



Pretreatment levels of the fatty acid handling proteins H-FABP and CD36 predict response to olanzapine in recent-onset schizophrenia patients



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ABSTRACT

Traditional schizophrenia pharmacotherapy remains a subjective trial and error process involving administration, titration and switching of drugs multiple times until an adequate response is achieved. Despite this time-consuming and costly process, not all patients show an adequate response to treatment. As a consequence, relapse is a common occurrence and early intervention is hampered. Here, we have attempted to identify candidate blood biomarkers associated with drug response in 121 initially antipsychotic-free recent-onset schizophrenia patients treated with widely-used antipsychotics, namely olanzapine ($n = 40$), quetiapine ($n = 23$), risperidone ($n = 30$) and a mixture of these drugs ($n = 28$). Patients were recruited and investigated as two separate cohorts to allow biomarker validation. Data analysis showed the most significant relationship between pre-treatment levels of heart-type fatty acid binding protein (H-FABP) and response to olanzapine ($p = 0.008$, $F = 8.6$, $\beta = 70.4$ in the discovery cohort and $p = 0.003$, $F = 15.2$, $\beta = 24.4$ in the validation cohort, adjusted for relevant confounding variables). In a functional follow-up analysis of this finding, we tested an independent cohort of 10 patients treated with olanzapine and found that baseline levels of plasma H-FABP and expression of the binding partner for H-FABP, fatty acid translocase (CD36), on monocytes predicted the reduction of psychotic symptoms ($p = 0.040$, $F = 6.0$, $\beta = 116.3$ and $p = 0.012$, $F = 11.9$, $\beta = -0.0054$, respectively). We also identified a set of serum molecules changed after treatment with antipsychotic medication, in particular olanzapine. These molecules are predominantly involved in cellular development and metabolism. Taken together, our findings suggest an association between biomarkers involved in fatty acid metabolism and response to olanzapine, while other proteins may serve as surrogate markers associated with drug efficacy and side effects.

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1. Introduction

Schizophrenia is a severe mental illness affecting at least 21 million people worldwide (World Health Organization, 2014).

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The disorder is treated primarily with antipsychotic medication but the exact mechanism of action of these drugs remains largely unknown. As a consequence, traditional schizophrenia pharmacotherapy can be a subjective trial and error process involving administration, titration and switching of drugs multiple times until an adequate response is achieved. This results in the problem that not all patients respond well to initial treatment, with up to 50% of patients not responding or experiencing relapse (Robinson et al., 1999).

Treatment with antipsychotic drugs has also been associated with adverse side effects which can lead to health problems and non-compliance (Dolder et al., 2002; Fenton et al., 1997; Tandon et al., 2008). The effects of second-generation (atypical)

antipsychotics, for example olanzapine, quetiapine and risperidone, include metabolic-related side effects such as weight gain, hyperglycaemia, insulin resistance and type II diabetes mellitus (Newcomer, 2005). Side effects of atypical antipsychotics have been associated with the high affinity of these compounds for histamine H₁, α_1 adrenergic, 5-hydroxytryptamine 2C (5-HT_{2C}) and 5-HT₆ receptors, which are known to be involved in appetite regulation (Kim et al., 2007; Kroeze et al., 2003). Considering the potential negative health impact of these side effects, clinicians are now required to monitor patients receiving atypical antipsychotics closely for their glucose, insulin and fatty acid blood levels.

In addition, these deficits have led academic and pharmaceutical industry researchers to launch biomarker discovery initiatives to facilitate treatment decisions and identify novel therapeutic approaches. It is anticipated that the application of biomarker strategies into the clinical decision making process could lead to benefits such as better stratification of patients, monitoring of therapeutic effects, or early detection of side effects (personalised medicine). There have already been some molecular studies which have investigated the association between antipsychotic treatment effects and biomarker readouts (Choi et al., 2009; Schwarz et al., 2012; Thomas, 2006). For example, changes in immune and metabolic markers have been associated with psychotic relapse in schizophrenia patients after treatment with atypical antipsychotics (Schwarz et al., 2012).

With this in mind, we have attempted to characterise serum molecular profiles in early stage schizophrenia patients, before and after treatment with some of the most widely-used atypical antipsychotics. We performed a comprehensive molecular analysis on serum collected from recent-onset, drug-free schizophrenia patients before and after treatment with olanzapine, risperidone, quetiapine or a mixture of these antipsychotics. Sera were analyzed using the Human MAP[®] multiplex immunoassay panel which targets a selection of hormones, growth factors, transport proteins and immune-related molecules, which may be relevant to the antipsychotic mechanism of action. Proteins which showed significant predictive value for treatment response or alterations after treatment were evaluated in an independent clinical cohort treated with the same medications. In addition, we carried out functional

follow-up study of the findings using blood cells isolated from an independent cohort of recent-onset drug-naïve patients.

2. Materials and methods

2.1. Clinical samples

Subjects were recruited from the Departments of Psychiatry at the Universities of Cologne (discovery cohort), Muenster (discovery cohort), Magdeburg (validation cohort) and the Erasmus Medical Centre in Rotterdam (follow-up cohort; Table 1). Schizophrenia was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). The medical faculty ethical committees of the respective universities approved the protocols of the study. Informed consent was given in writing by all participants and clinical investigations were conducted according to the Declaration of Helsinki.

