PGC GWAS (Ripke, 2014) and using a P-value threshold = 1, PRS were calculated multiplying the imputation dosage for each risk allele by the effect size for each genetic variant. A total number of 113,282 independent variants were included. The resulting values were summed up in an additive fashion obtaining an individual estimate of the genetic load for schizophrenia risk in each subject.

### 2.6. Genotype-Tissue expression dataset

The Genotype-Tissue Expression (GTEx) project data (Lonsdale, 2013) were used to investigate the correlation in gene expression of the selected markers between peripheral blood/Epstein-Barr virus (EBV)-transformed lymphocytes and 12 brain regions. Gene expression data (GTEx_Analysis_2017-06-05_v8_RNASeQCv1.1.9_gene_tpm) were downloaded from https://gtexportal.org/home/datasets and matched within donors using the GTEx_Analysis_v8_Annotations_SampleAttributesDS sample information file. Only *CD36*, *INSR* (insulin receptor) and *SLC2A1* (GLUT1) gene expression data were analysed.

### 2.7. Statistical data analysis

Flow cytometry data was analyzed in FCS 3.0 file format using Flow Jo v.10.0.8 software (Tree Star). Statistical analysis was conducted using R v.3.6.1 software (CoreTeam. R: A Language and Environment for Statistical, 2017). PBMC samples in which the lymphocyte gate contained < 60% of events, measured by forward scatter (FSC-A)/side scatter (SSC-A), were excluded from further analysis. Experimental variables including positional effects within and across 96-well plates, sample viability, cell counts, experimental day, clinical group and sample source were investigated using principal component analysis. Batch effects in median fluorescence intensities (MFIs) caused by running samples from different clinical centres (NS) or different experimental runs (ES) were normalized for each metabolic marker and PBMC subtype using median scaling based on control samples from each clinical centre (NS) or experimental run (ES). Matching of clinical groups was evaluated using the Wilcoxon rank-sum test (Kruskal-Wallis test for more than two groups) for continuous variables or the Fisher's exact test (chi-squared test for more than two groups) for categorical variables. All statistical tests were two-sided, unless specified otherwise. P values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure (shown as Q values). Samples with missing data were excluded from analysis.

The stain index of each antibody, per PBMC subtype, was calculated across PBMC samples for each study and clinical group individually as the median MFI of the antibody-stained sample divided by the median MFI of the corresponding unstained control. For comparison of metabolic marker expression across clinical groups, only metabolic markers with a median stain index greater than two in at least one of the comparison groups were used for analysis. Likewise, for each PBMC cell subtype only samples with a minimum of 100 live cells in the PBMC cell subtype gate were included in analysis. Control data from the NS study were combined across the cohorts to increase statistical power and allow more direct group comparisons. Association of metabolic marker expression in each PBMC subset with clinical group status was investigated using the analysis of covariance (ANCOVA) F-test ($P < 0.05$) after adjusting for covariates. Optional covariates, age, sex, BMI, treatment status (NS) and cell counts, were selected in a stepwise procedure for each marker and PBMC subtype separately using Bayesian Information Criterion. Pairwise *t*-test comparisons of adjusted group means were used to determine which clinical groups differed significantly from typical controls ($P < 0.05$). Additionally, a threshold fold change of at least 5% in either direction for at least one clinically significant group relative to controls was used to define metabolic markers and PBMC subsets with significantly altered expression. Box plots of disease-associated nodes show interquartile range with the median (horizontal line) and the minimum and maximum values (whiskers), excluding outliers (dots).

For follow-up analyses in the ES cohort, the updated Homeostasis Model Assessment (HOMA2) (Levy et al., 1998) was used to infer IR, β cell function, and insulin sensitivity from clinical measurements of fasting serum glucose and insulin levels. Associations of metabolic markers, significantly linked to SCZ in the ES cohort, with HOMA-2 metrics, SCZ polygenic risk score (PRS) and clinical measures of symptom improvement (ΔBPRS, ΔSAPS and ΔSANS) or side effects (ΔBMI, Δcholesterol, ΔHDL, ΔLDL, Δglucose, Δinsulin and Δtriglycerides) were investigated using multivariable linear regression. Changes in symptoms or side effects were calculated as scores at follow-up time point – scores at baseline (0 weeks) time point. For HOMA-2 and PRS analyses, the predictive variables of clinical group and HOMA2 or PRS were set as interaction terms, alongside main effect terms, within the model to determine whether the relationship between metabolic marker expression and HOMA-2 or PRS differed across the clinical groups ($P < 0.05$). Optional covariates, age, sex, BMI and cell counts, in addition to baseline measures (BPRS, SAPS, SANS, BMI, cholesterol, HDL, LDL, glucose, insulin and triglycerides), GWAS principal components 1 to 4 for PRS analysis (to account for ancestry), and treatment drug when predicting response to treatment, were selected in a stepwise procedure for each marker and PBMC subtype separately using Bayesian Information Criterion.

