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ARTICLE

Metabolomic profiling of schizophrenia patients at risk for metabolic syndrome

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Abstract

Second-generation antipsychotics (SGAs) are commonly used to treat schizophrenia. However, SGAs cause metabolic disturbances that can manifest as metabolic syndrome (MetS) in a subset of patients. The causes for these metabolic disturbances remain unclear. We performed a comprehensive metabolomic profiling of 60 schizophrenia patients undergoing treatment with SGAs that puts them at high (clozapine, olanzapine), medium (quetiapine, risperidone), or low (ziprasidone, aripiprazole) risk for developing MetS, compared to a cohort of 20 healthy controls. Multiplex immunoassays were used to measure 13 metabolic hormones and adipokines in plasma. Mass spectrometry was used to determine levels of lipids and polar metabolites in 29 patients and 10 controls. We found that levels of insulin and tumor necrosis factor alpha (TNF-α) were significantly higher ($p<0.005$) in patients at medium and high risk for MetS, compared to controls. These molecules are known to be increased in individuals with high body fat content and obesity. On the other hand, adiponectin, a molecule responsible for control of food intake and body weight, was significantly decreased in patients at medium and high risk for MetS ($p<0.005$). Further, levels of dyacylglycerides (DG), tryacylglycerides (TG) and cholestenone were increased, whereas α-Ketoglutarate and malate, important mediators of the tricarboxylic acid (TCA) cycle, were significantly decreased in patients compared to controls. Our studies suggest that high- and medium-risk SGAs are associated with disruption of energy metabolism pathways. These findings may shed light on the molecular underpinnings of antipsychotic-induced MetS and aid in design of novel therapeutic approaches to reduce the side effects associated with these drugs.

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Introduction

Schizophrenia is a severe psychiatric disorder that affects 1% of the population worldwide, with vast socio-economic consequences. Second-generation antipsychotics (SGAs) are the most common type of medication currently used to treat this disorder. However, SGAs are known to induce severe side effects, such as increase risk for metabolic syndrome (MetS) and diabetes. Based on this, SGAs have been classified according to their risk for inducing weight gain and increased body mass index (BMI), which are considered indicators of MetS according to the National Cholesterol Education Program (NCEP) (2001) and the International Diabetes Federation (IDF) (Alberti et al., 2006). Clozapine and olanzapine appear to be associated with the greatest metabolic side effects (high risk), followed by quetiapine and risperidone (medium risk), with ziprasidone and aripiprazole causing the least side effects (low risk) (Newcomer, 2007; Tschoner et al., 2009). Some studies indicate that the use of a low-risk antipsychotic such as ziprasidone can improve the metabolic side effects observed with the use of higher risk antipsychotics (Lindenmayer et al., 2012).

Although evidence of weight gain, increased BMI, and dyslipidemia in patients treated with these antipsychotics has been well documented (Fenton and Chavez, 2006; Martinez-Ortega et al., 2013), the mechanisms responsible for these metabolic effects have not been well characterized. There are interrelated hypotheses that have been proposed to explain antipsychotic-induced MetS. One hypothesis is that SGAs cause a dysregulation of hormones that control appetite and food intake such as insulin, leptin, adiponectin, and ghrelin (Sentissi et al., 2008; Teff and Kim, 2011; Stip et al., 2012). Most studies investigating this have focused on just a few metabolic mediators and

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have not established correlations with the different types of medications (high, medium, or low risk) used. Moreover, these studies are conflicting in that some have reported metabolic hormones such as insulin, leptin, and ghrelin to be increased in patients undergoing treatment with SGAs compared to controls (Zhang et al., 2004; Venkatasubramanian et al., 2010), whereas others have reported decreases or no changes in these hormones (Hosojima et al., 2006; Chiu et al., 2010; Tsai et al., 2011). These discrepancies may be attributable to differences in duration of antipsychotic treatment, previous antipsychotics used, and/or concurrent use of other medications at the time of study.

Evidence from a separate line of study has put forth the hypothesis that SGAs alter the function of mitochondrial enzymes involved in energy metabolism, and thus affect carbon metabolism. We have previously found that clozapine, a high-risk SGA, induces oxidation of enzymes important in carbon metabolism such as pyruvate kinase and mitochondrial malate dehydrogenase in neuroblastoma cells (Walss-Bass et al., 2008). We also found mitochondrial enzymes to be oxidized by clozapine in lymphoblastoid cells from schizophrenia patients (Walss-Bass et al., 2008; Baig et al., 2010). More recently, we found that clozapine causes alterations in mitochondrial structure and function in insulin-sensitive cell types (Contreras-Shannon et al., 2013). Other studies using animal models have shown that clozapine alters mitochondrial function and expression of mitochondrial proteins (Streck et al., 2007; Ji et al., 2009). In humans, alterations in electron transport were observed in peripheral blood cells from patients taking SGAs (Casademont et al., 2007), suggesting that mitochondrial function is altered in these patients. Using metabolomic approaches to understand the effect that medications may have on metabolism, studies found clear metabolic differences between pre- and post-treatment with risperidone, a medium-risk SGA for MetS, in schizophrenia patients (Xuan et al., 2011; Cai et al., 2012). Their findings point to metabolic changes in glycolysis and fatty acid synthesis as well as mitochondrial, phospholipid, amino acid and neurotransmitter metabolism (Xuan et al., 2011; Cai et al., 2012). Importantly, the hypotheses of altered mitochondrial function and dysregulation of metabolic hormones are not mutually exclusive. Alterations in mitochondrial function, leading to disruption of energy metabolism, may contribute to a dysregulation of metabolic hormones.

