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DNA methylation, insulin resistance and second-generation antipsychotics in bipolar disorder

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Abstract

Aims—This study aimed to assess the effect of second-generation antipsychotic (SGA) use and insulin resistance on a global measure of DNA methylation in patients diagnosed with bipolar disorder.

Materials & Methods—Subjects stable on medication (either mood stabilizer monotherapy or adjuvant SGAs) were assessed for insulin resistance. Global methylation levels were assessed in leukocyte DNA from whole blood using the Luminometric Methylation Assay. Multivariable linear regression was used to investigate the effect of insulin resistance and SGA use on DNA methylation.

Results—A total of 115 bipolar I subjects were included in this study. The average age was 43.1 ± 12.2 years and 73% were on SGAs. Average% global methylation was 77.0 ± 3.26 and was significantly influenced by insulin resistance, SGA use and smoking.

Conclusion—This is the first study to show a relationship between SGA use, insulin resistance and global DNA methylation. Further work will be needed to identify tissue- and gene-specific methylation changes.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Keywords

antipsychotic; bipolar; global DNA methylation; insulin resistance

Background

Co-morbid medical disorders within bipolar disorder are a risk factor for a more complicated course of psychiatric illness and higher rates of mortality [1–5]. In particular, the rates of insulin resistance, diabetes and cardiovascular disease are elevated in bipolar disorder and thought to contribute to shorter life expectancies in this group [5,6]. Second-generation antipsychotic (SGA) use doubles the risk of diabetes and metabolic syndrome in bipolar patients compared with SGA-naïve bipolar patients [7,8]. SGA use may further add to the baseline risk of comorbid disease burden in bipolar disorder due to their high propensity to cause metabolic side effects [9]. Although research has investigated the underlying mechanisms for SGA-induced insulin resistance in both bipolar and schizophrenia disorders [10–13] none have been able fully describe this link. SGA-induced insulin resistance is likely caused by several different factors and further mechanistic insight may be provided by looking at medication, environment and gene interactions with epigenetics.

Insulin resistance is considered the underlying pathophysiologic process that connects each of the metabolic syndrome components [14]. Identification of the underlying mechanism for SGA-induced insulin resistance is particularly important as it can be used for personalized medicine approaches aimed at preventing progression to diabetes, metabolic syndrome and cardiovascular disease. Furthermore, given the underlying cardiometabolic disease seen in bipolar disorder and its connection to psychiatric symptomatology, this may have important implications on the treatment approach to bipolar disorder. The direct molecular effects of SGA-induced metabolic side effects is complicated and has been associated with several changes in biochemical pathways in the body [10]. Genetic aberrations in the folate cycle have been linked to the risk of insulin resistance and metabolic syndrome in the schizophrenia and bipolar populations treated with SGAs [15–18]. Importantly, the production of methyl donors for DNA is one of the main endpoints of the folate cycle [19]. Thus, epigenetic changes through DNA methylation can be hypothesized to be linked to SGA-induced metabolic side effects, particularly insulin resistance. To date, global methylation of DNA in bipolar disorder has not been studied in this respect.

The chronic administration of SGAs may induce alterations in DNA methylation, profoundly influencing DNA regulation and expression. This change in genomic tone, or stable transcriptional repertoire of the cell [20], could be responsible for the subsequent progression to insulin resistance, a more severe course of psychiatric illness and higher mortality rates seen in bipolar disorder. Briefly, methylation of DNA occurs at CpG dinucleotides throughout the genome and it is most commonly associated with a repression in gene expression [21]. Epigenetic mechanisms and its role in the ‘metabolic memory’ hypothesis of obesity and diabetes pathophysiology is an active area of study [22] and so the investigation of epigenetics in SGA-induced metabolic side effects is a natural extension of current work. It is possible that environmental factors and disease processes (e.g., medications and their side effects) may influence DNA methylation and furthermore, certain

subsets of patients may have a higher propensity to have their epigenetic code altered by environmental influences. Thus, the combination of epigenetic, environmental and medication factors may serve to inform personalized medicine interventions aimed at minimizing the risk of SGA-induced insulin resistance and its long-term consequences in bipolar disorder.

