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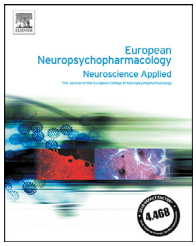

Skeletal muscle DNA methylation modifications and psychopharmacologic treatment in bipolar disorder

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Abstract
Both severe mental illness and atypical antipsychotics have been independently associated with insulin resistance and weight gain. Altered regulation of skeletal muscle DNA methylation may play a role. We aimed to evaluate DNA methylation modifications in human skeletal muscle samples to further understand its potential role in the metabolic burden observed in psychiatric patients and psychopharmacologic treatment. Subjects were included in our study if they had a bipolar diagnosis and were currently treated with a mood stabilizer or atypical antipsychotic. A healthy control group free of psychiatric or physical disease was also included for comparisons. Anthropometric, BMI and hemoglobin A1C (HbA1C%) were measured. Fasting skeletal muscle biopsies were obtained and methylation levels of 5-methycytosine (5-mC), 5-hydroxymethylcytosine (5-hmC) and 5-formylcytosine (5-fC) were measured. Skeletal muscle global methylation of 5-mC and 5-fC were significantly higher in bipolar subjects compared to healthy controls. 5-mC was significantly higher in the AAP group compared to the mood stabilizer group. Significant correlations were observed between 5-fC methylation and HbA1C%. Our findings suggest that psychiatric disease and treatment may influence some methylation

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measures in the skeletal muscle of patients with bipolar disorder, which may be further influenced by medication treatment.

1. Introduction

Bipolar disorder is a severe mental illness characterized by episodes of elevated and depressed mood that interfere with a patient's ability to function and thrive (Grande et al., 2016). The treatment of bipolar disorder includes both non-pharmacologic and pharmacologic modalities (Yatham et al., 2018). Pharmacologic strategies consist of acute treatment to resolve symptoms associated with a manic or depressive episode followed by maintenance treatment to prevent recurrence of future episodes. Maintenance treatment is generally achieved with a mood stabilizer such as lithium, valproic acid or lamotrigine or with an atypical antipsychotic (AAP) such as olanzapine, quetiapine or risperidone (Yatham et al., 2018).

Bipolar disorder and its treatment carries many risks that increase morbidity and mortality including suicide and drug side effects. Unique to bipolar disorder, and severe mental illness that includes schizophrenia, is an increased rate of obesity, glucose dysregulation and insulin resistance prior to drug treatment compared to healthy controls (Charles et al., 2016; Greenhalgh et al., 2017; Pillinger et al., 2017, 2018; Vancampfort et al., 2016). This is in addition to the well-documented disturbances in glucose metabolism and weight gain caused by drug treatment (primarily AAP) for bipolar disorder (Aly et al., 2015; Correll et al., 2015; Zhang et al., 2017). These drug effects have been observed in psychiatric disorders and independent of psychiatric disorders suggesting a direct effect of the drug that is not dependent on pathophysiological or environmental (e.g., diet, lifestyle, etc.) factors (Burghardt et al., 2018b). The metabolic burden observed with psychiatric diseases and caused by treatment is important as patients with severe mental illness have 10–20 year lower life expectancy compared to the general population, which is mainly attributable to cardiovascular disease (Laursen et al., 2019, 2013; Osby et al., 2016; Weiner et al., 2011; Westman et al., 2013). Dietary and lifestyle differences may account for some of this risk however; the molecular mechanisms remain to be elucidated. Some possible pathways that have been suggested to be involved in the glucose and metabolic dysregulation observed in psychiatric disease and treatment include the leptin pathway, insulin signaling pathways, obesogenic pathways and drug receptor-related pathways (e.g., histamine and serotonin receptor antagonism) which has been reviewed previously (Ballon et al., 2014; Boyda et al., 2010; Chen et al., 2017). A unified theory has not emerged which could be due to a lack of work in metabolic tissues involved in insulin resistance and metabolic disease.

The skeletal muscle is one of the major tissues responsible for insulin-stimulated glucose uptake and storage (DeFronzo et al., 1979; Thiebaud et al., 1982). Dysfunction in the skeletal muscle's ability to complete insulin-stimulated glucose uptake is thought to be the primary defect in the development of insulin resistance which is a major component in the development of diabetes (DeFronzo and Tripathy, 2009). The skeletal muscle molecular mechanisms underlying insulin resistance have been extensively studied in general, obese and diabetic populations. Such dysregulations include aberrant protein abundance and/or signaling as well as changes in DNA methylation modifications (Abdul-Ghani and DeFronzo, 2010; Caruso et al., 2014, 2015; Castellano-Castillo et al., 2019; Yi et al., 2008). Despite the appreciated role of skeletal muscle in insulin resistance, this tissue has not been studied for its role in the elevated metabolic co-morbidity (i.e., obesity and glucose dysregulation) observed in severe mental illness and with AAP treatment.