In an effort to unify these hypotheses and to clearly establish the relationship between different SGAs and metabolic markers in schizophrenia-associated MetS, we utilized a comprehensive metabolomic profiling approach to assess levels of key metabolic mediators in plasma from 20 healthy controls and 60 schizophrenia patients under treatment with SGAs that put them at high (n=22), medium (n=27), or low (n=11) risk for MetS, based on the antipsychotic regimen used. Levels of lipids and polar metabolites were assessed in a subset of subjects (29 patients and 10 controls) by mass spectrometry. We hypothesized that differences in levels of metabolic markers would be predicted by the type of SGA taken by schizophrenia patients. These studies may lead to understanding how atypical antipsychotics increase the risk for MetS and may lead to novel therapeutic approaches to reduce these side effects.

Methods

Subjects

This study was performed in accordance with the principles of the Declaration of Helsinki with approval from the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Written informed consent was obtained from all the participants after a complete description of the study was provided. Sixty patients with schizophrenia and 20 healthy subjects were studied. Patient participants were recruited from local community mental health centers and control participants were recruited through advertisements in the community. Patients and controls were matched for ethnicity, age and socioeconomic status. All of the patients met DSM-IV-TR criteria for schizophrenia by the Structured Clinical Interview (SCID) for DSM-IV. Patients were on the same antipsychotic treatment for at least three months prior to enrollment and blood sample collection. Age of disease onset was defined as the age at which patients were first treated with any antipsychotic to manage their psychiatric symptoms (e.g., hallucinations or delusions). This age of onset was used to calculate patients' total illness duration. Risk for MetS was classified based on the type of SGA taken at the time of the study. When more than one type of SGA was being taken, the risk level for that patient was based on the highest risk SGA. Therefore, three risk levels were established as: high (clozapine, olanzapine; N=22), medium (quetiapine, risperidone; N=27), or low (ziprasidone and aripiprazole; N=11) risk for developing MetS. Overall, the prescribed doses of all the antipsychotics were within the clinically recommended range for each of these agents, e.g., olanzapine 10–20 mg/d (except for one patient taking 2.5 mg/d and another 30 mg/d); seroquel 200–800 mg/d; risperidone 1–5 mg/d; and ziprasidone 20–80 mg/d. Healthy subjects were screened for DSM-IV axis I disorders by the SCID non-patient version. Subjects who had a history of diabetes or dyslipidemia were excluded from the study. Therefore, none of the subjects were being treated for type II diabetes or were under insulin treatment. Healthy control subjects who had current or past axis I DSM-IV psychiatric disorders or had first-degree relatives with any axis I psychiatric disorder were excluded. Weight and BMI was obtained from all subjects at the time of enrollment.

Processing of blood samples

Fasting blood was drawn by venipuncture from each subject. Blood samples were immediately processed by centrifugation at (3400 rpm for 10 min) and supernatant (plasma) was subsequently separated into aliquots and stored at −80 °C until biological measurements were performed.

Measurement of metabolic markers

50 μl of plasma were used for detection of levels of 10 metabolic hormones (insulin, leptin, ghrelin, total-amylin, C-peptide, glucagon, pancreatic polypeptide (PP) and peptide YY (PYY), tumor necrosis factor (TNF)-a, and MCP-1) and 3 adipokines (Adiponectin, Resistin and Plasminogen Activator Inhibitor-1 (PAI-1)) involved in regulation of metabolism. Analyte levels were determined using bead-based flow immunoassays in a Luminex 100 system (Luminex Corporation, USA) using Millipore bead arrays (EMD Millipore, USA). These multiplex microbead assays measure protein levels with sensitivity and range comparable to standard sandwich enzyme-linked immunosorbent assay (ELISA).

Metabolomic profiling of plasma samples

Sample preparation: 100 μl of plasma were extracted using 500 μl of cold (−20 °C) chloroform/methanol (2:1) and maintained on ice for 10 min, as described (Gao et al., 2012). After centrifugation at 13 800*g* for 10 min, the methanol layers were removed by pipetting and used for polar metabolite analysis. The chloroform layers were dried *in vacuo* and reconstituted in 200 μl of isopropanol before liquid chromatography-mass spectrometry (LC-MS) analysis. The protein precipitates in the middle were discarded.