Therefore, in this present study, we examine the relationship between SGAs, insulin resistance and degree of peripheral blood DNA methylation in a population of bipolar subjects on SGA therapy compared with bipolar subjects on mood stabilizer monotherapy. This work will be used to inform future investigations into the mechanisms behind SGA-induced insulin resistance in bipolar disorder.

Methods

Subjects

Eligible participants on antipsychotic therapy were screened from community health centers and in participation with the Prechter Longitudinal Study of Bipolar Disorder at the University of Michigan. Subjects were considered eligible if they met the following inclusion criteria:

- Aged 18–90 years old;
- Prescribed an antipsychotic or mood stabilizer as determined by their primary physician;
- No medication changes for the previous 3 months;
- Currently diagnosed with Bipolar I disorder.

Subjects were excluded from study participation if they:

- Were unwilling or unable to participate;
- Were currently diagnosed or receiving treatment for diabetes;
- Had an active substance abuse or dependence diagnosis (currently smoking was allowed).

Healthy controls were not collected for this study. The study was approved by the Institutional Review Board of the University of Michigan. Subjects were consented and then seen in the Michigan Clinical Research Unit for a single visit. Diagnoses were verified separately by chart records and a diagnostic interview at the time of study visit using the structured clinical interview for DSM-IV-TR diagnoses (SCID-IV) by a trained research assistant [23].

Demographic & metabolic measurements

At the clinic visit subjects were asked about current medication, alcohol and tobacco use. Smoking was assessed by use the Centers for Disease Control's 'Smoking Status' definition where subjects are asked two questions: "Have you smoked at least 100 cigarettes in your entire life?" and "Do you now smoke cigarettes every day, some days, or not at all?" After

collecting responses, subjects were categorized into two groups for our study: (1) current smoker and (2) former smoker or never smoker. Gender, race and ethnicity were self-reported and a detailed medical history was also collected. Medication profiles were verified via pharmacy records when possible. Subjects fasted for at least 8 hours prior to the morning clinic visit. A nurse measured height, weight and waist and hip circumference. Body mass index (BMI, kg/m²) was calculated and a blood draw was taken for epigenetic and insulin resistance assessment. Insulin resistance was calculated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) based on the following formula: (insulin [μIU/ml] × glucose [mmol/l] / 22.5) [24]. Although a single, cross-sectional measure of insulin resistance, HOMA-IR provides valuable information regarding glucose homeostasis and has been correlated to more invasive techniques [25,26].

DNA isolation & methylation measurement

Leukocyte DNA was isolated from whole blood using the salting out method. DNA was then cleaned using the Zymo Genomic DNA Clean and Concentrator kit (Zymo Research, Irvine, CA, USA) and quantified using a Qubit 2.0 Fluorometer (Thermo Scientific Waltham, MA, USA). Methylation of genomic DNA was assessed using a global method. Although this type of method does not give gene specific methylation information it provides a snapshot of the entire genome's methylation status and gives evidence for further, in-depth exploration of DNA methylation. The LUMInometric Methylation Assay (LUMA) is a restriction enzymebased method that detects methylation at 5'-CCGG-3' sequences throughout the entire genome and has been described previously [27–29]. In brief, two separate reactions are carried out, one containing approximately five units of methylation-insensitive *MspI* (New England Biolabs, Ipswich, MA, USA) and the other containing approximately five units of methylation-sensitive *HpaII* (New England Biolabs). Both reactions contained *EcoRI* (New England Biolabs) as an internal standard, 300 ng of genomic DNA and 10 times Tango with BSA Buffer (Thermo Scientific, Waltham, MA, USA) for differential digestion. Reactions were incubated at 37°C for 4 h after which 20 μl of annealing buffer was added and samples were pyrosequenced in AQ mode on a PyroMark MD 96 machine using the following dispensation order: GTG TCA CAT GTG TG. Upon measurement of normalized signal intensity, a ratio was set up between the methylation sensitive and insensitive individual reactions to calculate global DNA methylation based on the following equation: $1 - [(HpaII/EcoRI)/(MspI/EcoRI)] \times 100$. All samples were run in duplicate with negative DNA, low methylated and high methylated controls (Zymo Research).