All patients were antipsychotic-naïve ($n = 67$) or had been free of antipsychotic medication for at least 6 weeks at the start of the study ($n = 66$). Patients with co-morbidities, such as type II diabetes mellitus, hypertension, cardiovascular, autoimmune, inflammatory or infectious diseases, were excluded from the study. Patients were assessed for psychopathology by experienced clinicians using the Positive and Negative Syndrome Scale (PANSS) before and after 4–6 weeks treatment with antipsychotics.

2.2. Multiplex immunoassay profiling

Blood samples from the discovery and validation cohorts were collected between 8:00 and 12:00 am into S-Monovette 7.5 mL serum tubes (Sarstedt). Patients were not required to fast at the time of blood collection. The blood was left at room temperature for 2 h according to standard protocols to allow clotting and centrifuged at 4000g for 5 min to remove particulate material. Resulting supernatants were stored at -80°C in Low Binding microcentrifuge tubes (Eppendorf). Levels of 147 analytes were measured in 250 μL serum using the Human MAP[®] multiplexed immunoassay platform (Appendix A, Table A.1) in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory

Table 1

Demographic details of schizophrenia patients from discovery, validation and follow-up cohorts. BMI – body mass index, T_0 – baseline measurement, T_1 – follow-up measurement (4–6 weeks). Values are shown as mean \pm standard deviation. nk = not known.

	Discovery	Validation	Follow-up
Number	47	74	12
Treatment (ola/quet/risp/mix)	23/9/7/8	17/14/23/20	12/0/0/0
Gender (male/female)	35/12	50/24	12/0
Age (years)	29.0 \pm 10.8	35.4 \pm 11.0	26.4 \pm 6.2
Body mass index (kg/m ²)	22.6 \pm 3.1 ^a	25.3 \pm 4.6	24.0 \pm 4.8
Smoking (yes/no/nk)	6/16/25	49/25/0	8/4/0
Cannabis (yes/no/nk)	0/1/46	16/52/6	2/10/0
Treatment status (drug-naïve/free)	23/24	32/42	12/0
PANSS positive score T_0	19.9 \pm 6.8	23.1 \pm 7.2	20.5 \pm 5.3 ^b
PANSS negative score T_0	19.0 \pm 8.3	19.0 \pm 8.6	22.1 \pm 5.4 ^b
PANSS general psychopathology score T_0	39.6 \pm 10.3	42.9 \pm 10.4	39.6 \pm 6.0 ^b
PANSS total score T_0	78.5 \pm 22.2	85.0 \pm 20.3	82.2 \pm 13.9 ^b
PANSS positive score T_1	13.4 \pm 6.0	12.7 \pm 5.4	13.7 \pm 4.8 ^b
PANSS negative score T_1	16.1 \pm 7.9	14.1 \pm 6.9	19.8 \pm 5.1 ^b
PANSS general psychopathology score T_1	31.3 \pm 11.7	28.6 \pm 9.2	33.7 \pm 8.6 ^b
PANSS total score T_1	60.8 \pm 23.8	55.5 \pm 18.7	67.2 \pm 15.4 ^b
Cumulative chlorpromazine equivalent units T_1 (mg):			
Olanzapine	nk	19,211 \pm 8919	nk
Quetiapine	nk	28,191 \pm 16,404	–
Risperidone	nk	12,290 \pm 6293	–
Mixture	nk	17,716 \pm 12,998 ^c	–

^a Data available for 23 patients.

^b Data available for 10 patients.

^c Data available for 17 patients.

(Myriad RBM Inc.; Austin, TX, USA) as described previously (Schwarz et al., 2010).

2.3. Immunoassay analysis of human H-FABP

Whole blood was collected from fasting patients from the follow-up cohort into 7.5 mL heparin tubes (Sarstedt), centrifuged at 500 g for 10 min and the supernatants (plasma) were stored at -80°C . Plasma levels of H-FABP were determined using a human H-FABP enzyme-linked immunosorbent assay (ELISA) kit (Hycult Biotech) according to manufacturer's protocol.

2.4. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were prepared from blood collected into 7.5 mL heparin tubes from the follow-up cohort, as above. The pellets containing blood cells after the centrifugation step were diluted 1:1 with phosphate-buffered saline (PBS; Sigma–Aldrich) and centrifuged over Ficoll (GE Healthcare) at 750 g for 20 min. PBMCs were extracted from the interphase, washed three times with PBS at 300 g for 10 min and cryopreserved in foetal bovine serum (FBS) containing 10% dimethyl sulfoxide (Sigma–Aldrich) at 5×10^6 cells/mL. For analysis, PBMCs were thawed at 37°C and suspended in pre-warmed sterile RPMI-1640 medium (Sigma–Aldrich) containing 10% heat-inactivated FBS, 50 U/mL penicillin and 50 $\mu\text{g}/\text{mL}$ streptomycin, 2 mM L-alanyl-L-glutamine dipeptide (Life Technologies) and 20 $\mu\text{g}/\text{mL}$ DNase (Sigma–Aldrich). The cells were centrifuged at 500 g for 5 min and suspended at 1×10^6 cells/mL in 200 μL of FACS buffer (PBS with 0.5% bovine serum albumin; Sigma Aldrich) containing 20% human Fc receptor binding inhibitor (eBioscience) to block non-specific antibody binding sites. Following 20 min incubation at room temperature, 1×10^5 cells were stained with 0.5 μL anti-human CD3-PeCy7, 0.5 μL anti-human CD4-PerCP-eFluor710, 0.5 μL anti-human CD8-APC-eFluor780 (eBioscience) and 0.3 μL anti-human CD14-V500 (BD Biosciences) in a total volume of 135 μL . Another 1×10^5 cells were stained for CD36 expression using the above staining cocktail plus 2.5 μL anti-human CD36-eFluor660 (eBioscience) in a total volume of 135 μL . The cells were stained for 30 min in the dark at room temperature, washed twice at 500 g for 5 min with 3 mL FACS buffer and resuspended in 0.5 mL FACS buffer with 1 μM DAPI (Sigma–Aldrich). A minimum of 40,000 cells were acquired from each sample using an 8-colour BD FACSVerser flow cytometer (BD Biosciences).

