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

Jakub Tomasik a,b, Emanuel Schwarz a, Santiago G. Lago a, Matthias Rothermundt c, F. Markus Leweke d,e, Nico J.M. van Beveren d,f,g, Paul C. Guest a, Hassan Rahmoune a, Johann Steiner h, Sabine Bahn a,b,*

a Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, UK
b Department of Neurosciences, Eramus Medical Centre, Rotterdam, The Netherlands
c Department of Psychiatry, University of Muenster, Muenster, Germany
d Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany
e Department of Psychiatry, Erasmus Medical Centre, Rotterdam, The Netherlands
f Department of Psychiatry, University of Cologne, Cologne, Germany
g Delta Centre for Mental Health Care, Rotterdam, The Netherlands
h Department of Psychiatry, University of Magdeburg, Magdeburg, Germany

A R T I C L E   I N F O

Article history:
Received 24 July 2015
Received in revised form 15 October 2015
Accepted 27 October 2015
Available online 2 November 2015

Keywords:
Schizophrenia
Olanzapine
Biomarker
Personalised medicine
FABP3
CD36

A B S T R A C T

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, β = 70.4 in the discovery cohort and p = 0.003, F = 15.2, β = 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, β = 116.3 and p = 0.012, F = 11.9, β = −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.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

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

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, α1 adrenergic, 5-hydroxytryptamine 2C (5-HT2C) 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 and after treatment with some of the most widely-used 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 analysed 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 5-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 4000 g 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.
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 A2). 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 | ΔPANSS negative | 60.6 | 5.5 | 0.030 | 0.998 | 90.2 | 19.7 | 0.012 |
| Heart-type fatty acid binding protein | ΔPANSS total | 70.4 | 8.6 | 0.008 | 0.813 | 24.4 | 15.2 | 0.003 |
| Quetiapine:                   |                   |   |         |         |         |   |         |         |
| Angiotensinogen               | ΔPANSS negative | −43.7 | 8.3 | 0.022 | 0.540 | −51.9 | 11.8 | 0.039 |
| Insulin-like growth factor I  | ΔPANSS total | −21.7 | 9.6 | 0.018 | 0.432 | −71.0 | 5.7 | 0.042 |
| Interleukin-10                | ΔPANSS positive | −729.1 | 15.4 | 0.008 | 0.168 | −172.2 | 8.5 | 0.014 |
| Interleukin-10                | ΔPANSS total | −489.5 | 8.8 | 0.026 | 0.432 | −114.8 | 10.6 | 0.016 |
| Macrophage inflammatory protein-1 beta | ΔPANSS positive | 185.6 | 7.0 | 0.045 | 0.270 | 194.3 | 14.5 | 0.006 |
| Receptor tyrosine kinase AXL  | ΔPANSS general | −188.2 | 10.0 | 0.024 | 0.209 | −105.0 | 5.5 | 0.032 |
| T-cell-specific protein RANTES | ΔPANSS positive | 159.7 | 11.5 | 0.019 | 0.187 | 200.0 | 13.2 | 0.006 |
| Risperidone:                  |                   |   |         |         |         |   |         |         |
| Thyroxine-binding globulin    | ΔPANSS 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 (ΔPANSS positive; (d)). P, β and ΔPANSS 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 (ΔPANSS 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 \((p = 0.05, p = -0.50; \text{Fig. 1c})\). 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 \((p = 0.012, F = 11.9, \beta = -0.0054; \text{Fig. 1d})\).

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 \((P < 0.05, \text{same directional change})\) 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 group. 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 \((P \text{ values } 5.35E-09–3.64E-03; \text{with 9 or more molecules involved in each function}; \text{Appendix A, Table A.5})\). The top canonical pathway affected by the olanzapine treatment was atherosclerosis signalling \((P = 2.41E-07; 5 \text{ proteins involved})\) and the disorder that was most significant to the dataset was cardiovascular disease \((P \text{ values } 3.98E-08–3.28E-03; 9 \text{ out of 17 molecules involved})\).

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

| Analyte [by treatment] | UniProtKB ID | Discovery | Validation |
|------------------------|-------------|-----------|------------|
| **Olanzapine:**        |             | RC        | P value    | Q value    | RC | P value    |
| Angiotensin-converting enzyme | P12821     | 1.32      | 0.001     | 0.005     | 1.42 | <0.001   |
| Alpha-fetoprotein       | P02816      | 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                     | P00626      | 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    |
| **Mixture:**            |             | RC        | P value    | Q value    | RC | P value    |
| IGFBP2                  | P18065      | 0.74      | 0.035     | 0.338     | 0.79 | 0.035    |
| Prolactin               | P01236      | 4.64      | 0.016     | 0.315     | 2.04 | 0.011    |
| **Quetiapine:**         |             | RC        | P value    | Q value    | RC | P value    |
| Angiotensin-converting enzyme | P12821     | 1.28      | 0.024     | 0.694     | 1.28 | 0.002    |
| Prolactin               | P01236      | 3.84      | 0.016     | 0.394     | 3.96 | <0.001   |