Statistical analysis

The duplicate LUMA reactions were averaged for all analyses. Duplicates resulting in greater than 15% were deleted before statistical analysis leading to a loss of five subjects. After exclusion of these five subjects, the average percentage coefficient of variation (CV%) for the entire population as well as each group was <4% which resembles that of others [29–31]. Demographic data was compared using student t-tests for continuous variables and chi-square tests for dichotomous variables. The variable of race was compared using the Fisher's Exact Test. LUMA was initially investigated using ANOVA based on clinical variables. A multivariable linear regression was conducted to test the association between

SGA use, insulin resistance and global methylation while adjusting for smoking status, age, gender, race, folate level and waist-to-hip ratio (WHR). Smoking status was included as a covariate because of its identified influence on global methylation in our population and others [32], and race was included because of its identified differences between the SGA and mood stabilizer groups of this cohort (both detailed in results). Age and sex were included since they are commonly known to influence gene methylation. Folate level was included as different levels of folate status could confound the ability to produce methyl donors [33] and WHR was included as this has been identified as a strong factor that influences insulin resistance in SGA users [17]. A statistically significant result was set at $p < 0.05$ and analyses were ran using R version 3.1.0.

Results

Demographics & clinical statistics

A total of 115 bipolar I subjects stable on either adjuvant SGA therapy (73%) or mood stabilizer monotherapy (27%) were included in this study. The average age was 43.1 ± 12.2 years and 67% were female. The majority were Caucasian (81%) followed by African American (11%). The rest self-categorized as Asian, Hispanic or Latino or Other. Approximately 38% of subjects were taking quetiapine, 21% were taking aripiprazole and 12% were on risperidone. The remaining SGA treated subjects (29%) were taking olanzapine, clozapine, paliperidone or ziprasidone. In the mood stabilizer monotherapy group, 39% were on lamotrigine, 35% were on lithium and 26% were on valproic acid. A low percentage of both the mood stabilizer and SGA groups were also treated with non-psychiatric medications (e.g., statins, antihypertensives, blood thinners, etc.) which may be a reflection of excluding diabetic subjects from our study. The use of these nonpsychiatric medications did not differ between the mood stabilizer and SGA groups (all $p > 0.3$). None of our subjects had co-morbid infectious diseases. Table 1 shows the demographics and clinical characteristics of the entire study population and by treatment status (SGA or mood stabilizer). The only significant difference identified between the treatment groups was for global methylation (detailed below). The variables of race and folate status trended towards, but did not reach, statistical significance ($p = 0.05$ and $p = 0.08$, respectively). Race approached statistical significance because the mood stabilizer group had no subjects categorized as Asian, Hispanic or Latino or Other. To correct for confounding we included race and folate status in our primary outcome regression below. Finally, of note, the SGA group also used various mood stabilizers, however their distribution of mood stabilizer type did not significantly differ from the mood stabilizer monotherapy group ($p = 0.2$).

Global methylation (LUMA)

For the entire sample population the mean LUMA (% global methylation) was 77.0 ± 3.26 . Overall, HOMA-IR was negatively correlated to global methylation ($p = 0.0114$). In comparing bipolar subjects taking SGAs versus mood stabilizers, SGA users had lower global methylation values (76.2% vs 78.2%; $p = 0.04$). In looking at individual antipsychotic effects of global methylation, no individual antipsychotic reach statistical significance ($p = 0.33$). Of the other baseline factors in our population, only the variable of smoking status trended toward significance ($p = 0.06$) where subjects categorized as currently smoking had

lower global methylation (76.2%) compared with nonsmokers (77.4%). Of note, valproic acid, a known histone deacetylase inhibitor, did not significantly influence global methylation in the SGA or mood stabilizer groups, nor did its use significantly differ between each group (all $p > 0.1$). However, a lack of significant effect by valproic acid could be due to a small sample size. Finally, nonpsychiatric medication use did not have significant effects on global methylation (all $p > 0.45$).