2.5. Data analysis

R version 2.15.2 (R Core Team, 2014) was used for statistical analyses and FlowJo version 10 was used for analysis of flow cytometry data. Treatment efficacy was determined by comparing PANSS scores before and after treatment using Wilcoxon signed-rank tests. Multiplex immunoassays with more than 30% missing values (39 out of 147; Appendix A, Table A.1) were excluded from further analysis. To account for values below and above the lower and upper limits of detection, these were imputed with half the minimum and 1.5 the maximum values, respectively. Association of baseline molecular levels with response to treatment (ΔPANSS) was determined using linear regression models after adjusting for confounding factors. Relevant covariates were selected using Bayesian Information Criterion. Age, gender and baseline PANSS scores were included as optional covariates for all cohorts (discovery, validation and follow-up) and body mass index (BMI), smoking, cannabis use and treatment status (drug-free/naïve) were included as optional covariates in the validation and follow-up

cohorts only. ΔPANSS scores were calculated as percentages of the baseline score (T_0) after subtraction of the minimal possible PANSS score (Leucht, 2014; Leucht et al., 2007). For linear regression modelling, the values from the immunoassays were \log_{10} -transformed to approximate normality. Distribution of analyte levels was assessed using Shapiro–Wilk tests. P values from linear regression models were adjusted by unrestricted permutation of observations 1000 times (Manly, 2007). Due to known association, a one-tailed Spearman's rank correlation test was used to calculate the correlation between plasma H-FABP levels and monocyte CD36 expression. Wilcoxon signed-rank tests were used to identify changes in protein levels following antipsychotic treatment. Fold changes were calculated as the ratio of the mean levels after and before treatment. P values were controlled for false discovery rate (Q value) with the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). However, due to the exploratory nature of this study, unadjusted $P < 0.05$ were considered significant. *In silico* functional analysis was performed using the Ingenuity Pathway Analysis software (IPA; Ingenuity® Systems).

3. Results

3.1. Patient characteristics and treatment efficacy

Patient samples in the discovery and validation cohorts were matched for gender and the initial total PANSS scores (Table 1). Cumulative antipsychotic doses at the follow-up time point did not differ significantly between different treatment types in the validation cohort, except being significantly higher in the group treated with quetiapine than in the group treated with risperidone ($p < 0.01$, Kruskal–Wallis test with *post hoc* Dunn's multiple comparison tests). There was a significant decrease in PANSS general psychopathology and total scores in both cohorts for all treatment groups (Appendix A, Table A.2). However, the decrease in PANSS positive scores was significant in both cohorts for patients treated with olanzapine, risperidone and antipsychotic mixture, but not after quetiapine treatment. Furthermore, the decrease in PANSS negative scores was significant in both cohorts only after olanzapine treatment.

3.2. Prediction of response

Multiplexed immunoassay profiling of baseline molecular levels for the four treatment groups allowed reproducible identification of eight molecules, which could predict response to antipsychotic medication. After controlling for relevant confounding factors, 2 out of an initial 8 predictions were validated for response to olanzapine, 7 out of an initial 55 predictions were validated for response to quetiapine, 1 out of an initial 64 predictions was validated for treatment with risperidone and none out of an initial 67 predictions was validated for mixed antipsychotic treatment (Table 2). The most significant association across the discovery and validation cohorts was observed between heart-type fatty acid binding protein (H-FABP; UniProtKB ID P05413) and response to olanzapine indicated by a change in total PANSS scores. Higher baseline levels of H-FABP were associated with a greater symptom reduction in the discovery ($p = 0.008$, $F = 8.6$, $\beta = 70.4$; Fig. 1a) and validation ($p = 0.003$, $F = 15.2$, $\beta = 24.4$; Fig. 1b) cohorts. Two molecules were reproducibly associated with response in multiple symptom domains. H-FABP was predicting improvement in negative and total PANSS scores for treatment with olanzapine and interleukin-10 (IL-10) was predicting a change in positive and total PANSS scores for treatment with quetiapine (Table 2).

Table 2

Predictors of change (Δ) in positive, negative, general psychopathology and total PANSS scores significant across the discovery and validation cohorts. The table shows values adjusted for relevant confounding factors, as described in the Section 2. β – estimate from linear regression modelling; Q value – P value adjusted for false discovery rate.