Correlation in gene expression between peripheral blood/EBV-transformed lymphocytes and 12 brain regions in the GTEx project data was analysed using one-tailed Spearman's rank-order correlation ($H_0$: $\rho \le 0$; $H_1$: $\rho > 0$).

Data was visualized using Flow Jo, R software and Adobe Illustrator (Adobe Systems).

### 2.8. Code availability

Upon request.

## 3. Results

### 3.1. Expression of metabolic markers across neuropsychiatric spectrum

To explore peripheral markers of metabolic abnormalities across the neuropsychiatric spectrum, we compared the cell surface expression of GLUT1, IR and CD36 proteins on different PBMC subtypes, including $CD4^+$ T cells, $CD8^+$ T cells, $CD4^-$/$CD8^-$ T cells, $CD3^-$ cells (largely B and natural killer (NK) cells; labelled 'B cells') and monocytes, from individuals with autism spectrum condition (ASC), bipolar disorder (BD), major depressive disorder (MDD) and schizophrenia (SCZ) (n = 25 each) relative to typical controls (Ctrl; n = 100; Fig. S1 and S2; Tables S1 and S2). In addition to previously reported differences in the relative frequencies of immune cell subsets in specific neuropsychiatric conditions (Fig. S3), the data revealed that significant (ANCOVA $P < 0.05$, fold change > 5%) alterations in the expression of these metabolic

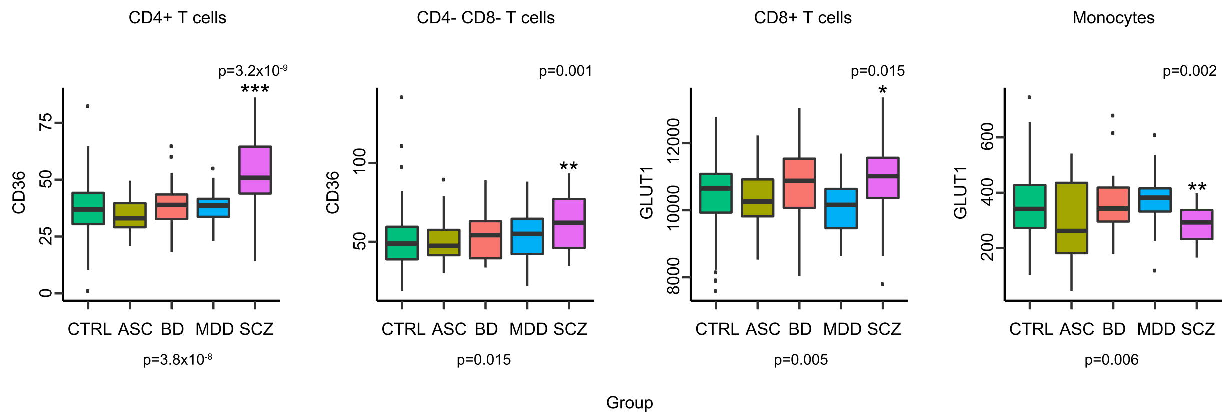


**Fig. 1.** Differential expression of metabolic markers in peripheral blood mononuclear cell (PBMC) subsets across the neuropsychiatric spectrum. Shows metabolic markers and PBMC cell subtypes with a significant association to clinical group status across the five comparison groups, autism spectrum condition (ASC; yellow), bipolar disorder (BD; red), major depressive disorder (MDD; blue), schizophrenia (SCZ; purple) (n = 25 each) and typical controls (CTRL; n = 100; green) ($P < 0.05$, ANCOVA F-test, lower P values), and additionally between at least one of the neuropsychiatric condition groups and controls ($P < 0.05$, pairwise *t*-test of adjusted means, upper P values). Plots show median fluorescence intensity (MFI) of epitope expression (y axis) per clinical group (x axis). Only markers with a median stain index (MFI of stained/MFI of unstained condition) greater than two and a fold change (MFI neuropsychiatric condition/MFI CTRL) of minimum 5% per PBMC subtype in at least one of the clinically significant groups are shown. Optional covariates included age, sex, body mass index, treatment status and cell count. GLUT1 - glucose transporter 1 and CD36 - fatty acid translocase. Asterisks denote P values within the plots as follows: $^{*}P < 0.05$ $^{**}P < 0.005$ $^{***}P < 0.0005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

markers were associated solely with SCZ. These included altered expression of CD36 in $CD4^{+}$ (adjusted fold change (FC) = 1.46) and $CD4^{-}/CD8^{-}$ (FC = 1.29) T cells and GLUT1 in monocytes (FC = 0.74) and $CD8^{+}$ T cells (FC = 1.06; Fig. 1; Table S4).