Multivariable linear regression of global methylation & insulin resistance

Our multivariable linear regression used global methylation as the dependent variable and SGA use, HOMA-IR, smoking status, age, gender, race, folate level and WHR as the independent variables. This model was significant due to the effect of HOMA-IR ($p = 0.0077$), smoking ($p = 0.0368$) and an interaction between WHR and SGA use ($p = 0.0204$). Increased HOMA-IR, smoking and SGA use (at a given WHR) was associated with lower global methylation, or hypomethylation. Table 2 details the model's parameters.

A secondary analysis was conducted only in SGA treated subjects in an effort to assess the effect of SGA-induced insulin resistance on global methylation. This analysis again used global methylation as the response variable and race, smoking status, WHR, HOMA-IR and folate level as predictors. This secondary analysis (Table 3) revealed that global methylation in subjects treated with SGAs was significantly predicted by HOMA-IR alone ($\beta(95\%CI)$: $-0.13 (-0.24, -0.02)$, $p = 0.020$). Of note, smoking status and WHR did not significantly influence global methylation in SGA-treated bipolar patients ($p = 0.1$ and $p = 0.08$, respectively). Finally, when adding antipsychotic exposure defined by chlorpromazine equivalents to the model, the model remained significant due to HOMA-IR (data not shown).

Discussion

This study is the first to identify associations between global DNA methylation, smoking, insulin resistance and SGA use within bipolar disorder. We have identified that the addition of a SGA to a mood stabilizer is associated with a decrease in global methylation in bipolar disorder subjects. Such a finding adds further support to environmental factors influencing global methylation and possibly genomic tone in bipolar disorder. It also begins to help understand how folate cycle abnormalities have been linked to SGA-induced insulin resistance in bipolar disorder. Future work will help identify the downstream effects of these methylation changes and how they may possibly lead to insulin resistance. We also identified associations between SGA-induced insulin resistance and global hypomethylation in our secondary analysis which may lend support for an epigenetic mechanism in this negative iatrogenic outcome. The difference in DNA methylation identified in this study may be one possible mechanism for increased risk for insulin resistance in bipolar patients exposed to SGAs, however, the total risk is likely due to a combination of factors that includes epigenetics, genetics, lifestyle and diet. Going forward, epigenetic studies in bipolar disorder must carefully assess the myriad of factors that play a role in this severe mental illness.

Although the pharmacoepigenetic association identified in this study is novel in bipolar subjects, the association between various measures of insulin resistance and global DNA methylation has been identified in other populations including animal models, monozygotic twins and obese population [34–37]. The current finding along with similar findings in various populations suggests that the interaction of genetics and the environment may play an important role in determining the risk of developing insulin resistance in bipolar disorder. To date, the study of SGA-induced side effects using epigenetics is still at its infancy in bipolar disorder and so there are, to our knowledge, no candidate epigene analyses that have looked at SGA treatment outcomes in the bipolar population. Further work is needed in this area to begin to truly analyze pharmacoepigenetic-directed DNA methylation changes in bipolar disorder. Within schizophrenia, the main utilizers of SGAs, candidate epigene analyses have yielded mixed results when correlating methylation differences to metabolic outcomes [19,38–40]. We previously identified global hypomethylation in female schizophrenia subjects using the linear interspersed nuclear elements 1 (LINE1) assay [39]. This population is not entirely comparable to that of this study since all of the schizophrenia subjects were on antipsychotics, however, the finding of a decrease in methylation with a cardiometabolic side effect does agree with this study's findings. Additionally, Melas and colleagues showed that risperidone and olanzapine-treated schizophrenia subjects had significantly lower global methylation (measured by LUMA) compared with haloperidol [41]. Subjects on haloperidol had global methylation values similar to control subjects. Finally, Melka and colleagues have added preclinical support to the hypothesis that antipsychotics influence methylation by showing that gene promoter regions of rat brains and liver have altered methylation after the administration of olanzapine [42].