LC-MS analysis: LC-MS analyses were conducted on a Thermo Fisher Q Exactive mass spectrometer (Thermo Fisher, USA) with on-line separation using either a Thermo Fisher/Dionex rapid separation liquid chromatography (RSLC) nano high-performance liquid chromatography (HPLC) (for lipids) or an Ultimate 3000 HPLC (for polar metabolites). HPLC conditions for lipid analysis were: column, Atlantis dC18, 3 μm, 300 μm × 150 mm (Waters); mobile phase A, acetonitrile/water (40:60) containing 10 mM ammonium acetate; mobile phase B, acetonitrile/isopropanol (10:90) containing 10 mM ammonium acetate; flow rate, 6 μl/min; gradient, 10% B to 40% B over 5 min, 40% B to 99% B over 35 min and held at 99% B for 10 min. HPLC conditions for polar metabolite analysis were: column, Luna NH2, 3 μm, 1 mm × 150 mm (Phenomenex); mobile phase A, 5% acetonitrile in water containing 20 mM ammonium acetate and 20 mM ammonium hydroxide, pH 9.45; mobile phase B, acetonitrile; flow rate, 50 μl/min; gradient, 85% B to 1% B over 15 min and held at 1% B for 13 min. For both analyses, data-dependent MS/MS scans were performed using one full MS scan (m/z 200–2000 for lipid analysis and m/z 85–1000 for polar metabolite analysis) followed by 6 MS/MS scans in the HCD collision cell with a normalized collision energy (NCE) of 35 arbitrary units. Both positive and negative ion detection were performed through Q Exactive MS at 70 000 resolution (m/z 300). Raw data were acquired under the control of Thermo Xcalibur software (Thermo Fisher, USA).

Data analysis: Progenesis CoMet (Nonlinear Dynamics) was used to process the raw data files acquired from the Q Exactive MS analyses to detect the metabolites that exhibit significant differences in the intensity among the different groups of plasma samples. Peak alignment and integration was performed and the relative abundance was generated for each metabolite among different sample types. The metabolites were identified with accurate mass through Metlin and Lipid Maps databases searching using a 5-ppm mass tolerance and manual interpretation of the MS/MS fragment patterns. The comparison with the retention times with commercially available standards was also performed for further confirmation.

Metabolomic analysis: A total of 2383 features for polar metabolite analysis and 8250 features for lipid analysis were generated through peak picking and quantified through peak integration using the software Progenesis CoMet. 445 features for polar metabolites and 1019 for lipids with significant changes among the four groups of samples after analysis of variance (ANOVA) analysis ($p<0.05$) were subsequently submitted for Metlin database, lipid maps database and human metabolome database (HMDB) searching. 141 polar metabolites and 205 lipids were initially identified through the databases. The putative metabolite IDs were manually identified through MS/MS spectra and in agreement with the retention time from available authentic standards. The confirmed IDs (total 5 polar metabolites and 7 lipids) were reported in this study. A major obstacle in global metabolomics is the identification of unknown metabolites; therefore future structural identification on the unknown metabolites will be performed to obtain a more comprehensive picture of the disturbed metabolites and metabolic pathways.

Statistical analyses

Variables were analyzed using statistical software from GraphPad PRISM 4, IBM SPSS version 20 and JMP 10. Data were log transformed to obtain normally distributed populations based on the Kolmogorov–Smirnov and Shapiro–Wilk tests. For comparison between controls and the three antipsychotic risk groups, Kruskall–Wallis followed by Dunn's post-hoc (for non-parametric data) and ANOVA followed by Bonferroni's post-hoc (for parametric data) tests were used. A Bonferroni adjustment was applied to correct for multiple testing; for metabolic hormone measurements (13 variables,

Table 1. Population demographic characteristics

Risk level*	Gender	Ethnicity	BMI	Weight (lb)	Age (yr)	Years of education (yr)
Control (20)	F (6) M (14)	AA (3) H (13) C (4)	28.55±1.5	188.4±9.36	41.1±2.6	12.6±0.2
Low (11) Aripiprazole (1) Ziprasizone (10)	F (1) M (10)	AA (2) H (8) C (1)	33.82±2.7	213.00±14.8	43.7±3.8	12.7±0.3
Medium (27) Quetiapine (5) Risperidone (22)	F (8) M (19)	AA (6) H (17) C (4)	32.41±1.3	200.81±7.99	41.9±2.2	11±0.3
High (*N*=22) Olanzapine (20) Clozapine (2)	F (5) M (17)	AA (4) H (14) C (4)	30.18±1.4	200.59±8.36	42.6±2.1	11±0.5
Total (*N*=80)	F (20) M (60)	AA (15) H (52) C (13)	31.03±0.8	199.33±4.69	42.1±1.2	11.6±0.2