### 3.2. Characterization of metabolic alterations in schizophrenia

To further investigate the metabolic abnormalities observed in SCZ, we assessed the same cell surface markers in PBMC subsets from an extended cohort of first-onset antipsychotic drug-naïve SCZ patients and controls (Fig. S4 and S5 and Table S3). This revealed significant (ANCOVA $P < 0.05$, fold change > 5%) alterations in the expression of CD36 in $CD4^{+}$ (FC = 1.64) and $CD4^{-}/CD8^{-}$ (FC = 1.22) T cells and GLUT1 (FC = 0.68) and IR (FC = 1.11) in monocytes associated with SCZ (Fig. 2; Table S5). Given the relevance of these markers to metabolic homeostasis, we investigated their relationship to key indicators of metabolic syndrome, including insulin sensitivity, insulin resistance and pancreatic β-cell function calculated using the Homeostasis Model Assessment (HOMA2) tool and fasting measures of serum glucose and insulin concentration across the clinical groups. This revealed that the expression of IR in monocytes was significantly associated with HOMA2 insulin sensitivity in the control group (β = 0.45, P = 0.005), but not the SCZ group (β = 0.16, P = 0.36; Fig. 3a; Table S6). We furthermore explored whether altered expression of metabolic markers in PBMCs was associated with whole-genome polygenic risk of SCZ across the clinical groups. This showed that the relationship between SCZ polygenic risk score and IR expression in monocytes differs significantly (interaction P = 0.027) between the SCZ (β = 19.5) and control (β = -20.1) groups (Fig. 3b; Table S7).

### 3.3. Prediction of clinical response and side effects in schizophrenia

To assess the clinical relevance of PBMC metabolic markers which were altered in SCZ, we explored their potential to predict early response to antipsychotic treatment, defined as changes in the Brief Psychiatric Rating Scale (ΔBPRS), Scale for the Assessment of Positive Symptoms (ΔSAPS) and Scale for the Assessment of Negative Symptoms (ΔSANS) scores at 6 weeks and 3 months, in addition to the principal side-effect of weight gain, defined by changes in body mass index (ΔBMI) at 3 months, through longitudinal clinical follow-up of the drug-naïve SCZ cohort (Fig. S6). This revealed that baseline IR expression in monocytes predicted improvement in general psychopathology (ΔBPRS; $\beta_{6weeks} = -0.031$, $P_{6weeks} = 0.007$; $\beta_{3months} = -0.023$, $P_{3months} = 0.039$; Fig. 4a,b; Table S8) and negative symptoms of SCZ (ΔSANS; $\beta_{6weeks} = -$

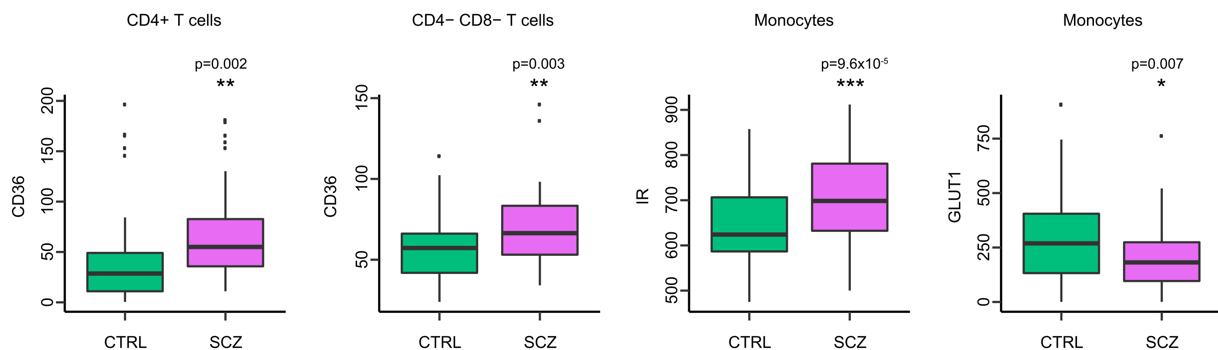


**Fig. 2.** Expression of metabolic markers in PBMC subsets in the extended schizophrenia cohort. Shows metabolic markers and PBMC cell subtypes which are significantly associated ($P < 0.05$, ANCOVA F-test) with first-episode antipsychotic-naïve schizophrenia (SCZ; n = 58) relative to typical controls (CTRL; n = 63). Plots show median fluorescence intensity (MFI) of epitope expression (y axis) per clinical group (x axis). Only markers with a median stain index (MFI of stained/MFI of unstained condition) greater than two and a fold change (MFI neuropsychiatric condition /MFI CTRL) of minimum 5% per PBMC subtype in at least one of the clinical groups are shown. Optional covariates included age, sex, body mass index and cell count. GLUT1 - glucose transporter 1, IR - insulin receptor and CD36 - fatty acid translocase. Asterisks denote P values as follows: $^{*}P < 0.05$ $^{**}P < 0.005$ $^{***}P < 0.0005$.