Our work has previously suggested that changes in peripheral blood DNA methylation may play a role in AAP-induced insulin resistance and that other molecular changes may be specific to diagnosis (Burghardt et al., 2015a, 2015b, 2016). DNA methylation modifications may arise in several forms. The most commonly studied form is 5-methylcytosine (5-mC) due to its established stability and role in gene regulation (Kumar et al., 2018). Although knowledge of other modifications has long been known, recent investigations into the "oxidized derivative" methylation modifications including 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) has increased due to their possible regulatory and signaling functions beyond simply an intermediate in the de-methylation pathway (Shi et al., 2017; Song et al., 2013; Spruijt et al., 2013; Wang et al., 2017). Previous work into the epigenetic mechanisms in psychiatric disease and treatment, including our own, has been limited by its measurement of gene methylation in the blood, the deficiency of assessing DNA methylation beyond 5-mC and the lack of comparisons to a healthy control group. We aimed to address some of these previous limitations by evaluating an *a priori* hypothesis that, global levels of DNA methylation modifications in a tissue with a known role in insulin resistance (i.e., the skeletal muscle) are associated with psychiatric disease and psychiatric treatment in a group of patients with elevated metabolic burden. Thus, we wanted to understand if psychiatric disease and/or treatment is associated with the overall levels of various methylation marks in DNA in a critical metabolic tissue, the skeletal muscle. We chose to analyze global levels of these methylation modifications since no previous work has been performed in this patient population within the skeletal muscle and this will allow for an unbiased assessment of each methylation modification versus concentrating a candidate gene or panel of genes. The identification of tissue-specific changes in DNA methylation may provide evidence regarding the molecular changes associated with metabolic burden in psychiatric disease and treatment. This work at a global methylation level may provide direction for future experiments aimed at understanding the gene level changes of the methylation modifications by concentrating efforts on particular methylation modifications that are

altered globally (e.g., 5-mC and 5-fC) with a genome-wide or candidate pathway approach. Therefore, although our hypothesis was established *a priori*, the information here can still be used to generate further hypotheses on methylation modification in psychiatric disease and treatment.

2. Experimental procedures

2.1. Inclusion/Exclusion criteria for patient and healthy subject selection

For the bipolar group, patients were recruited from the Metropolitan Detroit area with postings and flyers based on the following inclusion criteria: (1) 21–60 years old, (2) diagnosis of bipolar I, II or Not Otherwise Specified (NOS), (3) currently stable on an AAP or mood stabilizer therapy (defined as no dose changes >50% for at least 3 months) and (4) able to give full, informed consent. Patients were excluded if they had or were: (1) a diagnosis of diabetes, (2) a primary relative with diabetes, (3) a documented metabolic disease (e.g., obesity, dyslipidemia, etc.) prior to starting psychiatric drug therapy, (4) an allergy to lidocaine, (5) a bleeding disorder or inability to refrain from anti-coagulants for biopsy, (6) an active or past (1 year) diagnosis of substance abuse or dependence and (7) taking any medication (including herbal or over-the-counter) that may affect insulin sensitivity. Psychiatric diagnoses were initially asked during pre-screening and then confirmed by the Mini International Neuropsychiatric Interview (M.I.N.I) by a trained research assistant (Sheehan et al., 1998). A group of healthy control subjects were recruited from the Wayne State University campus and surrounding area via a separate protocol through web postings and flyers. Healthy control subjects were included if they were: (1) age 21–60 years, (2) had no current psychiatric or physical disease and (3) were able to give informed consent. Healthy control subjects were excluded if they: (1) had a primary relative with diabetes, (2) had an allergy to lidocaine, (3) were unable to refrain from anti-coagulants during study or (4) were taking any medication (including herbal or over-the-counter) that may affect insulin sensitivity. We purposely recruited healthy controls without physical disease as our interest was the metabolic burden that differentiates psychiatric patients from the general population. All protocols and procedures for the study were approved by the Wayne State University Institutional Review Board and carried out in accordance with the Declaration of Helsinki. All subjects gave full informed consent prior to undergoing any assessments or questionnaires in the study which included an explanation of the physical and all other risks associated with the study (including biopsy risks such as cramping or bruising at the site). All subjects were informed that their participation was voluntary and that they would expect no direct benefit from this study other than the knowledge obtained from the collection of their samples. Subjects were compensated for the time in the study.

2.2. Clinical assessments and skeletal muscle biopsy

After undergoing a phone pre-screening, eligible subjects were invited to the Wayne State University Clinical Research Services Center (CRSC) to obtain informed consent and undergo: (1) completion of a questionnaire that include demographic and medical/medication history, (2) anthropometric measurements and vital signs, (3) hemoglobin A1C% using the A1C Now+ system (PTS diagnostics) and (4) evaluation of fasting glucose by a bedside YSI 2300 Glucose Analyzer (Xylem, Yellow Springs, OH) (Shanely et al., 2014). Chlorpromazine equivalents were calculated for patients on an AAP (Leucht et al., 2014). Biopsied muscle was immediately cleaned of blood, fat and connective tissue and flash frozen until further processing.