*Risk of developing metabolic syndrome (MetS). The number of patients under treatment with each type of second-generation antipsychotics (SGA) is shown for each group. Females (F), Males (M), African-American (AA), Hispanic (H), Caucasian (C). (N) Denotes the number of individuals belonging to a specified group. Numbers indicating weight, body mass index (BMI) and age are the mean±S.E.M.

p=0.05/13=0.00384), for polar metabolites (5 variables, p=0.05/5=0.01) and for lipids (7 variables, p=0.05/10=0.00714). All data analyses were performed in a blinded manner without knowledge of patient status.

Results

Demographics

Blood samples for this study were collected from a cohort of 60 patients undergoing treatment for schizophrenia (75%) and 20 healthy controls (25%). The population consisted of 65% Hispanics, 16% Caucasians, and 19% African-Americans. The age range was between 19 to 59 yr old, with a patient average age of 42.5 yr±1.4 and control average age of 41.1 yr±2.6. The gender distribution was 75% male and 25% female (Table 1). No significant differences in gender, ethnicity, level of education, BMI, weight, or age were found between groups. Patient illness duration was 18 yr±10. Patients were classified as high, medium, or low risk for MetS, depending on the type of medication used for treatment at the time of recruitment. Several patients were under treatment with other medications not related to the study, such as anticonvulsants, analgesics, etc.; the distribution and classification of those medications are summarized in Supplementary Table S1.

Comparison of metabolic hormones between antipsychotic risk groups

Levels of metabolic hormones were assessed in plasma across risk groups. Several hormones (insulin, adiponectin, and TNF-α) were found to be significantly different in the risk groups compared to the control group (p=0.0014, 0.000042, 0.0037 respectively, Table 2, Fig. 1*a*). Even though Leptin and C-peptide had a significant p value between groups (ANOVA, p=0.0246 and 0.018), they did not pass the multiple test correction. Metabolic hormones that were not significantly different between groups are not shown. Levels of insulin and TNF-α were the highest, whereas levels of adiponectin were lowest, in the high-risk group. This was evident irrespective of the antipsychotic dose taken by the patients, indicating that there was a lack of dose-response in the metabolic disruptions induced by medications such as clozapine and olanzapine (data for olanzapine in Supplementary Table S2; data not shown for other antipsychotics). Likewise there was not a correlation between patients' illness duration and levels of TNF- α, insulin, or adiponectin (data not shown).

Analysis of lipids and polar metabolites among antipsychotic risk groups

Panels of lipids and polar metabolites, separately, were analyzed by mass spectrometry in a subset of the studied population (29 patients and 10 controls, selected randomly), revealing further molecular differences between groups (Table 2). In the lipid panel, levels of several dyacylglycerides (DG) and tryacylglycerides (TG) were increased in patients compared to controls; however, none of them passed the correction for multiple tests (Table 2 and Fig. 1*b*). Interestingly, for several of the lipids measured, the medium-risk group showed the most

Table 2. Distribution of metabolic mediators across antipsychotic metabolic syndrome (MetS) risk groups

	Control		Low		Medium		High	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Molecule (pg/ml)								
Insulin**	402.8	62.9	745.5	153.3	780.8	91.9	949.1	185
Leptin*	6462	1414.5	15788.3	4895	18191.7	4196.5	14723.3	2580.9
C-peptide*	1448.2	161.7	2426.5	425	2008.3	184	2358.9	275.1
TNF-a**	5.2	0.8	5.3	0.5	5.2	0.7	6.9	0.4
Adiponectin***	12.1 E6	1.60E+06	6.30E+06	7.00E+06	5.90E+06	6.00E+06	5.10E+06	5.80E+06
Molecule (abundance from LC-MS)								
Cholestenone	3.1	3.1	0	0	959.7	838.2	425.7	238.3
DG(30:2)*	1975.4	804.8	582.6	394.6	6159.7	2532.6	3146.6	1092.4
DG(34:6)*	1.20E+04	4.60E+02	1.00E+04	3.80E+02	1.20E+04	5.30E+02	1.20E+04	5.70E+02
DG(35:2)*	4.90E+05	7.50E+04	9.90E+05	2.10E+05	1.10E+06	1.70E+05	1.00E+06	2.00E+05
DG(42:2)	9.00E+03	8.80E+03	3.40E+04	2.60E+04	6.80E+04	3.20E+04	2.90E+04	1.30E+04
TG(52:1)*	1.10E+06	7.80E+05	1.10E+06	2.90E+05	3.40E+06	1.10E+06	1.50E+06	6.30E+05
(±)9- and/or (±)13-HODE*	941.6	72	783.2	52	824.7	69	668.9	61.9
2-Hydroxyglutarate*	466.7	90.4	349	44.6	276.7	48.9	167.4	67.7
α-Ketoglutarate*	4290.9	805.9	1654.2	315.5	3531.6	823.7	2933.3	690
Glutamate**	9220.4	2063.3	13443.9	1659.1	11659.2	848.3	16946.3	923.9
Malate**	8.30E+04	2.40E+03	6.60E+04	2.40E+03	7.00E+04	3.20E+03	7.70E+04	6.60E+03
Kynurenine*	6353.2	595.8	4320	440.8	5474.9	752	7211.4	556.3