In the only other study to investigating gene-specific methylation in SGA-induced metabolic side effects in severe mental illness [40], Moons and colleagues, found no associations between DNA methylation of *IGF* genes 1 or 2 and metabolic side effects. However, they did identify associations between variants within the *IGF* genes and *IGF* methylation which may suggest a link between DNA variation and DNA methylation. Their findings point to the many various factors, including genetic variation, that may be associated with gene methylation differences in human subjects. Further work using epigenome-wide association (EWAS) strategies will aid in the understanding of how epigenetics may modulate SGA-associated insulin resistance. Identifying differentially methylated regions in bipolar patients with insulin resistance and treated with SGAs will be an important strategy to developing personalized medicine approaches.

The average HOMA-IR values for the collective cohort (7.05) was considerably elevated when compared with other studies in the general population where HOMA-IR can range from 1–2 for healthy, nondiabetic populations, and to 5–6 for the diabetic population [43,44]. Such an elevated HOMA-IR could be the result of a lack of treatment of glucose abnormalities in patients on SGAs. Our study excluded subjects currently diagnosed with diabetes or treated with a diabetes medication and so such a heightened value may indicate a lack of awareness or focus on glucose homeostasis in bipolar disorder. Indeed, within the mentally ill population there is a documented lack of glucose testing and subsequent treatment after identification of glucose abnormalities [45]. However, attention to

comorbidities in mental illness is becoming a part of the holistic approach to treating psychiatric illness with findings linking psychiatric outcomes to metabolic comorbidities and the increasingly common use of interdisciplinary care teams. A further explanation for the highly elevated HOMA-IR values seen in this study could be the already elevated insulin resistance seen in medication-naïve bipolar disorder. Guha and colleagues compared insulin resistance and metabolic syndrome components in a cohort of newly diagnosed, medication-naïve bipolar I subjects to healthy controls [46]. The authors showed that insulin resistance was significantly higher in the bipolar disorder subjects compared with controls (HOMA-IR 3.16 vs 1.19, respectively) and that HOMA-IR was not predicted by age, sex, waist circumference or mood status. Their findings suggest an underlying insulin resistance pathology in bipolar disorder. Drug treatment may then elevate the risk of insulin resistance further, possibly through epigenetic mechanisms, as is seen for our cohort on mood stabilizer monotherapy (average HOMA-IR of 5.69) and SGAs (average HOMA-IR of 7.55). Taken together, this demonstrates the need to find the mechanism(s) behind SGA-induced insulin resistance in bipolar disorder as this group is already at an elevated risk for insulin resistance before beginning necessary psychopharmacotherapy. Adequate control or prevention of progression to insulin resistance may also lead to better psychiatric treatment outcomes and reduced mortality, however further work is needed in this area.

Limitations

Several limitations of our study require discussion. The natural course of bipolar disorder often requires several pharmacotherapeutic treatment strategies. Indeed, in our cohort, psychiatric polypharmacy was commonplace with 43% on an antidepressant and 72% using benzodiazepines (data not shown). Briefly, antidepressant use was no different between the mood stabilizer and SGA groups. Benzodiazepine use was as needed for all subjects included so we do not believe that sporadic use would have significant effects on methylation. Indeed, both antidepressants and benzodiazepines did not significantly influence methylation in this population. This cohort also included several SGAs and not a single agent. Furthermore, mood stabilizer use was common in the SGA group and it is not known which medication was the original base of therapy (e.g., started with SGA or SGA was added on). Nevertheless, our study demonstrates that having an SGA on a therapeutic regimen likely has significant effects on global methylation. Ideally, future work should endeavor to assess DNA methylation changes before and after the addition of an SGA in order to draw causal conclusions, however, this may be very difficult to accomplish in the severely mentally ill. Additionally, using a more 'naturalistic' cohort increases the translatability and applicability to bipolar disorder patient populations seen in the clinic. The measure of insulin resistance used in this study was a single time point clinical measure. In order to assess the true effect of DNA methylation changes on whole body glucose homeostasis in bipolar subjects on SGAs, a more invasive measure, such as the hyperinsulinemic euglycemic clamp, are needed to draw mechanistic conclusions. We also used peripheral blood to assess DNA methylation differences and did not estimate cellular composition. The use of peripheral blood mononuclear cells (PBMC) is controversial in epigenetic assessments as it is a heterogeneous cell type and not of the organ of interest, which in this study would be the brain and pancreas. Using peripheral blood retains clinical