Predictor (by treatment)	Response variable	Discovery				Validation		
		β	F value	P value	Q value	β	F value	P value
Olanzapine:								
Heart-type fatty acid binding protein	Δ PANSS negative	60.6	5.5	0.030	0.998	90.2	19.7	0.012
Heart-type fatty acid binding protein	Δ PANSS total	70.4	8.6	0.008	0.813	24.4	15.2	0.003
Quetiapine:								
Angiotensinogen	Δ PANSS negative	-43.7	8.3	0.022	0.540	-51.9	11.8	0.039
Insulin-like growth factor I	Δ PANSS total	-21.7	9.6	0.018	0.432	-71.0	5.7	0.042
Interleukin-10	Δ PANSS positive	-729.1	15.4	0.008	0.168	-172.2	8.5	0.014
Interleukin-10	Δ PANSS total	-489.5	8.8	0.026	0.432	-114.8	10.6	0.016
Macrophage inflammatory protein-1 beta	Δ PANSS positive	185.6	7.0	0.045	0.270	194.3	14.5	0.006
Receptor tyrosine kinase AXL	Δ PANSS general	-188.2	10.0	0.024	0.209	-105.0	5.5	0.032
T-cell-specific protein RANTES	Δ PANSS positive	159.7	11.5	0.019	0.187	200.0	13.2	0.006
Risperidone:								
Thyroxine-binding globulin	Δ PANSS negative	834.8	92.7	0.015	0.324	415.7	8.1	0.026

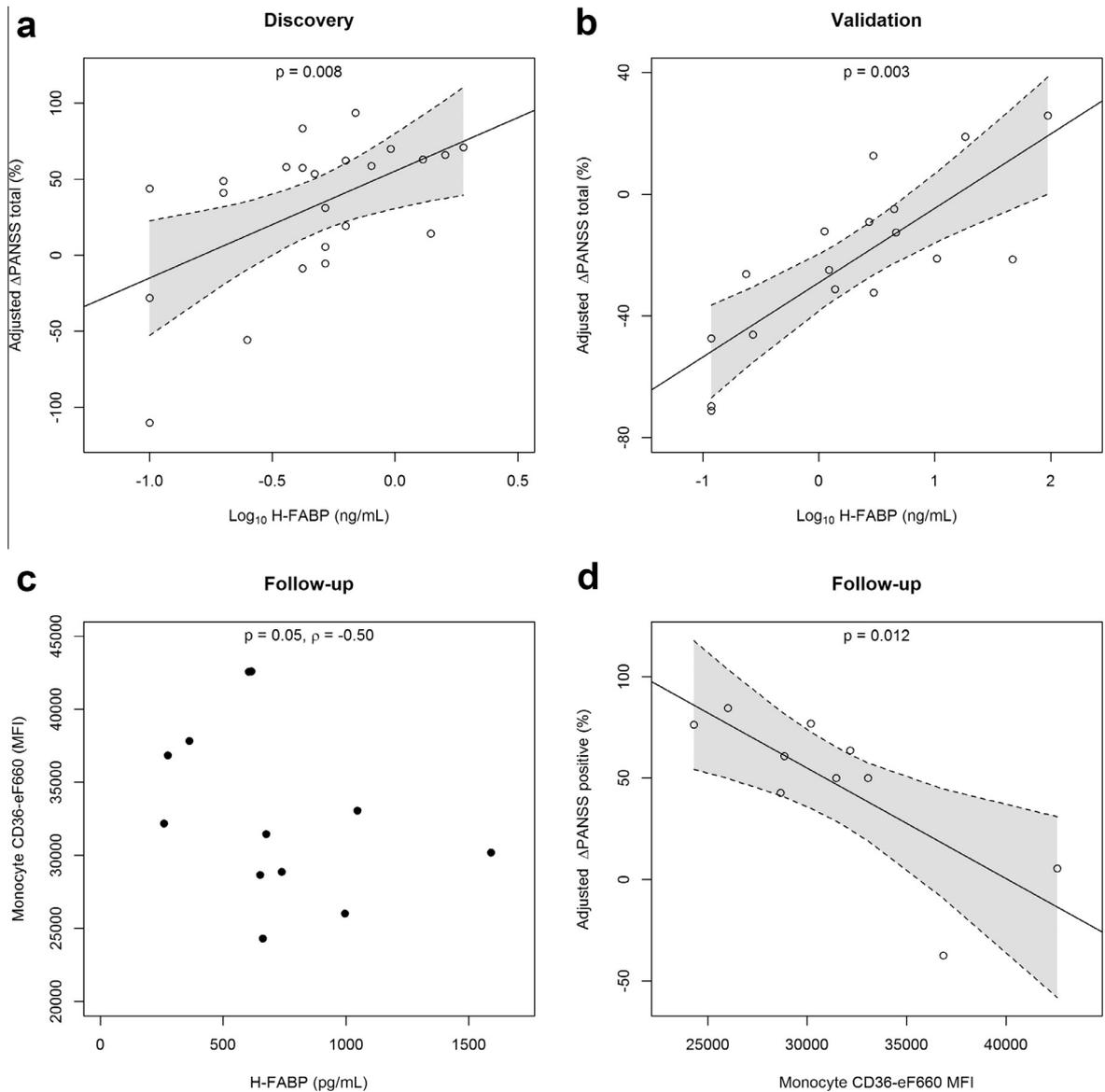


Fig. 1. The most significant predictor of response identified across the discovery and validation cohorts. Baseline H-FABP levels were associated with a reduction of total PANSS scores in the discovery (a) and in the validation (b) cohorts of schizophrenia patients treated with olanzapine. In a follow-up study, monocyte CD36 expression was found to be inversely correlated with plasma levels of H-FABP (c) and was predictive of improvement in psychotic symptoms (Δ PANSS positive; (d)). P, β and Δ PANSS values were adjusted for relevant confounding factors as described in the Section 2. Black line and grey area represent the fitted model and its 95% confidence intervals, respectively. ρ – Spearman's rank correlation coefficient.