2.3. Extraction of DNA and methylation assessment

Approximately 30 mg of biopsied muscle was homogenized with a Minilys (Precellys, Atkinson, NH) homogenizer in RLS buffer. DNA was extracted from the homogenate using the AllPrep DNA/RNA/miRNA kit (Qiagen, Hilden, Germany) on an automated Qiacube system (Qiagen). Extracted DNA was measured on a Qubit Fluorometer (Thermo Fisher, Waltham, MA) with the applicable kit. Methylation of 5-mC, 5-hmC and 5-fC was measured via two assay types. Global levels of 5-mC was measured using a previously validated protocol of Linear Interspersed Nuclear Element 1 (LINE-1) that utilizes methylation sensitive high-resolution melting (MS-HRM) on bisulfite-converted DNA along with a set of methylation standards (mixed ratios of methylated and unmethylated DNA) on a Roche LightCycler 480ii (Tse et al., 2011). We used the same primers as reported by Tse et al. along with Roche Lightcycler High Resolution Master Mix. Samples were ran in duplicate within a single batch (e.g., plate) for the LINE-1 analysis with an average coefficient of variation of $5.52 \pm 3.24\%$. Global levels of 5-hmC and 5-fC were measured using MethylFlash Epigentek ELISA kits according to manufacturer instructions (5-hmC Kit#P-1032 and 5-fC Kit#P-1041) including the provided negative and positive ontrols. These kits allow for relative quantification of the methylation modifications from DNA by utilizing a standard curve of known methylation along with the amount of sample input. For the 5-hmC and 5-fC analysis, only single samples were ran due to the limited amount of available muscle tissues available from our biopsies on human subjects.

2.4. Statistical analysis

Demographic and clinical data were compared between the three groups using chi-square, ANOVA or Kruskal–Wallis, where appropriate. A p-value <0.05 was considered significant for demographic and clinical comparisons. Methylation measurements were analyzed for normality using the Shapiro-Wilk Test. 5-mc measurements were considered normal however 5-hmC and 5-fC data were non-normal ($p < 0.05$). Log transformation of these variables resulted in normalized data by Shapiro-Wilk. Methylation variables were compared between the psychiatric and healthy control groups or between treatment groups using student t-tests (for treatment groups) or linear regression (for healthy volunteer comparisons). Any comparison between the psychiatric or specific treatment group and healthy volunteer included age in the model to statistically control for a significant difference observed between the psychiatric and healthy control groups. We did not control for these phenotypic factors in comparisons between the treatment groups as they were not significantly different. Exploratory correlations between methylation variables and HbA1C% were performed using Pearson correlation. To correct for multiple testing the false discover rate (FDR) method was applied and an FDR q-values <0.05 were considered statistically significant in this study (Benjamini and Hochberg, 1995). Analyses were performed in JMP 14.0 (SAS, Cary, NC).

3. Results

3.1. Patient characteristics

Forty-one participants were included consisting of 28 bipolar patients (16 on AAPs and 12 on mood stabilizers) and 13 healthy controls. The bipolar patients were 75% bipolar I, 18% bipolar II and 7% Bipolar NOS. The average chlorpromazine equivalent dose for patients on AAPs was 456.8 mg/day. The average dose for lithium was 630 mg/day,

Table 1 Demographic and clinical variables of psychiatric and healthy control groups.

Variable	Bipolar ($n=28$)			Healthy Control ($n=13$)	*P*-Value
	All ($n=28$)	Atypical Antipsychotic Subgroup ($n=16$)	Mood Stabilizer Subgroup ($n=12$)		Comparisons between three groups
Age	43.7 ± 13.9	44.4 ± 15.1	42.8 ± 12.6	25.3 ± 4.0	0.0002
% Male	43	37.5	50	61	0.4
Race (% Caucasian)[a]	61	56	67	39	0.4
BMI (kg/m^2)	31.6 ± 7.32	31.1 ± 8.2	32.4 ± 6.3	21.7 ± 2.3	0.0004
WHR	1.0 ± 0.09	1.0 ± 0.1	1.0 ± 0.06	1.0 ± 0.1	0.9
HbA1C%	5.6 ± 0.4	5.6 ± 0.5	5.6 ± 0.4	4.9 ± 0.3	0.0002
Fasting Glucose (mg/dL)	93.4 ± 9.5	94.1 ± 10.7	92.4 ± 7.9	83.7 ± 4.6	0.0033
Antipsychotic or Mood Stabilizer (% name)	NA	37.5% quetiapine; 18.75% olanzapine; 18.75% risperidone; 12.5% asenapine; 6.25% lurasidone; 6.25% ziprasidone	42% lamotrigine; 42% lithium; 16% valproic acid	NA	NA

The table gives the demographic and clinical statistics for the healthy control group, the combined bipolar group and for the break down by psychopharmacologic treatment. The right column presents the comparisons between the three groups (atypical antipsychotics, mood stabilizers and healthy controls) using chi-square (sex, race), ANOVA (BMI, HbA1C%) or Kruskal-Wallis (age, WHR and fasting glucose) Values presented in mean ± s.d. or%.