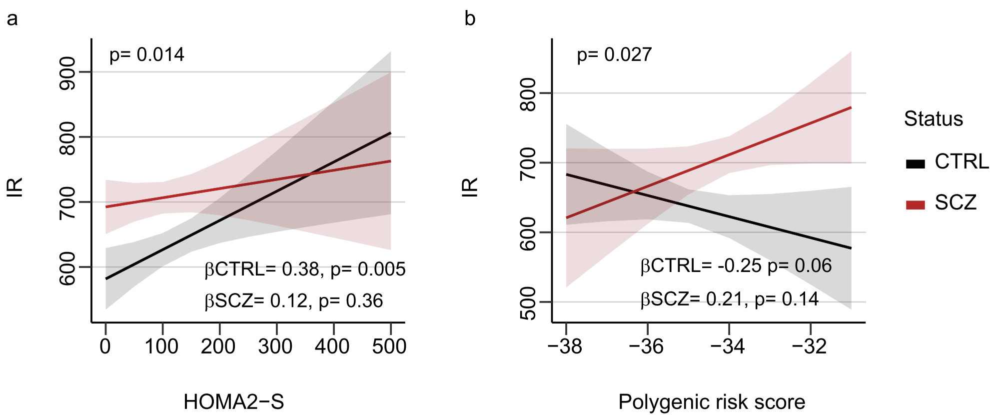


**Fig. 3.** Characterization of PBMC metabolic markers associated with schizophrenia in the context of clinical insulin sensitivity and schizophrenia polygenic risk. Shows (a) the significant association ($P < 0.05$, multivariable linear regression) of insulin receptor (IR) expression in monocytes (y axis) with clinical insulin sensitivity based on the updated Homeostasis Model Assessment (HOMA2-%S; x axis) and (b) the significant interaction ($P < 0.05$, multivariable linear regression) of insulin receptor (IR) expression in monocytes (y axis) and whole genome schizophrenia (SCZ) polygenic risk score (x axis) with clinical group status. Plots show marginal effects with 95% confidence intervals and estimates (β; calculated from scaled predictor and outcome values, to improve interpretability) based on values adjusted for optional covariates age, sex, body mass index, GWAS principal components (for the polygenic risk score analysis) and cell counts for first-episode antipsychotic-naïve SCZ (n = 58) and typical control (CTRL; n = 63) groups. P values represent the significance of (a) association for both groups combined (upper) or each group separately (lower) and (b) interaction between clinical groups (upper) derived from multivariable linear regression.

0.017, $P_{6weeks} = 0.017$; $\beta_{3months} = -0.016$, $P_{3months} = 0.028$; Fig. 4c,d; Table S8) at clinically relevant time points early in the treatment course, six weeks and three months. Moreover, CD36 expression in CD4$^+$ T cells predicted the side-effect of increased BMI at three months (ΔBMI; $\beta = 0.015$, $P = 0.005$; Fig. 4e; Table S8). Consistent with weight gain, the cohort also displayed long-term indicators of metabolic syndrome (up to 3 years) such as increased serum glucose, triglycerides, cholesterol, LDL and decreased HDL. Exploratory analysis of other PBMC cell types which strongly express CD36 (stain index > 10) showed that CD36 expression in monocytes additionally predicted changes in BMI (ΔBMI; $\beta = 5.5\times10^{-5}$, $P = 0.035$) and HDL (ΔHDL; $\beta = -0.0004$, $P = 0.046$) at three months.

### 3.4. Gene expression correlation between blood and brain

Exploratory correlation of *CD36*, *INSR* (IR) and *SLC2A1* (GLUT1) gene expression in whole blood and EBV-transformed lymphocytes with gene expression in 12 brain regions using GTEx project data based on independent samples from the GTEx database showed that gene expression of *CD36* in peripheral whole blood significantly correlated with *CD36* expression in the frontal cortex (Brodmann area 9; N = 159, $\rho = 0.22$, $P = 0.003$), amygdala (N = 109, $\rho = 0.19$, $P = 0.024$), caudate (N = 186, $\rho = 0.13$, $P = 0.035$) and cerebellum (N = 193, $\rho = 0.12$, $P = 0.050$), in addition to significant correlation in *CD36* expression between EBV-transformed lymphocytes and nucleus accumbens (N = 22, $\rho = 0.37$, $P = 0.046$). No other significant correlations were observed.