Analysis of variance (ANOVA) followed by Bonferroni's test (parametric) or Kruskal–Wallis followed by Dunn's test (non-parametric) performed between groups. Statistical significance at *$p<0.05$, **$p<0.01$ or ***$p<0.001$ between groups.
DG, diacylglycerides; TG, triacylglycerides; HODE, the co-eluted lipids 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid.

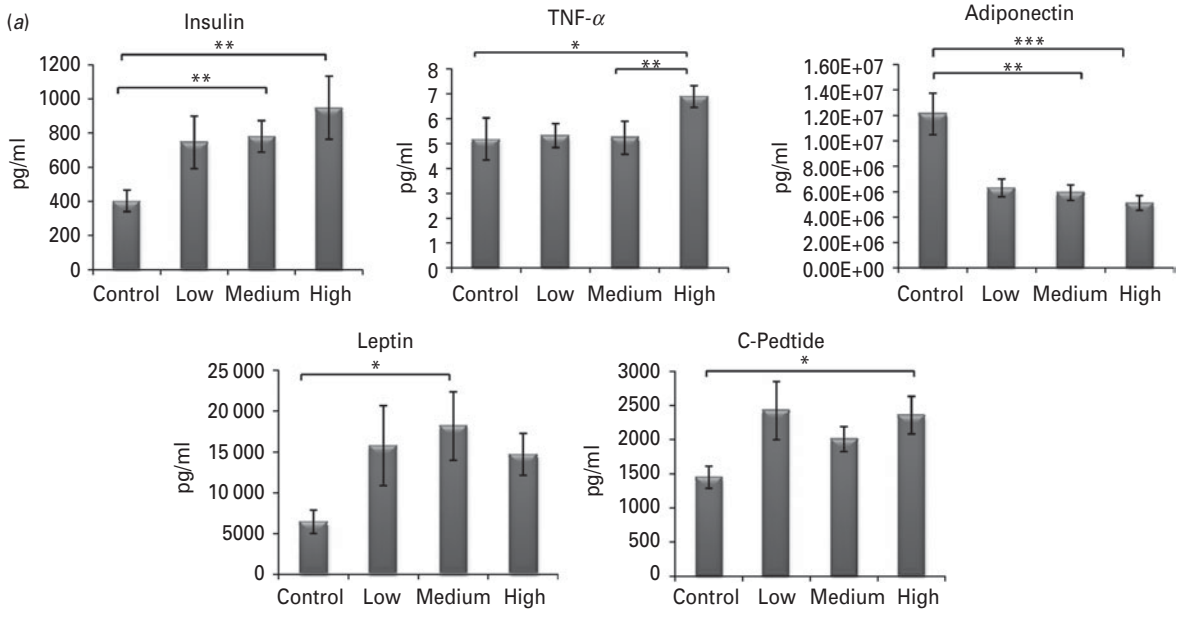

Fig. 1. For legend see next page.

differences compared to the low risk group (DG 30:2) or to controls (DG 35:2, DG 42:2, and TG 52:1). Additionally, for cholestenone and DG 34:6, there was a trend for differences between the low- and high-risk groups. For cholestenone and DG 42:2, a high number of subjects had no detectable levels of these two lipids in the control and low-risk groups, compared to medium and high risk, suggesting a clear relationship of SGAs