Table 3
Consistency of changes revealed by multiplexed immunoassays between discovery and validation cohorts for four different treatments. Only significant changes \((P < 0.05, \text{Wilcoxon signed-rank test})\) with the same direction of change are shown. Q value \(\text{– } P\) value adjusted for false discovery rate; RC \(\text{– ratio change}\).
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, which affects receptor function, or in impaired signal transduction from dopamine D\(_2\) (D\(_2\)R) and 5-hydroxytryptamine 2 (5-HT\(_2\)R) 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, PLA\(_2\) – phospholipase A\(_2\), PLC – phospholipase C.

**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 D\(_2\) (D\(_2\)R) and 5-hydroxytryptamine 2 (5-HT\(_2\)R) 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, PLA\(_2\) – phospholipase A\(_2\), PLC – phospholipase C.

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 (Moulle 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; Moulle 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., 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 correlated 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 D2 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, 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 RI, 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 (Nizzei 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 Tomaski is a consultant for Psynova Neurotech Ltd, Santiago G. Lago is partly supported by Psynova Neurotech Ltd and Prof. Sabine Bahn was 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.

Acknowledgements

This work was supported by the Stanley Medical Research Institute (SMRI); the European Union FP7 SchizDX research programme (grant reference 223427); the European Union FP7 funding scheme: Marie Curie Actions Industry Academia Partnerships and Pathways (nr. 286334, PSYCH-AID project); by the Virgo consortium, funded by the Dutch government (project number FES0908); by the Netherlands Genomics Initiative (project number 050-060-452); by the Dutch Fund for Economic Structure Reinforcement, the NeuroBasic PharmaPhenomics project (no. 0908) and by the Engineering and Physical Sciences Research Council UK (EPSRC CASE studentship and Impact Acceleration Award).

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.