[a] The remaining self-categorized race were combined as "other" BMI=Body Mass Index; HbA1C% = glycated hemoglobin; WHR = Waist-to-Hip Ratio.

for lamotrigine was 190 mg/day and for valproic acid was 750 mg/day. The groups differed in age, BMI, fasting glucose and HbA1C% (all $p<0.001$) which was mainly driven by differences in the healthy control group. Of note, the psychiatric groups tended to have worse glucose control (higher HbA1C%) and fall within or near the cutoff of pre-diabetes as defined by an HbA1C% of 5.70 - 6.49 (American Diabetes Association, 2019). No significant differences (all $p>0.05$) were observed between the AAP and mood stabilizer groups. Table 1 presents the cohort's characteristics based psychiatric diagnosis and by treatment group (AAP versus mood stabilizer).

3.2. Comparisons of skeletal muscle 5-methylcytosine, 5-hydroxymethycytosine and 5-formylcytosine

Our investigation of DNA methylation modifications within the skeletal muscle of bipolar patients and healthy controls identified several differences. First, significantly higher levels of 5-mC and 5-fC methylation were observed in bipolar patients compared to healthy controls when controlling for age. When further stratifying the bipolar patients based on maintenance psychiatric treatment (i.e., AAP or mood stabilizer), significantly higher levels of 5-mC and 5-fC were observed in the AAP group compared to healthy controls. The AAP and mood stabilizer groups had significantly different 5-mC levels (AAP higher) and a possible trend for a difference in 5-fC. Many of the differences between the healthy control group were moderated by age but remained significant. There were no statistically significant correlations of treatment length on current AAP with methylation measures except for a positive trend ($p=0.09$, $r^2=0.19$) with 5-fC. Overall, no differences were observed for 5-hmC methylation however there was a significant correlation between AAP dose (CPZE equivalents) and 5-hmC ($p=0.0015$, $r^2=0.52$). Table 2 presents that comparisons of methylation measures between the groups and depicts adjustment for age in comparisons to the healthy control group and the adjustment for multiple testing.

3.3. Correlations of skeletal muscle methylation with HbA1C%

Since the skeletal muscle is a primary tissue in insulin-mediated glucose uptake and our sample were on long-term AAP or mood stabilizer treatment, we analyzed correlations between measured methylation variables with a measure of long-term glucose management (i.e., HbA1C%) using Pearson correlations (Table 3). A significant, moderate correlation (correlation between 0.2 and 0.5) was observed for 5-hmC and 5-fC with HbA1C%. No correlation was observed for 5-mC.

4. Discussion

4.1. Methylation changes in psychiatric disease and psychopharmacological treatment

To date, our study is the first to demonstrate associations between psychiatric disease, treatment and methylation modifications of DNA in human skeletal muscle. Studies have investigated these effects in the peripheral blood and post-mortem brain samples in psychiatric patients and have shown variable changes in methylation levels depending on the psychiatric disease or treatment being evaluated (Backlund et al., 2015; Lockwood and Youssef, 2017;

Table 2 Methylation comparisons between psychiatric, treatment and healthy control groups.

Comparison	Statistic	Methylation Measure		
		5-methylcytosine (5-mC)	5-hydroxy-methycytosine (5-hmC)	5-formylcytosine (5-fC)
Psychiatric versus Healthy Control	Raw Fold Change	1.10	1.01	3.13
	Unadjusted *p*-value	4.83×10^{-5}	0.49	0.0077
	Adjusted *p*-value	0.0011	0.38	0.0014
	FDR *q*-value	0.0056*	0.41	0.0056*
Atypical Antipsychotic versus Healthy Control	Raw Fold Change	1.12	1	3.91
	Unadjusted *p*-value	2.32×10^{-5}	0.90	0.0048
	Adjusted *p*-value	0.0004	0.79	0.0084
	FDR *q*-value	0.0048*	0.79	0.0252*
Mood Stabilizer versus Healthy Control	Raw Fold Change	1.07	1.03	2.09
	Unadjusted *p*-value	0.0067	0.30	8.7×10^{-5}
	Adjusted *p*-value	0.12	0.24	0.07
	FDR *q*-value	0.18	0.3	0.12
Atypical Antipsychotic versus Mood Stabilizer	Raw Fold Change	1.05	0.97	1.87
	p-value[a]	0.0156	0.25	0.06
	FDR *q*-value	0.037*	0.3	0.12

Comparisons performed between (1) psychiatric and healthy control groups, (2) atypical antipsychotic and healthy control groups, (3) mood stabilizer and healthy control groups and (4) atypical antipsychotic and mood stabilizer groups. Fold change represents relative changes to healthy control group (or mood stabilizer group) using raw data. Comparison *p*-values estimated with normalized data for 5-hmC and 5-fC. The table shows the *p*-values before and after adjustment for age in the comparisons to the healthy control group and the final FDR-adjusted q-value.

* Indicates significance of FDR $q < 0.05$;.

[a] Indicates no adjustment was made for age in comparison between the treatment groups.

Table 3 Pearson correlations between HbA1C% and methylation measures.