3.3. Functional follow-up study

In an independent pilot study performed in 12 recent-onset initially drug-naïve patients treated with olanzapine (Δ PANSS data was available for 10 of them), plasma levels of H-FABP predicted change in total PANSS scores with borderline significance ($p = 0.089$, $F = 3.6$, $\beta = 60.8$), significantly predicted the change in PANSS positive scores ($p = 0.040$, $F = 6.0$, $\beta = 116.3$) and were negatively correlated with CD36 expression in monocytes ($p = 0.05$, $\rho = -0.50$; Fig. 1c). CD36 (UniProtKB ID P16671) is a fatty acid translocase known to interact with H-FABP. Among the tested T helper cells, T cytotoxic cells, B cells and monocytes, CD36 molecules were predominantly expressed in the latter (Appendix B, Fig. B.1). After controlling for relevant confounding variables, monocyte CD36 expression predicted the reduction in PANSS total scores with borderline significance ($p = 0.080$, $F = 4.0$, $\beta = -0.0033$) and significantly predicted PANSS positive score reduction ($p = 0.012$, $F = 11.9$, $\beta = -0.0054$; Fig. 1d).

3.4. Treatment effects

In the discovery cohort, multiplexed immunoassay profiling led to identification of 50 molecules with altered levels after treatment with olanzapine (Appendix A, Table A.3), whereas only 5, 10 and 15 analytes were changed after the quetiapine, risperidone and mixed antipsychotic treatments, respectively (Appendix A, Table A.4). The highest number of consistent changes ($P < 0.05$, same directional change) between the discovery and validation cohorts was observed for the olanzapine treatment group. In both cohorts, the levels of 14 proteins were increased and 3 were decreased (Table 3). In addition, one protein [angiotensin-converting enzyme (ACE)] which met the same criteria, was identified for the quetiapine treatment and one (prolactin) for the risperidone treatment groups. The mixed antipsychotic therapy resulted in significant changes in two proteins, insulin-like growth factor binding protein 2 (IGFBP2) and prolactin. Finally, two proteins were reproducibly

increased after treatment with different antipsychotic medications: prolactin (after treatment with olanzapine, risperidone and antipsychotic mixture) and ACE (after treatment with olanzapine and quetiapine).

Pathway analysis of combined multiplexed immunoassay data from olanzapine-treated patients revealed that the most significantly altered molecular and cellular functions were cell-to-cell signalling, cellular development, cellular growth, proliferation and movement (P values $5.35E-09$ – $3.64E-03$; with 9 or more molecules involved in each function; Appendix A, Table A.5). The top canonical pathway affected by the olanzapine treatment was atherosclerosis signalling ($P = 2.41E-07$; 5 proteins involved) and the disorder that was most significant to the dataset was cardiovascular disease (P values $3.98E-08$ – $3.28E-03$; 9 out of 17 molecules involved).

4. Discussion

This is the first comprehensive protein profiling study to identify and validate serum biomarkers in recent-onset schizophrenia patients associated with response prediction and molecular effects of different antipsychotic treatments. Serum was collected from 121 drug-naïve and drug-free patients following four different treatment regimes. On average, the patients showed reduction in positive, negative and cognitive symptoms, with the most significant changes observed following olanzapine treatment. Multiplexed immunoassay analysis of 147 serum analytes before and after therapy resulted in identification of 8 molecules significantly associated with response to treatment, with the most significant finding for H-FABP as a baseline predictor of response to olanzapine. This was followed-up at the functional level using samples from 10 drug-naïve patients with the finding that plasma H-FABP levels and monocyte expression of the H-FABP molecular partner, CD36, predicted changes in psychotic symptoms after treatment with olanzapine. Importantly, the analyses were controlled for a number of potential confounding factors, including

Table 3
Consistency of changes revealed by multiplexed immunoassays between discovery and validation cohorts for four different treatments. Only significant changes ($P < 0.05$, Wilcoxon signed-rank test) with the same direction of change are shown. Q value – P value adjusted for false discovery rate; RC – ratio change.