translatability and comparative studies of blood and brain showed both methylation patterns that are tissue-specific and conserved across tissues [47–49]. Although still debated, one study suggests epigenetic loci are generally conserved across different cell types [50]. Finally, as SGAs act not only on the brain but on the whole body, it could be hypothesized that there might be a significant overlap between methylation changes in the brain and blood due to medication use, despite the cell-type specific nature of DNA methylation patterns. Regardless, it is possible that cell composition from the blood, which was altered by SGA use, could have a significant influence on DNA methylation and be an alternative explanation to the differences observed in this study. The lack of cell counts in this study is a limitation and so cell composition measurements are needed in future studies to investigate tissue-specific DNA methylation and expression changes in SGA-induced insulin resistance in order to draw mechanistic conclusions.

Conclusion

Our study is the first to find an association between SGA use, insulin resistance, smoking and global DNA methylation in a cohort of stable bipolar subjects. Insulin resistance has been implicated in bipolar disorder pathophysiology and the addition of an SGA may be further causing glucose dysregulation through epigenetic mechanisms. As this is the first study to identify this relationship using a cross-sectional design causal inferences cannot yet be drawn. This finding should be replicated in a larger, well-defined cohort and future studies need to identify tissue and gene specific methylation for further investigation in SGA-induced insulin resistance using gold standard methods to analyze glucose homeostasis. The use of global methylation as a biomarker is unlikely to be of benefit due to its many potential confounding factors, however, this work begins to lay the foundation for identifying gene-specific methylation differences that could be associated with SGA-induced insulin resistance. We believe that studying a single antipsychotic in future studies will yield more powerful and causal results. Quetiapine is an ideal clinical choice given its high use in bipolar disorder and we are currently running a study to collect these particular patients. Future work would also be needed to see if particular SGAs influence the epigenome more or if this is indeed a class effect. Such data will be pivotal for advancing the mission of personalized medicine in bipolar disorder treatment.

Future perspective

This study has identified relationships between global DNA methylation, SGA use, insulin resistance and smoking in bipolar disorder. Going forward, the true implications of these findings must be replicated and further assessed by finding gene regions that are differentially methylated with SGA-induced insulin resistance in a tissue relevant to insulin resistance pathophysiology. Future studies must also be designed to draw causal conclusions. It could be possible that over time this type of work could offer insight into the factors and mechanisms, beyond just weight-gain, that are causing SGA-induced insulin resistance in bipolar disorder. With such knowledge, the effective use of precision medicine with epigenetics may become possible. Bipolar disorder patients have an increased risk of diabetes due to their diagnosis and so it is pivotal that we develop tools that will allow

clinicians to continue to use effective medications like antipsychotics, without further increasing a patient's risk of diabetes.

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Executive summary

- Bipolar disorder patients have an elevated risk for insulin resistance and diabetes due to their diagnosis alone. Antipsychotics increase this risk further.
- The interaction of lifestyle, medications and genetics may provide a better understanding of the mechanisms behind antipsychotic-associated insulin resistance in bipolar disorder.
- Subjects in this study had substantially elevated insulin resistance values compared with studies in the general population, Type II diabetes and medication naive bipolar patients.
- Regression analysis found that global methylation was influenced by smoking status, insulin resistance and an interaction of antipsychotic use and waist hip ratio.
- An increase in each of the influencing variables of the regression led to lower global methylation.
- A secondary analysis to assess antipsychotic-induced insulin resistance found that global methylation values were lower in antipsychotic-treated subjects with higher insulin resistance.
- The findings of this study are from a cross-sectional study and so causal conclusions cannot be drawn. Future prospective study designs must be made in order to draw causal and mechanistic conclusions.
- Future gene-specific work must be done in well-characterized, nonobese bipolar subjects using gold-standard insulin sensitivity assessments in tissues known to influence insulin sensitivity (e.g., skeletal muscle, adipose tissue).
- This work is the first to identify possible epigenetic changes in antipsychotic-induced insulin resistance.

Table 1

Demographics and clinical characteristics of study population – entire sample broken down by medication use.