References

Akhondzadeh, S., Tabatabaeae, M., Amini, H., Ahmadi Abhari, S.A., Abbasie, S.H., Behnam, B., 2007. Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. Schizophr. Res. 96, 179–185.
Atmaca, M., Kugluoglu, M., Tezcan, E., Canatan, H., Gecici, O., 2002. Quetiapine is not associated with increase in prolactin secretion in contrast to haloperidol. Arch. Med. Res. 33, 562–565.
Bailie, A.C., Coburn, C.T., Abumard, N.A., 1996. Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog. J. Membr. Biol. 153, 75–81.
Bartzokis, G., Lu, P.H., Nuechterlein, I.H., Gitlin, M., Doi, C., Edwards, N., Lieu, C., Altshuler, L.L., Mintz, L., 2007. Differential effects of typical and atypical antipsychotics on brain myelination in schizophrenia. Schizophr. Res. 93, 13–22.
Ben-Jonathan, N., Hugo, E.R., Brandebourg, T.D., LaPensee, C.R., 2006. Focus on prolactin as a metabolic host in a host-microbe model. Trends Endocrinol. Metab. 17, 110–116.
Ben-Jonathan, N., Mershon, J.L., Allen, D.L., Steinmetz, R.W., 1996. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocr. Rev. 17, 639–669.
Benjamin, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300.
Bocchio Chiavetto, L., Boin, F., Zanardini, R., Popoli, M., Michelato, A., Bignotti, S., Tura, G.B., Gemmarelli, M., 2002. Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. Biol. Psychiatry 51, 480–484.
Cheon, M.S., Kim, S.H., Fountoulakis, M., Lubeck, G., 2003. Heart type fatty acid binding protein (H-FABP) is decreased in brains of patients with Down syndrome and Alzheimer’s disease. J. Neural Transm. Suppl., 225–234.
Choi, K.H., Higgs, B.W., Weis, S., Song, J., Llorens, I.C., Duly, J.R., Yolken, R.L., Webster, M.J., 2009. Effects of typical and atypical antipsychotic drugs on gene expression profiles in the liver of schizophrenia subjects. BMC Psychiatry 9, 57.
Daumit, G.L., Golf, D.C., Meyer, J.M., Davis, V.G., Nascallah, H.A., McEvoy, J.P., Rosenheck, R., Davis, S.M., Hsiao, J.K., Stroup, T.S., Lieberman, J.A., 2008. Antipsychotic effects on estimated 10-year coronary heart disease risk in the CATIE schizophrenia study. Schizophr. Res. 105, 175–187.
de Vries, S.J., van der Post, J., Pieters, M.S., Cohen, A.F., van Gerven, J.M., 2001. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br. J. Clin. Pharmacol. 51, 119–132.
de Witte, I., Tomaski, J., Schwarz, E., Guest, P.C., Rahmoune, H., Kahn, R.S., Bahn, S., 2007. Cytochrome P450 metabolite and cytokine alterations in first episode schizophrenia patients before and after antipsychotic treatment. Schizophr. Res. 154, 23–29.
Dolder, C.R., Lacro, J.P., Dunn, L.B., Jeste, D.V., 2002. Antipsychotic medication adherence: is there a difference between typical and atypical agents? Am. J. Psychiatry 159, 103–108.
Dutta-Roy, A.K., Gordon, M.J., Campbell, F.M., Crosbie, L.C., 1996. Arachidonic acid uptake by human platelets is mediated by CD36, Platelets 7, 291–295.
Fenton, W.S., Blier, C.R., Heinsen, K.R., 1997. Determinants of medication compliance in schizophrenia: empirical and clinical findings. Schizophr. Bull. 23, 637–651.
Furuhashi, M., Hotamisligil, G.S., 2008. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat. Rev. Drug Discovery 7, 489–503.
Gururajan, P., Gurumurthy, P., Nayar, P., Srinivasa Nageswara Rao, G., Babu, S., Chersian, K.M., 2010. Heart fatty acid binding protein (H-FABP) as a diagnostic biomarker in patients with acute coronary syndrome. Heart Lung Circ. 19, 660–664.
Hajri, T., Han, X.X., Bomen, A., Abumard, N.A., 2002. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. J. Clin. Invest. 109, 1381–1389.
Hanhoff, T., Lucke, C., Spener, F., 2002. Insights into binding of fatty acids by fatty acid binding proteins. Mol. Cell. Biochem. 239, 45–54.
Hedbaeker, K., Bisroky, K., Wysocki, R.W., Aslimar, E., Ahima, R.S., Farooqi, I.S., Friedman, J.M., 2010. Antidiabetic effects of IGFBP2, a leptin-regulated gene. Cell Metab. 11, 121–132.
Kaddurah-Daouk, R., McEvoy, J., Baillie, R.A., Lee, D., Yao, J.K., Doraiswamy, P., Krishnan, K.R., 2007. Metabolomic mapping of atypical antipsychotic effects in schizophrenia. Mol. Psychiatry 12, 934–945.
Kapur, S., Zipursky, R., Jones, C., Remington, G., Hourle, S., 2000. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am. J. Psychiatry 157, 514–520.
Khan, R.A., Mican, L.M., Sues, B.T., 2009. Effects of olanzapine and risperidone on metabolic factors in children and adolescents: a retrospective evaluation. J. Psychiatr. Pract. 15, 320–328.
Kim, S.F., Huang, A.S., Snowman, A.M., Teuscher, C., Snyder, S.H., 2007. From the cover: antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. Proc. Natl. Acad. Sci. U. S. A. 104, 3456–3459.
Klug, M., Schuld, A., Schacht, A., Himmerich, H., Dalal, M.A., Wehmeier, P.M., Hinze-Selch, D., Krau, P., Dittmann, R.W., Pollmacher, T., 2009. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. Psychoneuroendocrinology 34, 118–128.
Kroczek, W., Hufsen, S.J., Popali, B.A., Reneker, J., 2007. A double-blind, randomized and placebo-controlled trial. Schizophr. Res. 56, 179–185.
Krishnan, K.R., 2007. Metabolomic mapping of atypical antipsychotic effects in schizophrenia. Mol. Psychiatry 12, 934–945.
Kreyenbuehl, G., Hufsen, S.J., Popali, B.A., Reneker, J., 2007. A double-blind, randomized and placebo-controlled trial. Schizophr. Res. 56, 179–185.
prüfung: antipsychotic drug-induced weight gain medi...
Leucht, S., 2014. Measurements of response, remission, and recovery in schizophrenia and examples for their clinical application. J. Clin. Psychiatry 75 (Suppl 1), 8–14.

Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Orey, D., Richter, F., Samara, M., Barbui, C., Engel, R.R., Geddes, J.R., Kissling, W., Stadl, M.P., Lassig, B., Salanti, G., Davis, J.M., 2013. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet 382, 951–962.

Leucht, S., Davis, J.M., Engel, R.R., Kane, J.M., Wagenpfeil, S., 2007. Defining ‘response’ in antipsychotic drug trials: recommendations for the use of scale-derived cutoffs. Neuropsychopharmacology 32, 1903–1910.

Liu, R.Z., Mita, R., Beaulieu, M., Gao, Z., Godbout, R., 2010. Fatty acid binding proteins in brain development and disease. Int. J. Dev. Biol. 54, 1229–1239.

Manly, B.F.J., 2007. Randomization, Bootstrap, and Monte Carlo Methods in Biology, 3rd ed. Chapman & Hall, London.