Analyte (by treatment)	UniProtKB ID	Discovery			Validation	
		RC	P value	Q value	RC	P value
<i>Olanzapine:</i>						
Angiotensin-converting enzyme	P12821	1.32	0.001	0.005	1.42	<0.001
Alpha-fetoprotein	P02771	1.37	<0.001	0.002	1.66	0.008
Beta-2 microglobulin	P61769	1.28	<0.001	0.002	1.15	0.006
EN-RAGE	P80511	0.67	0.033	0.069	0.59	0.005
Factor VII	P08709	1.09	0.029	0.064	1.27	0.004
Fas (FASLG receptor)	P25445	1.24	0.001	0.004	1.25	0.001
ICAM-1	P05362	1.28	0.001	0.004	1.28	0.005
MDC	O00626	1.20	0.002	0.009	1.31	0.001
MMP-3	P08254	0.75	0.028	0.063	0.38	<0.001
Prolactin	P01236	1.52	0.015	0.037	4.04	<0.001
Prostatic acid phosphatase	P15309	1.30	0.001	0.007	1.43	0.002
Resistin	Q9HD89	0.78	0.006	0.019	0.83	0.001
Stem cell factor	P21583	1.16	0.013	0.034	1.24	0.006
Thyroid-stimulating hormone	P01215	1.28	0.031	0.067	1.83	<0.001
TNF-alpha	P01375	1.50	<0.001	0.002	2.03	0.013
TNF RII	Q92956	1.58	<0.001	<0.001	1.22	0.001
VCAM-1	P19320	1.23	<0.001	0.002	1.13	0.016
<i>Mixture:</i>						
IGFBP2	P18065	0.74	0.035	0.338	0.79	0.035
Prolactin	P01236	4.64	0.016	0.315	2.04	0.011
<i>Quetiapine:</i>						
Angiotensin-converting enzyme	P12821	1.28	0.024	0.694	1.28	0.002
<i>Risperidone:</i>						
Prolactin	P01236	3.84	0.016	0.394	3.96	<0.001

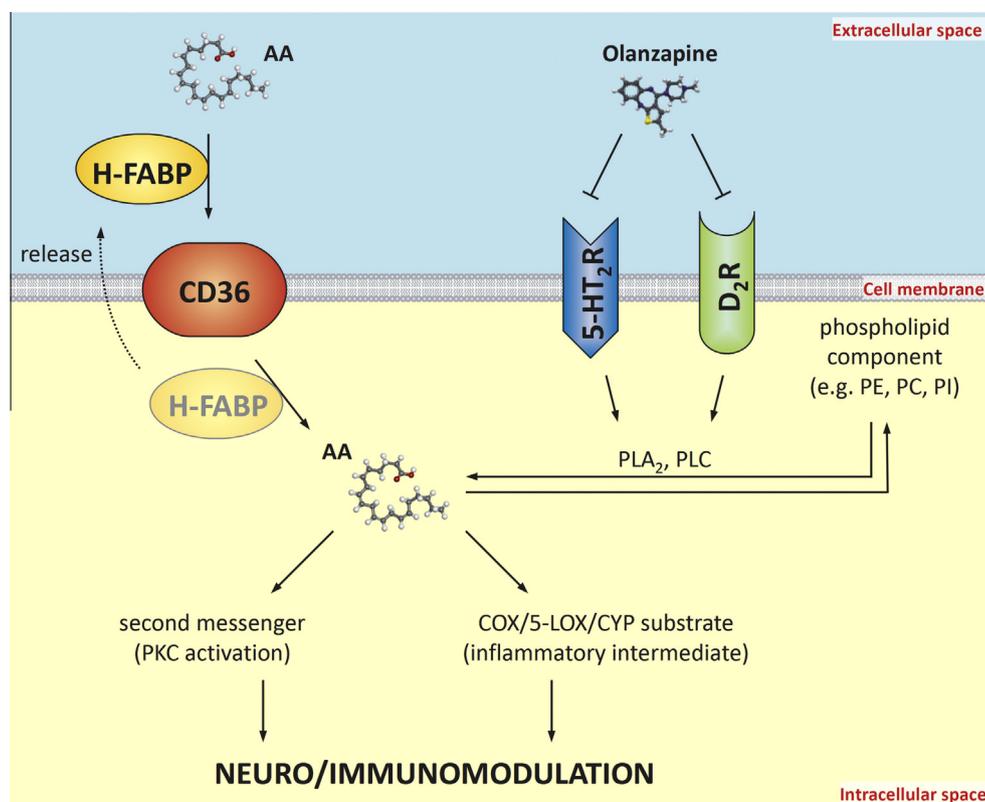


Fig. 2. Hypothetical mechanism of the interaction between fatty acid handling proteins H-FABP and CD36 and response to olanzapine. H-FABP is a critical factor for arachidonic acid (AA) transport and metabolism (Hanhoff et al., 2002), especially in the brain (Shioda et al., 2014). Also CD36 is known to mediate AA uptake inside the cell (Dutta-Roy et al., 1996). Alterations in AA transport may result in altered membrane fluidity, which affects receptor function, or in impaired signal transduction from dopamine D₂ (D₂R) and 5-hydroxytryptamine 2 (5-HT₂R) receptors, for which AA acts as a second messenger (Piomelli et al., 1991; Qu et al., 2005). This could diminish response to antipsychotic treatment and other processes. Other abbreviations: 5-LOX – 5-lipoxygenase, COX – cyclooxygenase, CYP – cytochrome P450, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PI – phosphatidylinositol, PKC – protein kinase C, PLA₂ – phospholipase A₂, PLC – phospholipase C.

baseline symptom severity, age, gender, BMI, smoking, cannabis use and medication status. Moreover, the identified associations were also significant without correcting for covariates, except the association between H-FABP and change in positive symptoms in the follow-up cohort (uncorrected $p = 0.076$). It should be noted that the groups treated with quetiapine, risperidone and mixed antipsychotics in the discovery cohort had low sample sizes, therefore results for these three treatment types should be considered with more caution.