## 4. Discussion

The present findings extend a growing body of evidence that peripheral metabolic alterations are a feature of SCZ even before the administration of antipsychotic drugs with known metabolic side effects (Pillinger, 2017; Schwarz, 2014; Herberth, 2011; Guest, 2010). However, while the majority of studies have focused on alterations of secreted metabolic factors such as insulin and insulin-related peptides, the present study suggests that circulating immune cells also bear the imprint of metabolic alterations in the form of abnormal cell surface protein expression of key metabolic regulators, such as GLUT1, IR and CD36.

Interestingly, metabolic abnormalities were only observed in SCZ patient-derived PBMCs and not in PBMCs from patients with other major neuropsychiatric conditions. This is notable given recent reports of genetic overlap and bidirectional epidemiological associations between mood disorders and cardiometabolic diseases (Amare et al., 2017; Vancampfort, 2015). Potential explanations for this discrepancy include that fact that many shared risk genes (e.g. *CACNB2*; *GSK3B* and *CREB1*) are highly pleiotropic (Amare et al., 2017) with incompletely characterized functions or lack of phenotypic specificity in the context of polygenic conditions. It could also be that the shared genetic variants in these conditions impact metabolism by indirect means such as altering circadian rhythms and abnormal hypothalamic–pituitary-adrenal axis function (Amare et al., 2017) and these changes are not reflected as metabolic abnormalities in blood cells, at least for the proteins measured. A final consideration is that putative metabolic alterations in

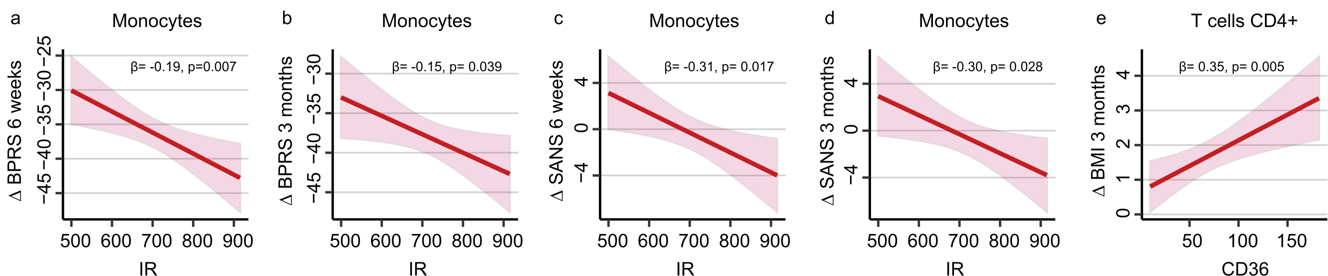


**Fig. 4.** Prediction of antipsychotic efficacy and side effects using PBMC metabolic markers associated with schizophrenia. Prediction of changes (Δ) in clinical symptoms, measured using the Brief Psychiatric Rating Scale (BPRS; a,b) and the Scale for the Assessment of Negative Symptoms (SANS; c,d), and the side effect of weight gain, measured using body mass index (BMI; e), in a longitudinal follow-up of first-episode antipsychotic-naïve schizophrenia (SCZ) patients, treated initially with antipsychotic drugs aripiprazole (n = 28) or risperidone (n = 29), using insulin receptor (IR) expression in monocytes (a-d) and fatty acid translocase (CD36) expression in CD4$^+$ T cells (e). Plots shows significant marginal effects ($P < 0.05$, multivariable linear regression) with 95% confidence intervals and estimates (β; calculated from scaled predictor and outcome values, to improve interpretability) based on values adjusted for optional covariates (baseline value, age, sex, BMI, cell counts and treatment status).

mood disorders may be more indicative of ‘disease state’ rather than ‘disease trait’, as suggested by differential GLUT1 methylation patterns in blood collected from acute versus remitted patients with MDD (Kahl, 2016; Shibata et al., 2013). Given that the SCZ patients in the present study were all drug-naïve while the other cohorts had mixed medication status, this finding requires further exploration in wider drug-naïve patient populations. These results suggest that while metabolic and immune abnormalities might be a feature of other neuropsychiatric conditions, the interaction between the two features is likely to be more prominent in SCZ. However further testing is required across the neuropsychiatric spectrum using other key metabolic markers, beyond those tested in the present study, to confirm this hypothesis.