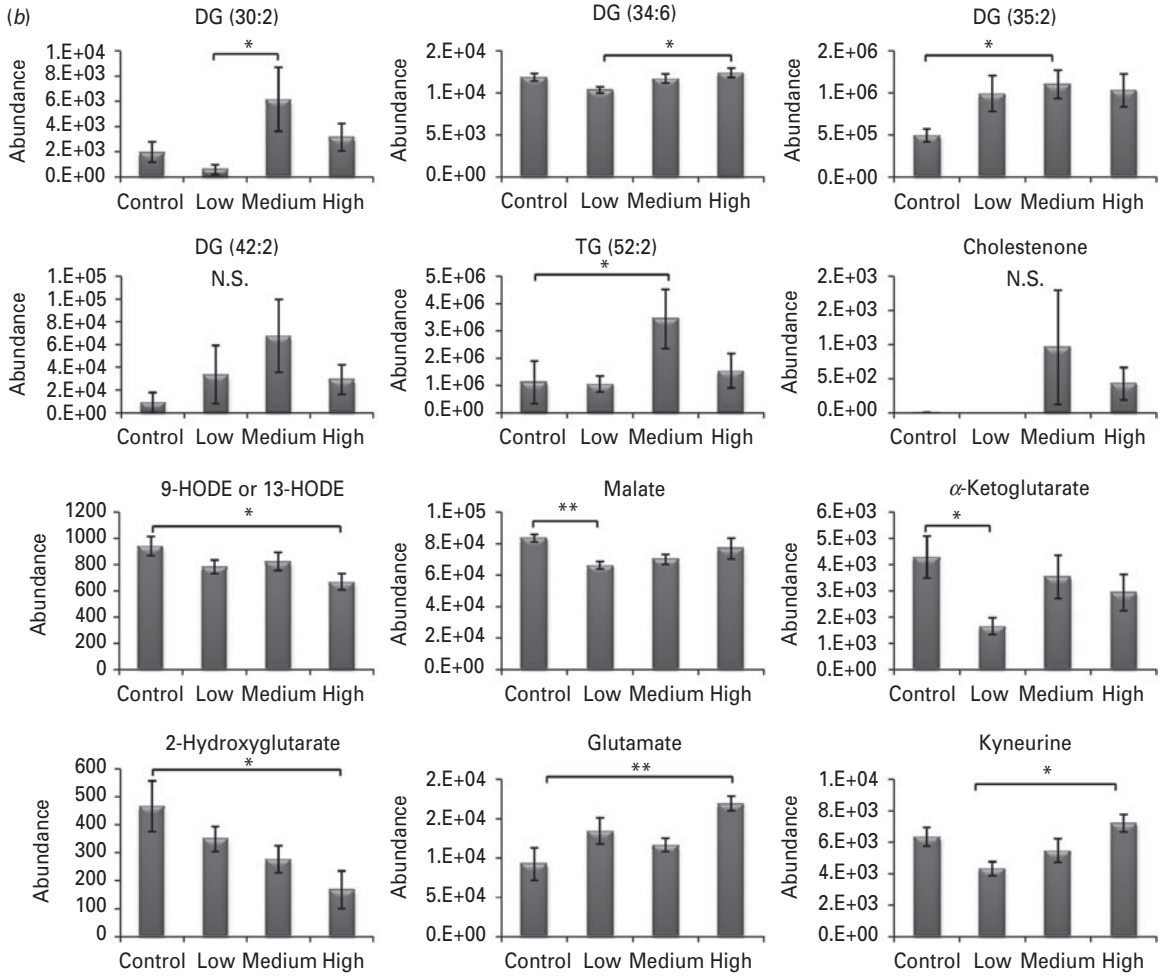

Fig. 1. Metabolic markers and metabolites differentially expressed across antipsychotic risk groups. (*a*) Levels of hormones were measured in plasma using Millipore bead arrays. Control (no risk, $N=20$), low (low-risk, $N=11$), medium (medium-risk, $N=27$), high (high-risk, $N=22$). Barr diagrams represent concentrations (pg/ml) as the mean±S.E.M. Analysis of variance (ANOVA) followed by Bonferroni's test (parametric) or Kruskal–Wallis followed by Dunn's test (non-parametric) performed between groups. Statistical significance at $^{*}p<0.05$, $^{**}p<0.01$ or $^{***}p<0.001$ between groups. (*b*) Lipids and polar metabolites were assessed in plasma by mass spectrometry. Control (no risk, $N=10$), low (low-risk, $N=10$), medium (medium-risk, $N=10$), high (high-risk, $N=9$). Barr diagrams represent the mean normalized abundance (sum of peak areas) of metabolites±S.E.M. For diacylglycerides (DG) and triacylglycerides (TG) numbers in parenthesis denote the chain length (total number of acyl carbons) followed by total double bonds. ANOVA followed by Bonferroni's test (parametric) or Kruskal–Wallis followed by Dunn's test (non-parametric) performed between groups. Statistical significance at $^{*}p<0.05$, $^{**}p<0.01$ or N.S. (not significant) between MetS risk groups.

in the medium- and high- risk groups (Table 2, Fig. 1*b*). In the polar metabolite panel, levels of important mediators of the mitochondrial tricarboxylic acid (TCA) cycle, malate and α-Ketoglutarate, were decreased (significantly for malate, $p<0.0070$) in patients using low-risk SGAs compared to controls (Fig. 1*b*). In contrast, a trend in the opposite direction was observed for levels of glutamate ($p<0.0085$) and kynurenine ($p<0.006$), where significantly increased levels were seen in the high-risk group compared to controls. Similarly, the co-eluted lipids 9-hydroxyoctadecadienoic acid (9-HODE) and 13-hydroxyoctadecadienoic acid (13-HODE) showed a trend that was inversely proportional to the risk level between high risk and controls.