The most significant predictor of response, H-FABP, is a lipid carrier protein expressed primarily in cardiac and skeletal muscles, brain, and mammary gland (Furuhashi and Hotamisligil, 2008). At concentrations >17.7 ng/ml, this protein has been used as a biomarker for acute coronary syndrome (Gururajan et al., 2010). However, across the 52 patients treated with olanzapine in the current study, we found that samples from only 3 individuals had H-FABP concentrations exceeding this threshold. Furthermore, patients with a diagnosis of cardiovascular disease had been excluded from analysis in this study, making it unlikely that these effects were associated with such conditions. Changes in H-FABP levels have also been identified in *post-mortem* studies of other brain disorders. Decreased levels of H-FABP have been found in brain tissues from patients with Down's syndrome and Alzheimer's disease (Cheon et al., 2003). The same researchers suggested that H-FABP may affect brain function by altering membrane fluidity and, therefore, signal transduction in neuronal cells (Cheon et al., 2003). More recently, H-FABP has been shown to be highly expressed in dopaminergic neurons (Shioda et al., 2014) and regulate dopamine D₂ receptor function (Shioda et al., 2010) as well as

synaptogenesis and myelination (Liu et al., 2010) in animal models. This is interesting considering the proposed role of elevated dopamine levels in schizophrenia (Meltzer and Stahl, 1976).

CD36 (or fatty acid translocase) is a membrane protein expressed by many cell types, which binds to a variety of ligands including thrombospondin, long-chain fatty acids, oxidised low density lipoproteins and pathogen associated molecular patterns (Mouille et al., 2012). It is involved in fatty acid and glucose metabolism, and has been linked to insulin resistance (Hajri et al., 2002). CD36 and H-FABP are known to physically interact *in vivo* (Spitsberg et al., 1995) and contain similar fatty acid-binding domains (Baillie et al., 1996). We showed that levels of H-FABP and monocyte CD36 were inversely correlated and patients with low concentrations of H-FABP and high expression of CD36 on monocytes were less likely to respond to olanzapine. We suggest that low treatment response may be due to a deregulation of signal transduction in these patients involving fatty acid transport or metabolism (Fig. 2). This is consistent with studies which have shown that cell membrane fatty acids can be depleted in some schizophrenia patients and higher intake of unsaturated fatty acids has been associated with less severe symptoms (Laugharne et al., 1996). Pre-treatment lipid levels have also been shown to be different between responders and non-responders to atypical antipsychotic treatment (Kaddurah-Daouk et al., 2007). Taken together, these findings suggest that fatty acid metabolism affects signal transduction in the brain and peripheral H-FABP and monocyte CD36 levels could provide a good indicator of response to olanzapine treatment and represent potential therapeutic targets (Furuhashi and Hotamisligil, 2008; Mouille et al., 2012).

Our results also suggest a potential role for the immune system in response to antipsychotic medication. It is interesting that cyclooxygenase (COX) is one of the proteins downstream the identified fatty acid metabolism pathway (Fig. 2). COX is an enzyme that produces prostaglandins and mediates inflammation. It has been shown that modulation of COX activity with anti-inflammatory agents such as aspirin and celecoxib may improve symptoms in schizophrenia patients (Akhondzadeh et al., 2007; Laan et al., 2010; Muller et al., 2010). In addition, a number of immune-related molecules in the present study were directly associated with response to quetiapine. In particular, pre-treatment levels of IL-10 predicted changes in positive and total PANSS scores. IL-10 is gaining increased attention in schizophrenia because of its genetic association with the disease (Bocchio Chiavetto et al., 2002; Ozbey et al., 2009) and the fact that serum levels of IL-10 correlate with psychopathology in schizophrenia patients (de Witte et al., 2014; Xiu et al., 2014). Our findings add to this evidence and support current efforts to increase response rate to antipsychotic medication with add-on immunomodulatory agents.

Although all tested antipsychotics belonged to the atypical class of neuroleptics, the findings did not reproduce between different treatment types. This suggests that the identified predictors may be specific to a specific medication and interact with the drug mechanism of action. In this context, it is not surprising that no predictors were identified for treatment with mixed antipsychotic medication, where patients were prescribed multiple antipsychotics, either simultaneously (polypharmacy) or sequentially. Nevertheless, the exact mechanisms of interaction between the identified predictors and different treatments remain unclear and require further evaluation.

Multiplexed immunoassay profiling of patient serum revealed also molecular changes associated with different atypical antipsychotic treatments. Common effects of therapy with different atypical antipsychotics included increased prolactin and ACE levels. The highest number of consistent changes was observed after treatment with olanzapine, which resulted in changes in the levels of 17 proteins. Pathway analysis indicated that these proteins were most significantly involved in cell signalling, development, growth and cardiovascular system regulation. We propose that these effects may be associated with response to treatment in terms of therapeutic efficacy and metabolic side effects, and that the identified biomarkers could have utility for critical decision making during antipsychotic treatment of first and early-onset schizophrenia patients.