The increased expression of IR and CD36 and decreased expression of GLUT isoforms in PBMC subsets in SCZ is consistent with alterations in the expression of these proteins in tissue types linked with insulin resistance, such as adipose and muscle tissue, in metabolic syndrome (Boucher et al., 2014; Cignarelli et al., 2019; Glatz et al., 2010; Guo, 2014; Kahn, 1992). In this respect, increased IR expression in monocytes in SCZ might reflect a compensatory response to decreased peripheral IR sensitivity. This is supported by the finding that the relationship between IR expression and insulin sensitivity (HOMA2-%S) is disrupted in SCZ relative to controls in the present study. Likewise, decreased expression of GLUT1 in monocytes is consistent with impaired glucose uptake and increased circulating glucose concentrations observed in both metabolic syndrome and SCZ (Boucher et al., 2014; Guo, 2014; Kahn, 1992; Mitchell et al., 2013; Pillinger, 2017). Although GLUT1 is not considered to be directly regulated by IR, as is the case for GLUT4, its high affinity for glucose and widespread expression mean that it is vital for the constitutive uptake of glucose into a range of tissues. Notably, GLUT1 is the principal glucose transporter in the blood–brain barrier and, in addition to GLUT3, one of the main glucose transporters in the brain (McDermott and de Silva, 2005; Xiuli et al., 2005). The current results align with previous findings suggesting that deficits in brain glucose uptake and energy metabolism could represent pathophysiological features of SCZ. These include decreased GLUT1 and GLUT3 mRNA expression in pyramidal neurons and decreased activity of key glycolytic enzymes, hexokinase and phosphofructokinase, in the dorsolateral prefrontal cortex of individuals with SCZ *post mortem* (Sullivan, 2019); decreased availability of brain high-energy phosphates in drug-naïve SCZ patients in vivo (Pettegrew et al., 1991) and subsets of SCZ-like behavioural symptoms in GLUT knockout mice (De Silva, 2011). Specifically, these alterations have been proposed to participate in the aetiology of SCZ by altering synaptic pruning and affecting neurotransmission through glutamine-glutamate cycling and are suggested to contribute to treatment resistant symptoms such as cognitive deficits (De Silva, 2011; McDermott and de Silva, 2005). Interestingly, GLUT1 showed increased expression in $CD8^+$ T cells in the cross-disorder study although this finding did not replicate in the extended SCZ cohort. This indicates that the GLUT1 expression may be subject to cell subtype-specific dysregulation at least in subgroups of patients. This hypothesis warrants further investigation in larger patient cohorts. Taken together, the present findings suggest that GLUT1 expression in monocytes might provide an accessible surrogate model for investigating systemic abnormalities in glucoregulatory pathways including the brain.

Increased expression of CD36 in $CD4^+$ and $CD4^-CD8^-$ T cells in SCZ is consistent with upregulation of CD36 in human and animal models of insulin resistance (Glatz et al., 2010). In fact, permanent relocation of CD36 in muscle and adipose tissue is considered an early event in the development of metabolic syndrome, with subsequent accumulation of intracellular fatty acids contributing to increased lipid storage, inhibition of insulin signalling by fatty acid metabolites, such as diacylglyceroles and ceramides, and reduced insulin-sensitive GLUT4 expression (Glatz et al., 2010). Interestingly, studies involving CD36 knock-out or knock-in animal models and human subjects with CD36 genetic variants suggest that both ablation and up-regulation of CD36 can result in insulin resistance depending on the context and tissue type affected (Silverstein and Febbraio, 2009). CD36 also has a range of other functions serving as a receptor for thrombospondin-1, scavenging oxidized phospholipids and LDL, recognizing bacterial and fungal pathogens and participating in sensory perception (Silverstein and Febbraio, 2009). Importantly, the binding of oxidized LDL by CD36 on platelets and macrophages contributes to platelet activation and the formation of atherosclerotic plaques resulting in a prothrombotic state, while the binding of thrombospondin-1 to CD36 on endothelial cells regulates angiogenesis (Silverstein and Febbraio, 2009; Silverstein, 2009). These features are particularly relevant to SCZ, given the increased risk of cardiovascular disease. Interestingly, CD36 has also been found to regulate astrocyte activation in the brain suggesting that its effect may also extend to the CNS (Bao, 2012). The potential relevance of our findings for the CNS is further supported by the observation that expression of *CD36* in peripheral blood and EBV-transformed lymphocytes correlated with *CD36* expression in multiple brain regions in the GTEx dataset. Taken together, this data suggests that further studies are required to explore the role of CD36 in SCZ including tissue-specific alterations in expression and their potential functional role in comorbidities such as cardiovascular disease and insulin resistance.