	HbA1C%		
	Correlation	*p*-value	FDR *q*-value
5-mC Methylation	0.16	0.35	0.35
5-hmC Methylation	0.34	0.037	0.056*
5-fC Methylation	0.50	0.0018	0.0054*

This table shows the Pearson correlation values between HbA1C%, 5-mC, 5-hmC and 5-fC methylation with their corresponding raw *p*-value and FDR-adjusted *q*-value.

* indicates significance of FDR $q < 0.1$.

Mill et al., 2008; Ovenden et al., 2018; Swathy and Banerjee, 2017). Much of this previous work pertains to the effects of disease and/or treatment on 5-mC at the global and gene-specific level. Furthermore, work in animal and cell models has also demonstrated 5-mC methylation changes globally and in genes such as *Igf2/H19*, *CDKN p21*, and *CDH* within the brain and liver (Goud Alladi et al., 2018; Melka et al., 2013, 2014; Seo et al., 2018). Although variable in their location of 5-mC assessments (some also provided epigenome-wide assessments identifying hundreds of differences in specific genes), these studies consistently point to an effect of both disease and treatment on 5-mC levels peripherally and in tissues relevant to the disease (i.e., brain) or treatment (e.g., brain, liver, etc.). It may be possible drug treatment changes brain DNA methylation in specific pathways leading to efficacy while simultaneously leading to methylation dysregulation within tissues like the skeletal muscle that play a role in drug side effects and metabolic disease. The findings demonstrated here add to this literature by suggesting that psychiatric disease or treatment may influence 5-fC in addition to 5-mC levels in insulin responsive tissues that are relevant to the baseline metabolic burden and glucose dysregulation observed in severe mental illness as well as the side effects of psychiatric drugs that occur independent of psychiatric illness (Burghardt et al., 2018b; Greenhalgh et al., 2017; Pillinger et al., 2017, 2018). Furthermore, our differences between the psychiatric and healthy control groups appear to be mainly driven by differences observed between the AAP and healthy control groups. This may be particularly important as AAPs likely have a higher risk of metabolic side effects compared to mood stabilizers. Therefore, these findings may point to methylation differences underlying the metabolic side effects of AAPs which will require further investigation. Functionally and potentially clinically important changes in 5-mC will likely occur in the promoter regions of genes given the evidence of 5-mC changes in these regions being associated with downstream gene expression (Curradi et al., 2002). It will be important for future studies to address this question on 5-mC changes in psychiatric disease and treatment by focusing on regions of methylation that are most likely associated with changes in gene expression such as promoter regions.

Investigations into alternative methylation modifications beyond 5-mC in psychiatric disease or treatment are less frequent. However, changes in these alternative forms have been observed in blood and other tissues. Zong and colleagues demonstrated increased 5-mC and 5-hmC methylation levels of the *GABRB2* gene in the peripheral blood of schizophrenia patients compared to controls which further was influenced by drug treatment (Zong et al., 2017). Swathy and colleagues demonstrated effects from antipsychotics on global 5-mC, 5-hmC and 5-fC using the same 5-hmC and 5-fC methodology we detail here in liver cells

in vitro and further validated these findings in peripheral blood from schizophrenia patients (Swathy et al., 2018). Finally, work has also identified increased hydroxymethylation of mRNA editing genes in the brains of psychotic patients compared to depressed or control patients (Dong et al., 2012). Our study identified increased levels of 5-mC and 5-fC global methylation in the skeletal muscle psychiatric subjects treated with AAPs compared to controls, which partially supports some of the findings described above. We did not identify a significant change in 5-hmC methylation between the groups. Reasons for these discrepant findings could be (1) different tissues under investigation, (2) using gene-specific versus global assessments of methylation, (3) population specific differences, (4) lack of power to identify an effect from limited sample sizes or (5) methodological differences. Further work is needed to investigate DNA modifications outside of 5-mC as our understanding of their role in psychiatric disease and/or psychiatric treatment within various tissues remains incomplete. Additionally, as with 5-mC above, it will be important to describe the genomic regions of differential alternative methylation modifications as these could be distinct from 5-mC and play alternative or partnering roles in gene control.

4.2. Methylation and de-methylation markers present in the skeletal muscle of psychiatric patients

The observed increased 5-mC methylation and 5-fC methylation compared to healthy controls may suggest that there are processes that are causing increased methylation (5-mC) and pathways of increased de-methylation (5-fC). The reasons for this are unknown. However, an increase in active or passive de-methylation may be present as suggested by the increased presence of 5-fC. This may be further supported by the previous work identifying significance increases in global and gene-specific 5-hmC in psychiatric patients discussed above (Swathy et al., 2018; Zong et al., 2017). Whether or not this is a compensatory molecular mechanism or merely a correlation due to higher 5-mC levels requires further work. Additionally, 5-fC may serve a role beyond just an intermediate to de-methylation through signaling or alternative regulation, however, these alternative roles are still being debated (Breiling and Lyko, 2015; Song et al., 2013). Future studies will need to assess the consequences of methylation and de-methylation (e.g., changes in protein abundance/activation, gene expression, etc.) to better understand the dynamic interplay between 5-mC and its oxidized modifications (5-hmC, 5-fc and 5-carboxylcytosine).