Meltzer, H.Y., Stahl, S.M., 1976. The dopamine hypothesis of schizophrenia: a multiple mechanisms in the pathology and treatment of psychiatric disorders. Mol. Psychiatry 34, 109–128.

Muller, N., Krause, D., Dehning, S., Musil, R., Schennach-Wolff, R., Obermeier, M., Muller, H.J., Klaus, V., Schwarz, M.J., Riedel, M., 2010. Celecoxib treatment in an early stage of schizophrenia: results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. Schizophr. Res. 121, 118–124.

Newcomer, J.W., 2005. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 19 (Suppl 1), 1–93.

Newcomer, J.W., 2007. Antipsychotic medications: metabolic and cardiovascular risk. J. Clin. Psychiatry 68 (Suppl 4), 8–13.

Nizeki, T., Takeishi, Y., Takahatake, N., Shibata, Y., Konta, T., Kato, T., Kawata, S., Kubota, I., 2007. Circulating levels of heart-type fatty acid-binding protein in a general Japanese population: effects of age, gender, and physiologic characteristics. Circ. J. 71, 1452–1457.

Ozbe, Y., Tug, E., Namli, M., 2009. Interleukin-10 gene promoter polymorphism in patients with schizophrenia in a region of East Turkey. World J. Biol. Psychiatry 10, 461–468.

Piomelli, D., Pilone, C., Giros, B., Sokoloff, P., Martres, M.P., Schwartz, J.C., 1991. Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism. Nature 353, 164–167.

Qu, Y., Villacreses, N., Murphy, D.L., Rapoport, S.I., 2005. 5-HT2A/2C receptor signaling via phospholipase A2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. Psychopharmacology 180, 12–20.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/).

Robinson, D., Woerner, M.G., Alvir, J.M., Bilder, R., Goldman, R., Geisler, S., Koreen, A., Sheitman, B., Chakos, M., Mayerhoff, D., Lieberman, J.A., 1999. Predictors of relapse following response from a first episode of schizophrenia or schizoaffective disorder. Arch. Gen. Psychiatry 56, 241–247.

Rosenberg, G.A., 2009. Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. Lancet Neurol. 8, 205–216.

Rosenberg, G.A., Sullivan, N., Espiri, M.M., 2001. White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 32, 1162–1168.

Schwarz, E., Guest, P.C., Steiner, J., Bogerts, B., Bahn, S., 2012. Identification of blood-based molecular signatures for prediction of response and relapse in schizophrenia patients. Transl Psychiatry 2, e82.

Schwarz, E., Imaizono, R., Spain, M., Barnes, A., Mapes, J., West, P.C., Rahmoune, H., Piettsch, S., Leewke, F.M., Rothermundt, M., Steiner, J., Kranaster, L., Ohrmann, P., Suslow, T., Levin, Y., Bogerts, B., van Beveren, N., McAllister, G., Weber, N., Niebuhr, D., Cowan, D., Yolken, R.H., Bahn, S., 2010. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. Biomark Insights 5, 39–47.

Shioda, N., Yabuki, Y., Kobayashi, Y., Onozato, M., Owada, Y., Furukawa, K., 2014. FABP3 protein promotes alpha-synuclein oligomerization associated with 1-methyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. J. Biol. Chem. 289, 18957–18965.

Shioda, N., Yamamoto, Y., Watanabe, M., Binas, B., Owada, Y., Furukawa, K., 2010. Heart-type fatty acid binding protein regulates dopamine D2 receptor function in mouse brain. J. Neurosci. 30, 3146–3155.

Spitsberg, V.L., Mattilashvili, E., Grewot, R.C., 1995. Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. Eur. J. Biochem. 230, 872–878.

Tandon, R., Belmaker, R.H., Gattaz, W.F., Lopez-Ibor Jr., J.J., Okasha, A., Singh, R., Stein, D.J., Olie, J.P., Fleischhacker, W.W., Moeller, H.J., 2008. World Psychiatric Association Pharmacopsychiatry Section statement on comparative effectiveness of antipsychotics in the treatment of schizophrenia. Schizophr. Res. 100, 20–38.

Thomas, E.A., 2006. Molecular profiling of antipsychotic drug function: convergent mechanisms in the pathology and treatment of psychiatric disorders. Mol. Neurobiol. 34, 109–128.

World Health Organization, 2014. What is schizophrenia? <http://www.who.int/mental_health/management/schizophrenia/en/> (accessed 18 Sep 2014).

Xiu, M.H., Yang, C.G., Tan, Y.L., Chen da, C., Tan, S.P., Wang, Z.R., Yang, F.D., Okusaga, O., Soares, J.C., Zhang, X.Y., 2014. Decreased interleukin-10 serum levels in first-episode drug-naive schizophrenia: relationship to psychopathology. Schizophr. Res. 156, 9–14.