Given the close functional relationship and reciprocal regulation between GLUT isotypes, IR and CD36, it is possible to hypothesise about which of the observed abnormalities could represent a causative aetiological feature in SCZ. The interaction between polygenic risk for SCZ and IR expression across clinical groups in the present study suggests that altered IR expression might represent an initial pathophysiological insult, at least in individuals with high genetic risk. This finding is supported by associations of genetic risk for SCZ with insulin resistance in drug-naïve patients, shared susceptibility genes between SCZ and type 2 diabetes and a higher prevalence of type 2 diabetes in relatives of patients with SCZ (Pillinger, 2017; Tomasik et al., 2019). Given that polymorphisms in the IR itself have not been reported among common genetic risk loci for SCZ (Ripke, 2014), it is possible that genetic loading behind this phenotype might involve multiple downstream genetic variants which cause decreased IR sensitivity and consequent upregulation of the receptor. In this respect, alterations in members of the insulin signalling pathway, such as previously reported changes in Akt in the brain and periphery, are noteworthy (Emamian et al., 2004; Lago et al., 2018; Melkersson and Persson, 2011; Zhao et al., 2006). Decreased IR sensitivity, resulting in reduced glucose uptake by insulin-sensitive tissues and hyperglycaemia, might in turn explain the observed downregulation of GLUT1. Alternatively, as the brain uses 20–50% of available glucose, depending on activity, downregulation of GLUT1 and reduced glucose uptake in the brain, as has been proposed for SCZ, might result in a physiological ‘backlog’ of blood glucose (De Silva, 2011), systemic hyperglycaemia and consequent upregulation of IR expression. Finally, increased expression of CD36 and resulting accumulation of free fatty acids within the cell could bias the expression of both IR and GLUT1, consistent with the concept that alterations in fatty acid homeostasis are initial events in the metabolic disruption preceding insulin-resistance.

The present results also integrate parallel hypotheses of immune and metabolic dysfunction in SCZ. Nutrient utilization mediated by GLUT isotypes, IR and CD36 is required to maintain a healthy immune response both in the brain and periphery (Howie et al., 2018; Matarese and La Cava, 2004), and alterations in the expression of these proteins might contribute to previously reported changes in immune cell reactivity, subset ratios and cytokine secretion profiles in SCZ (Miller et al., 2011; Rahmoune et al., 2013; Rapaport and Bresee, 2010; Steiner, 2010). For example, expression of GLUT1 and GLUT4 proteins have been shown to mediate the progression of monocyte-derived macrophages and brain microglia towards the M1 activated proinflammatory phenotype fuelled by increased anaerobic glycolysis (Freemerman, 2014; Orihuela et al., 2016; Wang, 2019). Although the simplistic representation of M1 vs. M2 microglial polarization has

recently been challenged (Ransohoff, 2016), it is likely that the balance of GLUT1 and GLUT4 expression plays a key role in defining both the spatial and temporal distribution of diverse microglial phenotypes in the brain. This is relevant in the case of SCZ as changes in microglial activation status have been postulated to mediate cognitive symptoms and regional changes in cortical brain volume in subsets of patients (Fillman, 2013; Fillman et al., 2016). Although the current findings of decreased GLUT1 expression in monocytes would suggest a less reactive set-point for related myeloid cell types, this could be offset by a greater GLUT4 shuttling capacity due to increased IR expression. Further studies are needed to explore the functional effects of potential changes in metabolic marker expression in brain microglia in SCZ. Conversely, altered CD36 expression in T cell subsets in the current study could contribute to changes in T cell differentiation, function, and survival via altered fatty acid metabolism. Fatty acid uptake via CD36 has been shown to play a role in T cell activation, proliferation and differentiation into Th1, Th2, Tregulatory (Treg) and Th17 lineages via engagement of peroxisome proliferator-activated receptor subtypes (Howie et al., 2018). This is consistent with alterations in these subtype ratios, their cytokine secretion profiles and proliferation in SCZ. Specifically, increased Treg cells have been reported as a compensatory mechanism for proinflammatory status in SCZ and are associated with fewer negatives symptoms (Kelly, 2018). Concurrently, abnormal Th17 activation in SCZ has been suggested to result in immune infiltration across the blood brain barrier (Debnath and Berk, 2014). Finally, differences in proliferation and naïve vs. memory T cell proportions (Craddock, 2007) or Th1 vs. Th2 cytokine secretion (Rapaport and Bresee, 2010) has been observed in SCZ T cells following antigenic stimulation. Interestingly, reports that both immune and metabolic alterations, for example in the glycolysis pathway proteome and cell surface metabolic protein expression, in SCZ are more readily observable under conditions of immune stimulation (Herberth, 2011) suggest that functional testing is likely necessary to fully explore the potential for divergent nutrient utilization in immune cells in SCZ. Likewise testing of other key regulators of metabolism and metabolic syndrome, such as inducible GLUT3 and GLUT4 proteins, under conditions of antigenic stimulation might be warranted in future studies.