4.3. DNA methylation within the skeletal muscle as a mechanism of glucose dysregulation observed in psychiatric disease or with psychiatric drug treatment

With the skeletal muscle being the primary tissue involved in insulin-stimulated glucose uptake, dysregulation of the molecular pathways that facilitate this process could possibly be involved in the metabolic dysregulation observed with psychiatric disease (prior to drug treatment) or caused directly by AAPs (Burghardt et al., 2018b; Greenhalgh et al., 2017; Pillinger et al., 2017, 2018). Our findings demonstrate increased 5-mC and 5-fC methylation of DNA at a global level in psychiatric patients. Although these psychiatric patients had higher metabolic burdens (increased BMI, higher HbA1C% and fasting glucose) compared to controls our correlation analysis only observed a statistically significant effect for 5-hmC and 5-fC. Future work will need to understand if these associations of psychiatric disease or treatment have any correlation with the metabolic burden and side effects observed in psychiatric populations.

Although our study is in human skeletal muscle, preclinical work has established molecular effects of psychiatric treatment, specifically AAPs, in metabolic tissues such as adipose and muscle in pathways that are critical to glucose homeostasis and insulin resistance. Engl and colleagues demonstrated the effect of olanzapine on protein kinase b (AKT) activation in the skeletal muscle of rats during hyperinsulinemia suggesting that olanzapine exerts some of its glucose dysregulation through effects on this muscle pathway (Engl et al., 2005). This work was extended by Rimessi and colleagues with their work on identifying new treatments that could prevent AAP-induced weight gain in rats (Rimessi et al., 2017). Specifically, they provided evidence of dysregulation in RNA transcripts and proteins in the adipose and skeletal muscle following AAP treatment that was corrected with a protein kinase C inhibitor. Our own work has suggested that AAPs may influence gene-specific methylation of protein kinase b isoforms (Burghardt et al., 2018a). Differential methylation beyond 5-mC (such as 5-fC, suggested by the global differences in our study) could be localized to pathways involved in glucose uptake (such as AKT/protein kinase b described above) meaning reduced expression of the genes and activation of this pathway. Future work should specifically focus on analyzing additional methylation marks of these candidate genes. It is also possible that alternative methylation modification (e.g., 5-fC) may occur in these pivotal pathways of glucose uptake offering additional insight into the methylation changes that underlie psychiatric and treatment-associated insulin resistance. Future work will be needed to replicate and expand on these findings by analyzing gene-specific methylation modifications with functional gene or protein expression analyses while co-analyzing the expression and protein abundance/regulation of the DNA de-methylation pathway (e.g., ten-eleven translocation enzymes). Functional validation would provide further critical mechanistic information by informing whether the methylation changes observed actually cause downstream differences in gene expression, protein abundance or metabolite levels within pathways of interest.

4.4. Limitations and future directions

To our knowledge, this is the first report of methylation modifications beyond 5-mC in the skeletal muscle from psychiatric patients. Nevertheless, some limitations should be considered when interpreting these findings. First, the cross-sectional design also does not rule out the effect of confounding or bias due to patient-specific factors that were clearly different with the healthy control group. However, we did statistically control for age in our comparisons to the

healthy control group while allowing the intended metabolic differences to remain. Nevertheless, these differences in groups do limit the conclusions that can be drawn when considering the comparisons to the healthy control group and future work should match groups more closely. Additionally, we excluded patients with a family history of diabetes to enable us to concentrate on the metabolic side effects of psychopharmacologic treatment. This could reduce our ability to observe the links between bipolar disorder and diabetes and future work should consider including these patients. The sample size was limited so results and conclusions should be considered preliminary and require further replication and follow-up in other psychiatric populations on pharmacological treatment. The measure of glucose management is clinically useful however, gold-standard measurements of whole body insulin sensitivity such as the hyperinsulinemic euglycemic clamp should be used in future studies. The methylation measures were in the skeletal muscle and can only be interpreted as potentially important to the metabolic burden observed in severe mental illness and its treatment. Future work would need to assess brain tissues in various models to make conclusions on the mechanisms of psychiatric disease development or treatment efficacy. We had only limited skeletal muscle for DNA methylation analysis allowing for single analyses of the 5-hmC and 5-fC kits however this is the first report of these measurements in skeletal muscle of psychiatric patients. Future work must be performed to replicate these measurements and assess their validity. Additionally, functional validation of the methylation changes are required to understand their significance (i.e., determining if methylation has a functional effect on gene regulation by measuring changes in gene expression or protein abundance). A correction for multiple testing was performed using the false discovery method but given the limited sample size and preliminary nature of the study, some findings may not have had sufficient power to reach statistical significance. The post-hoc power for the 5-mC, 5-hmC and 5-fC were 0.9. 0.3 and 0.9, respectively. This suggests that the 5-hmC analysis was potentially underpowered and future studies required larger sample sizes to know if there is really no difference between the groups studied here. For example some findings above an FDR of 0.1 may be in an acceptable range based on other studies (FDR of 0.1 to 0.25) (Geetha et al., 2011; Rahimov et al., 2012; Steele et al., 2015; Zheng et al., 2010). Our study is not able to separate out disease versus drug effects on skeletal muscle DNA methylation modifications. Prospective studies identifying methylation changes following treatment are required to establish cause and effect. The use of peripheral DNA samples from studies of patients not yet treated with AAPs may provide insight from a clinically-accessible source. Finally, the analyses performed only assess a snapshot of genomic methylation status and provide no information on gene- or region-specific methylation changes. Future work is required to understand these gene-level changes in order to identify potential pathways or mechanisms of interest.