Variable	Complete cohort	Cohort based on maintenance pharmacologic treatment	
	Bipolar I subjects (n = 115)	Subjects on SGAs (n = 84)	Subjects on mood stabilizer monotherapy (n = 31)
Age (years)	43.1 ±12.2	42.8 ±12.0	43.7 ±12.7
% female	67	67	67
% Caucasian/% African-American/% other [†]	81/11/8	81/10/9	84/16/0 [‡]
% Currently smoking	32	33	26
BMI (kg/m ²)	32.2 ±8.76	32.4 ±9.06	31.8 ±8.00
WHR	0.91 ±0.09	0.90 ±0.09	
HbA1C (%)	5.69 ±0.704	5.62 ±0.655	5.82 ±0.782
Glucose (mg/dl)	99.9 ±23.1	100 ±20.0	99.4 ±30.4
Insulin (μU/ml)	26.7 ±22.4	28.9 ±24.7	20.9 ±12.6
HOMA-IR	7.05 ±7.34	7.56 ±7.62	5.69 ±6.44
Folate (ng/ml)	18.7 ±5.76	18.2 ±5.90	20.3 ±5.12 [‡]
Global methylation (%)	77.0 ±3.26	76.2 ±3.51	78.2 ±2.02 [§]

[†] Other includes self-defined race/ethnic categories of Asian, Hispanic or Latino or Other.

[‡] Variable used to compare SGA and mood stabilizer monotherapy approached statistical significance (race: p = 0.05 and folate: p = 0.08).

[§] Significant difference between subjects on SGAs and subjects on mood stabilizer therapy based on t-test (p = 0.05 for race/ethnicity and p = 0.0451 for global methylation).

Values expressed as mean ± s.d. or %. Global methylation measured by the Luminometric methylation assay.

HbA1C: Glycated hemoglobin; HOMA-IR: Homeostatic model assessment of insulin resistance; s.d: Standard deviation; SGA: Second generation antipsychotic; WHR: Waist to hip ratio.

Table 2

Predictors for bipolar global DNA methylation based on medication and environmental factors.

Independent variables	β (95% CI)	p-value
Age	0.003 (−0.06, 0.06)	0.9
Gender [†]	−0.17(−0.91, 0.57)	0.7
HOMA-IR	−0.12 (−0.21, −0.03)	0.0077 [‡]
SGA use [§]	−0.68 (−1.41, 0.06)	0.06
WHR	−1.8(−9.98, 6.12)	0.6
SGA use WHR [‡]	−7.94 (−14.6, −1.27)	0.0204 [‡]
Smoking status [#]	−0.75 (−1.45, −0.05)	0.0368 [‡]
Race [¶]	−1.22 (−2.84, 0.40)	0.3
Folate level	0.005 (−0.11, 0.11)	0.9
r ²	0.24	

[†] Females compared with males.

[‡] p-value < 0.05.

[§] Mood stabilizer monotherapy compared with SGA use.

[#] Nonsmokers compared with subjects currently smoking.

[¶] African–Americans and all others compared with caucasians.

HOMA-IR: Homeostatic model assessment of insulin resistance; SGA: Second-generation antipsychotic; WHR: Waist to hip ratio.

Table 3

Predictors for secondary analysis of second-generation antipsychotic-induced insulin resistance on global methylation in bipolar disorder.

Independent variables	β (95% CI)	p-value
Age	0.02(−0.06, 0.10)	0.7
Gender [†]	−0.14(−1.09, 0.82)	0.8
HOMA-IR	−0.13 (−0.24, −0.02)	0.02 [‡]
WHR	−9.21 (−19.64, 1.22)	0.08
Smoking status [§]	−0.62 (−1.58, 0.34)	0.1
Race [¶]	−1.05 (−3.20, 1.11)	0.4
Folate level	−0.02 (−0.16, 0.13)	0.80
r ²	0.16	

[†] Females compared with males.

[‡] p-value < 0.05.

[§] Nonsmokers compared with subjects currently smoking.

[¶] African-Americans and all others compared with caucasians.

WHR: Waist-to-hip ratio.