Discussion

Our results show overall metabolic dysregulation in schizophrenia patients at risk for metabolic syndrome compared to controls. Levels of insulin, C-peptide (a precursor of insulin), and leptin, hormones known to be increased in individuals with high body fat content and obesity, were higher in the schizophrenia cohort. Levels of adiponectin, a key hormone that regulates glucose and fatty acid metabolism, and whose levels are known to be inversely correlated with body fat and BMI, were significantly lower in schizophrenia compared to controls (Fig. 1). These results are in agreement with previous studies suggesting that schizophrenia is associated with

metabolic disturbance, evidenced by elevated levels of insulin and leptin (Beumer et al., 2012; Panariello et al., 2012; Sugai et al., 2012). However, our study is unique in that, by grouping the schizophrenia patients based on antipsychotic-induced risk level for MetS, we found several important mediators of MetS, namely insulin, TNF-α, and adiponectin, to be significantly different between the SGA risk groups. Specifically, patients treated with high- and medium-risk SGAs had significantly higher levels of insulin and lower levels of adiponectin compared to controls, while patients treated with low-risk antipsychotics were not significantly different from controls (Fig. 1). In addition to metabolic hormones, patients treated with high-risk antipsychotics exhibited elevated levels of the pro-inflammatory cytokine TNF-α, compared to the other groups. Notably, the differences observed between risk-groups in the levels of TNF-α, insulin, and adiponectin were not dependent on the dose of the antipsychotics used or the total illness duration. Different lines of study have shown that TNF-α, which also functions as an adipokine, is highly expressed in obese humans as well as in animal models of obesity (Hotamisligil et al., 1995; Smith and Minson, 2012). This categorization of SGAs by level of risk for MetS gives us insight into the mechanisms behind SGA-induced MetS in schizophrenia.

Patients treated with SGAs also showed a trend for increased levels of diacylglycerides (DG) and triacylglycerides (TG), as well as significantly decreased levels of the TCA-cycle intermediates α-ketoglutarate and malate, compared to controls (Fig. 1). The opposite pattern found between these two types of metabolites (lipids and mitochondrial TCA intermediates) is important because of the intimate connection between lipid metabolism and mitochondrial energy metabolism, and suggests an overall impairment of energy homeostasis. During energy consumption, TGs are transformed into free fatty acids and under the process of lipolisis are further broken down to form Acetyl CoA, which fuels the mitochondrial TCA cycle. In a situation of mitochondria impairment, as may be case for SGA-treated patients, the TCA cycle is disrupted and lipids are shifted into TG synthesis (lipogenesis), which are then deposited in tissues, leading to increased body fat and weight gain. This scenario may explain the increase in DG and TG levels, and the decrease in TCA intermediates in patients compared to controls in our study, as well as the increased levels of cholestenone, an intermediate metabolite in the degradation of cholesterol, particularly in the medium-risk and high-risk groups compared to low-risk and controls. Overall, our findings suggest a shift in the balance between mitochondrial energy metabolism, fatty acid breakdown, and lipid synthesis in SGA-treated patients. This is in agreement with previous reports of dyslipidemia in patients taking antipsychotics (Watanabe et al., 2013) and evidence of increased lipids and tryacylglycerols in a metabolomic study of patients after treatment with olanzapine and risperidone, high- and medium-risk SGAs, but not aripiprazole, a low-risk SGA (Kaddurah-Daouk et al., 2007). Also in support of this hypothesis, we have found that treatment with clozapine, a high-risk antipsychotic, affects overall mitochondrial integrity and function in insulin-responsive cells (Contreras-Shannon et al., 2013). We have also shown that treatment with clozapine causes oxidation of mitochondrial proteins involved in energy metabolism, including malate dehydrogenase (Walss-Bass et al., 2008; Baig et al., 2010). Drug-induced oxidation of this enzyme may be the reason for our current findings of decreased levels of malate in patients undergoing treatment with SGAs. Other groups have also shown SGA-induced mitochondrial alterations. In human SHSY5Y neuroblastoma cells and in rat brain, treatment with antipsychotics reduces mitochondrial respiration by inhibiting complex I activity (Rosenfeld et al., 2011). This was also observed in human peripheral blood mononuclear cells (PBMCs), where both mitochondrial complex I activity and oxygen consumption were reduced in patients treated with antipsychotics (Streck et al., 2007). Levels of the mitochondrial enzyme succinate dehydrogenase were decreased in rat brain upon treatment with atypical antipsychotics (Streck et al., 2007).