In addition to nutrient utilization, proteins such as CD36 also have a direct role in eliciting an immune response, for example by targeting activated macrophages to atherosclerotic plaques or recognizing bacterial and fungal pathogens (Silverstein and Febbraio, 2009). The reverse is also true in the sense that immune activity is necessary for the development of metabolic syndrome. This is demonstrated by the absence of insulin resistance in knockout mice with mutations in SCZ-associated (Lago et al., 2018, 2019; Ripke, 2014) JAK-STAT immune cell signalling pathways relative to wild-type controls (Dodington et al., 2018) and links between macrophage activation status and metabolic syndrome in animal models of obesity (Debnath and Berk, 2014). Moreover, the fact that alterations in CD36, GLUT1 and IR in the present study were specific to different cell subsets suggests that different PBMC subtypes may represent more relevant surrogate models for different metabolic abnormalities and consequently their implications for immune cell function might be cell subtype-specific. In this respect, the relatively high expression of all three markers and enrichment of SCZ associations on monocytes highlights this cell type for future investigation. Interestingly, no metabolic alterations were found on B cells despite intermediate expression of CD36 and IR on this cell type. This is notable given the enrichment of SCZ-associated risk loci in B cell subtype-specific gene expression enhancers in GWAS analyses (Ripke, 2014).

Finally, the current results support the concept that alterations in peripheral metabolic markers at disease-onset can predict subsequent response to treatment or side effects. The association of monocyte IR expression with changes in BPRS and SANS scores at 6 weeks and 3 months is notable as there is an urgent need to predict response to refractory symptom subtypes such as negative symptoms early in the disease progression. Interestingly, CD36 expression in monocytes which has previously been found to predict improvement in positive symptoms in SCZ (Tomasik, 2016), was not significant, although this may relate to the small sample size ($n = 10$) and the drug treatment used (olanzapine) in the previously reported cohort. The observed prediction of weight gain and changes in HDL using increased CD36 is consistent with the role of CD36 in lipid storage and lipoprotein homeostasis (Glatz et al., 2010). Moreover, the fact that CD36 is associated with weight gain early in the course of treatment, before the average onset of severe metabolic side effects such as type 2 diabetes and cardiovascular disease, is consistent with the hypothesis that CD36 is an early mediator of metabolic syndrome (Glatz et al., 2010). Nevertheless, the difficulties in obtaining antipsychotic drug-naïve patient PBMC samples mean that the sample numbers reported in the present study remain relatively low compared to the likely complexity of the phenotype. In this respect further studies are needed in larger drug-naïve patient cohorts with carefully controlled baseline clinical parameters, such as BMI, to determine the clinical utility of these markers to predict metabolic effects of neuropsychiatric treatment.

These results also allow us to refine the potential implications of treatment with atypical antipsychotic medications. The majority of studies have focused on metabolic disturbances as side effects of antipsychotic treatment, including direct effects of atypical antipsychotic drugs at cellular and physiological levels. These include reports of increased glucose uptake in adipocytes (Vestri et al., 2007), orexigenic effects of histamine 1 antagonism in the brain (Kroeze, 2003), inhibition of insulin signalling proteins (Zhao et al., 2006) and direct GLUT antagonism in the blood brain barrier (McDermott and de Silva, 2005). However, the present results suggest that the development of metabolic syndrome over the course of antipsychotic therapy might represent an interaction between predisposition to metabolic disturbances, at least in subgroups of SCZ patients, and the effects of the drugs themselves. In this respect, the present results provide a prospective means to stratify patients in terms of metabolic risk using accessible cellular tissue. Given the variable metabolic side effect profile of different antipsychotics, this could potentially serve to inform the personalized choice of antipsychotic administered and to facilitate closer monitoring of metabolic side effects in at-risk individuals. This type of data could also provide rationale for recent interest in the use of adjunctive medications, such as glucagon-like peptide-1 agonists, to manage side effects of antipsychotic medication (Larsen et al., 2017). Finally, these findings suggest that metabolic parameters in SCZ may not solely be linked to side effects, but might also be related to clinical efficacy. While clinical trials aimed at repurposing metabolic drugs for SCZ have met with mixed results (Lago and Bahn, 2019), these findings suggest that further testing, combined with improved patient stratification, might be warranted to explore the possibility of metabolic compounds with efficacy in subgroups of SCZ patients.

Taken together the present findings suggest that key indicators of metabolic syndrome are present in PBMCs from subgroups of SCZ patients at disease onset and that these are associated with both side effects and the efficacy profile of currently used medications. Identification of such features at disease onset could potentially facilitate improved strategies for the personalized management of debilitating side effects, such as an increased risk of type 2 diabetes and cardiovascular disease, and better long-term treatment outcomes for individuals with SCZ.

## Declaration of Competing Interest

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

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## Conflict of interest

J.T. was consultant for Psynova Neurotech Ltd. until April 2016. S.L. was part funded by Psynova Neurotech Ltd. until October 2015. S.B. is a director of Psynova Neurotech Ltd. and Psyomics Ltd. The remaining authors declare that they have no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.07.043.

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