Prolactin and ACE were altered after treatment with different atypical antipsychotics, which could be related to the clinical effects of these compounds. Prolactin levels were increased significantly following treatment with olanzapine, risperidone and mixed antipsychotic drugs. Prolactin is a peptide hormone produced by the pituitary gland and lymphocytes (Ben-Jonathan et al., 1996). It is involved in many functions including lactation, immune modulation and behavioural modification. It is also known to be involved in stimulation of insulin release from pancreatic β cells, which may explain why many patients treated with these compounds develop insulin resistance (Ben-Jonathan et al., 2006). An increase in prolactin levels following typical antipsychotic treatment is well known and has also been ascribed to the antagonistic activity of some atypical antipsychotics such as olanzapine on dopamine D_2 receptors in the anterior pituitary (Kryzhanovskaya et al., 2009). These receptors regulate the prolactin production and release and, therefore, high levels of this hormone could be related to therapeutic outcome or metabolic side effects of antipsychotic treatment (de Visser et al., 2001; Kapur et al., 2000). Our study also confirmed previous reports that treatment with quetiapine does not affect prolactin levels (Atmaca et al., 2002; Leucht et al., 2013).

The finding that ACE was increased after treatment with olanzapine and quetiapine is interesting as this protein normally facilitates vasoconstriction by catalysing conversion of angiotensin I to angiotensin II. Therefore, the increased levels of this protein may be involved in adverse cardiovascular side effects which are sometimes associated with antipsychotic treatment, in particular olanzapine (Daumit et al., 2008; Newcomer, 2007). In addition, the *in silico* pathway analysis identified cardiovascular disease as the top disorder associated with the molecular changes observed after treatment with olanzapine, and atherosclerosis signalling as the top canonical pathway related to those changes. Further studies should be undertaken to determine the potential use of ACE measurement as an early indicator of cardiovascular side effects associated with antipsychotic treatment.

Although the affected proteins were measured in the blood, some may also regulate function of cells within the central nervous system. For example, MMP-3 is produced by microglia and macrophages and is known to induce white matter injury in patients with vascular dementia (Rosenberg et al., 2001) and also to cause de-myelination (Rosenberg, 2009). Therefore, the decreased levels of MMP-3 observed following olanzapine treatment may be associated with improved myelination. This is consistent with the pro-myelination effects of some atypical antipsychotics, which have been described previously (Bartzokis et al., 2007).

We also identified biomarkers that may be related to the metabolic side effect profile of olanzapine. This included increased levels of thyroid-stimulating hormone, which stimulates appetite and may be at least partly responsible for drug-induced weight gain (Khan et al., 2009). The olanzapine treatment also showed changes in the levels of tumour necrosis factor (TNF)-alpha and TNF RII, which have been implicated in weight gain during antipsychotic treatment (Kluge et al., 2009). Additionally, treatment with a mixture of antipsychotics resulted in decreased levels of IGFBP2, which is known to have anti-diabetic effects in animal models (Hedbacker et al., 2010). Taken together, these findings indicate that there is considerable scope for further studies in the identification of serum biomarkers which are associated with metabolic side effects of atypical antipsychotics.

There are potential limitations which should be considered in this study. Firstly, strict inclusion criteria pose an issue for the external validity of the study and potential personalised medicine applications. Findings presented here should not be generalised before validating them in a more naturalistic setup and larger patient cohorts. Secondly, the fact that olanzapine showed superior therapeutic efficacy compared to the other antipsychotics could be due to the smaller number of patients in other treatment groups. In addition, it is conceivable that this would also lead to a greater number of changes in serum biomarkers. Thirdly, response to antipsychotics may be affected by many additional factors not controlled for in these analyses, such as concomitant medication or thyroid status. It should also be noted that patients in the discovery and validation cohorts were not required to fast at the time of blood collection. This might have affected levels of some of the measured analytes, including H-FABP. However, fasting blood sugar levels have been shown before to have negligible effect on serum H-FABP levels (Niizeki et al., 2007).

5. Conclusions

The present results constitute the first comprehensive protein profiling study to identify and validate the effects of treatment with different atypical antipsychotics in recent-onset schizophrenia patients. This revealed that fatty acid handling proteins, H-FABP and its binding partner monocyte CD36, could be used as potential pre-treatment predictors of response to olanzapine. The

results also showed that prolactin and ACE levels were increased in common by different treatment protocols and the highest number of consistent changes was observed after treatment with olanzapine. We suggest that these effects may be associated with response to treatment and that the identified biomarkers could be useful to guide critical decision making for the selection of antipsychotic treatments of schizophrenia patients. Further molecular profiling studies, such as the one described here, can help to increase our understanding of the molecular basis of drug action as well as disease processes. Furthermore, they have the potential to enhance the effectiveness of schizophrenia treatment approaches by helping to identify those patients who are most likely to respond to a given treatment using surrogate markers of treatment response. In this way, future interventions can be tailored to specific patient populations, thereby facilitating early intervention and reducing medication non-response, adverse side effects and non-compliance. The identified biomarkers could also serve as candidate drug targets to improve efficacy of current antipsychotic medications by add-on or novel treatments.

Conflict of interest

Dr. Jakub Tomasik is a consultant for Psynova Neurotech Ltd, Santiago G. Lago is partly supported by Psynova Neurotech Ltd and Prof. Sabine Bahn is a consultant for Myriad Genetics Inc. and is director of Psynova Neurotech Ltd, although this does not interfere with policies of the journal regarding sharing of data or materials. Dr. Emanuel Schwarz, Prof. Matthias Rothermundt, Prof. F. Markus Leweke, Dr. Nico J.M. van Beveren, Dr. Paul C. Guest, Dr. Hassan Rahmoune and Prof. Johann Steiner declare no potential conflict of interest.

Role of the funding source

None of the funding agencies had a further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbi.2015.10.019>.

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