5. Conclusions

The findings here suggest that DNA methylation may be influenced by psychiatric disease and psychopharmacologic treatment. Although correlational analyses were performed, future work will need to specifically account for metabolic effects versus medication/psychiatric effects on 5-mC, 5-hmC and 5-fC in the muscle through the use of alternative inclusion criteria and statistical modeling. With future work, this could aid in understanding the elevated rates of metabolic disease in psychiatric populations which are thought to cause the increased rates of cardiovascular mortality in this vulnerable population. Identification of these factors and mechanisms may one day allow for interventions that reduce morbidity and mortality observed in psychiatric patients.

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Contributors

All authors assisted in the preparation and editing of the report. Author KB conceived the study design and oversaw the research and assisted in all aspects of the research. Author ZY provided oversight for the research and funding for the physician biopsies. Authors BS, ZM and AM performed muscle biopsies, oversaw safety of the study and provided clinical interpretation of findings. Authors ES, SD and BH provided management of the study protocol, collected data and analyzed samples.

Conflict of interest

The authors declare no conflicts of interest.

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References

American Diabetes Association, 2019. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. Diabetes Care 42, S13–s28.

Abdul-Ghani, M.A., DeFronzo, R.A., 2010. Pathogenesis of insulin resistance in skeletal muscle. J. Biomed. Biotechnol. 2010, 476279.

Aly, R.H., Amr, N.H., Saad, W.E., Megahed, A.A., 2015. Insulin resistance in patients on valproic acid: relation to adiponectin. Acta Neurol. Scand. 131, 169–175.

Backlund, L., Wei, Y.B., Martinsson, L., Melas, P.A., Liu, J.J., Mu, N., Östenson, C.G., Ekström, T.J., Schalling, M., Lavebratt, C., 2015. Mood stabilizers and the influence on global