The observed impairment of energy homeostasis may in part account for the metabolic imbalances observed in patients treated with SGAs. During cellular energy storage, several metabolic processes take place to maximize the storage and expenditure of available energy resources. Metabolic hormones such as insulin play a key role in energy homeostasis by metabolizing glucose and triglycerides from the bloodstream into cells. In cases of insulin resistance, diglycerides are converted into triglycerides which accumulate into adipose tissue and are deposited as fat (Meegalla et al., 2002; Zhao et al., 2008; Cao et al., 2011). These studies are in accordance with our current findings of increased insulin, and a trend for higher TGs and DGs in patients under treatment with medium- and high-risk SGAs compared to low-risk and controls. Additionally, it is interesting to note the relationship between levels of leptin and TCA cycle intermediates in the schizophrenia group. Whereas leptin levels are high in schizophrenia patients, malate and α-ketoglutarate are low in patients compared to controls. A possible explanation could be the role of leptin in regulation of gene transcription. Increased leptin levels in mice have been shown to reduce transcription of genes coding for enzymes of the TCA cycle (Soukas et al., 2000).

Another important finding from our study is that glutamate levels were elevated in plasma from SGA-treated patients compared to controls, particularly in the high-risk SGA group. This is of interest given the important function of glutamate as a neurotransmitter in the overall brain circuitry, and its role as a substrate for the *N*-methyl D-Aspartate (NMDA) receptor. Altered NMDA activity has been postulated as a causative factor in schizophrenia

(Field et al., 2011; Schwartz et al., 2012; Snyder and Gao, 2013). In mitochondria, the enzyme glutamate dehydrogenase (GDH) catalyzes the conversion of glutamate into α-ketoglutarate, an intermediate metabolite in the mitochondrial TCA cycle. In control subjects, we found low levels of glutamate and high levels of α-ketoglutarate, indicative of successful conversion by GDH. In contrast, higher levels of glutamate and lower levels of α-ketoglutarate were observed in SGA-treated patients, suggesting impairment in the GDH reaction, which could then affect feeding into the TCA cycle. This is in line with the decrease of another TCA intermediate, malate, in the patients in our study. However, we don't exclude the possibility that other mitochondrial non-TCA related pathway may also be affected owing to the fact that other metabolites not directly involved in the TCA cycle such as kynurenine were significantly different between controls and patients. Levels of kynurenine and kynurenic acid (products of tryptophan metabolism) have been found to be increased in postmortem brain samples (Schwarcz et al., 2001) and cerebrospinal fluid from patients with schizophrenia (Nilsson et al., 2005; Linderholm et al., 2012). It has been postulated that the increased levels of kynurenine observed in schizophrenia are independent of medication (Schwarcz et al., 2001; Sathyasaikumar et al., 2011). In this study we have classified the schizophrenia cohort by MetS risk groups based on medication and we show that plasma levels of kynurenine are significantly increased in patients treated with high risk SGAs compared to those treated with low risk SGAs. Both of these mechanisms, i.e. medication dependent or independent, could contribute to kynurenine levels.

There are several limitations to this study. First, there is no available information on smoking history, which may influence levels of metabolic hormones, or waist circumference measurements, which some may consider to be a better indicator of adiposity compared to BMI. However, most previous metabolomic studies have utilized BMI as an indicator of metabolic dysregulation (Cai *et al*, 2012). Second, some of the patients were taking other psychotropic medications, as is common in the treatment of psychiatry illnesses, and these other medications may also influence levels of metabolic mediators. However, these additional medications were of a large variety and not in common within subjects. Ideally, although it is difficult to recruit antipsychotic naïve patients, future studies using larger cohorts with patients before and after treatment with SGAs should be conducted to validate the current findings. A third limitation pertains to the cross-sectional data obtained which does not allow for conclusions regarding causal relations and calls for the need for further longitudinal studies. Finally, given that the mean duration of illness in this patient sample was 18±10 yr, prior medications taken by patients may confound the current measurements. Nevertheless, aiming to reduce the confounding effects of treatment history, in this study we only included patients that were in their current antipsychotic regimen for at least 3 months.

In conclusion, we have used a novel approach of grouping schizophrenia patients into three risk levels for MetS, based on the type of antipsychotic medication used. Our results suggest an overall metabolic dysregulation in schizophrenia patients, especially in those treated with SGAs that induce medium and high risk for MetS. These results underline the necessity to implement measures to reduce the risk for MetS in patients under SGA treatment. Understanding how antipsychotic treatment increases risk for metabolic syndrome may lead to development of novel therapeutic approaches to reduce these side effects, and may also provide insight into the molecular mechanisms of development of metabolic disease in the general population.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000157.

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Statement of Interest

Dr. Velligan conflicts of interest are: Ad Board and Honoraria: Genentech, Abbvie, Amgen, Otsuka Lundbeck, Research grants: Genentech, Amgen and Speakers Bureau: Otsuka Lundbeck. All other authors declare no conflict of interest.

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