leukocyte DNA methylation in bipolar disorder. Mol. Neuropsychiatry 1, 76–81.
Ballon, J.S., Pajvani, U., Freyberg, Z., Leibel, R.L., Lieberman, J.A., 2014. Molecular pathophysiology of metabolic effects of antipsychotic medications. Trends Endocrinol. Metab. 25, 593–600.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B (Methodol.) 57, 289–300.
Boyda, H.N., Tse, L., Procyshyn, R.M., Honer, W.G., Barr, A.M., 2010. Preclinical models of antipsychotic drug-induced metabolic side effects. Trends Pharmacol. Sci. 31, 484–497.
Breiling, A., Lyko, F., 2015. Epigenetic regulatory functions of dna modifications: 5-methylcytosine and beyond. Epigenetics Chromatin 8, 24.
Burghardt, K.J., Evans, S.J., Wiese, K.M., Ellingrod, V.L., 2015a. An untargeted metabolomics analysis of antipsychotic use in bipolar disorder. Clin. Transl. Sci. 8 (5), 432–440.
Burghardt, K.J., Goodrich, J.M., Dolinoy, D.C., Ellingrod, V.L., 2015b. DNA methylation, insulin resistance and second-generation antipsychotics in bipolar disorder. Epigenomics 7, 343–352.
Burghardt, K.J., Goodrich, J.M., Dolinoy, D.C., Ellingrod, V.L., 2016. Gene-specific DNA methylation may mediate atypical antipsychotic-induced insulin resistance. Bipolar Disord. 18, 423–432.
Burghardt, K.J., Seyoum, B., Dass, S., Sanders, E., Mallisho, A., Yi, Z., 2018a. Association of protein kinase B (AKT) DNA hypermethylation with maintenance atypical antipsychotic treatment in patients with bipolar disorder. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 38 (4), 428–435.
Burghardt, K.J., Seyoum, B., Mallisho, A., Burghardt, P.R., Kowluru, R.A., Yi, Z., 2018b. Atypical antipsychotics, insulin resistance and weight; a meta-analysis of healthy volunteer studies. Prog. Neuropsychopharmacol. Biol. Psychiatry 83, 55–63.
Caruso, M., Ma, D., Msallaty, Z., Lewis, M., Seyoum, B., Al-Janabi, W., Diamond, M., Abou-Samra, A.B., Højlund, K., Tagett, R., Draghici, S., 2014. Increased interaction with insulin receptor substrate 1, a novel abnormality in insulin resistance and type 2 diabetes. Diabetes 63 (6), 1933–1947.
Caruso, M., Zhang, X., Ma, D., Yang, Z., Qi, Y., Yi, Z., 2015. Novel endogenous, insulin-stimulated akt2 protein interaction partners in L6 myoblasts. PLoS One 10, e0140255.
Castellano-Castillo, D., Moreno-Indias, I., Sanchez-Alcoholado, L., Ramos-Molina, B., Alcaide-Torres, J., Morcillo, S., Ocaña-Wilhelmi, L., Tinahones, F., Queipo-Ortuño, M.I., Cardona, F., 2019. Altered adipose tissue DNA methylation status in metabolic syndrome: relationships between global DNA methylation and specific methylation at adipogenic, lipid metabolism and inflammatory candidate genes and metabolic variables. J. Clin. Med. 8 (1), 87.
Charles, E.F., Lambert, C.G., Kerner, B., 2016. Bipolar disorder and diabetes mellitus: evidence for disease-modifying effects and treatment implications. Int. J. Bipolar Disord. 4, 13.
Chen, J., Huang, X.F., Shao, R., Chen, C., Deng, C., 2017. Molecular mechanisms of antipsychotic drug-induced diabetes. Front. Neurosci. 11, 643.
Correll, C.U., Detraux, J., De Lepeleire, J., De Hert, M., 2015. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. World Psychiatry, Official Journal of the World Psychiatric Association (WPA) 14, 119–136.
Curradi, M., Izzo, A., Badaracco, G., Landsberger, N., 2002. Molecular mechanisms of gene silencing mediated by DNA methylation. Mol. Cell. Biol. 22, 3157–3173.
DeFronzo, R.A., Tobin, J.D., Andres, R., 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol. 237, E214–E223.
DeFronzo, R.A., Tripathy, D., 2009. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32 (Suppl 2), S157–S163.
Dong, E., Gavin, D.P., Chen, Y., Davis, J., 2012. Upregulation of TET1 and downregulation of apobec3a and apobec3c in the parietal cortex of psychotic patients. Transl. Psychiatry 2, e159.
Engl, J., Laimer, M., Niederwanger, A., Kranebitter, M., Starzinger, M., Pedrini, M.T., Fleischhacker, W.W., Patsch, J.R., Ebenbichler, C.F., 2005. Olanzapine impairs glycogen synthesis and insulin signaling in L6 skeletal muscle cells. Mol. Psychiatry 10, 1089–1096.
Geetha, T., Langlais, P., Luo, M., Mapes, R., Lefort, N., Chen, S.C., Mandarino, L.J., Yi, Z., 2011. Label-free proteomic identification of endogenous, insulin-stimulated interaction partners of insulin receptor substrate-1. J. Am. Soc. Mass Spectrom. 22, 457–466.
Goud Alladi, C., Etain, B., Bellivier, F., Marie-Claire, C., 2018. DNA methylation as a biomarker of treatment response variability in serious mental illnesses: a systematic review focused on bipolar disorder, schizophrenia, and major depressive disorder. Int. J. Mol. Sci. 19.
Grande, I., Berk, M., Birmaher, B., Vieta, E., 2016. Bipolar disorder. Lancet 387, 1561–1572.
Greenhalgh, A.M., Gonzalez-Blanco, L., Garcia-Rizo, C., Fernandez-Egea, E., Miller, B., Arroyo, M.B., Kirkpatrick, B., 2017. Meta-analysis of glucose tolerance, insulin, and insulin resistance in antipsychotic-naive patients with nonaffective psychosis. Schizophr. Res. 179, 57–63.
Kumar, S., Chinnusamy, V., Mohapatra, T., 2018. Epigenetics of modified DNA bases: 5-Methylcytosine and beyond. Front. Genet. 9, 640.
Laursen, T.M., Plana-Ripoll, O., Andersen, P.K., McGrath, J.J., Toender, A., Nordentoft, M., Canudas-Romo, V., Erlangsen, A., 2019. Cause-specific life years lost among persons diagnosed with schizophrenia: is it getting better or worse? Schizophr. Res. 206, 284–290.
Laursen, T.M., Wahlbeck, K., Hallgren, J., Westman, J., Osby, U., Alinaghizadeh, H., Gissler, M., Nordentoft, M., 2013. Life expectancy and death by diseases of the circulatory system in patients with bipolar disorder or schizophrenia in the Nordic countries. PLoS One 8, e67133.
Leucht, S., Samara, M., Heres, S., Patel, M.X., Woods, S.W., Davis, J.M., 2014. Dose equivalents for second-generation antipsychotics: the minimum effective dose method. Schizophr. Bull. 40, 314–326.
Lockwood, L.E., Youssef, N.A., 2017. Lockwood LE, Youssef NA. Systematic review of epigenetic effects of pharmacological agents for bipolar disorders. Brain Sci. 7 (11), 154.
Melka, M.G., Castellani, C.A., Laufer, B.I., Rajakumar, R.N., O'Reilly, R., Singh, S.M., 2013. Olanzapine induced DNA methylation changes support the dopamine hypothesis of psychosis. J. Mol. Psychiatry 1, 19.
Melka, M.G., Laufer, B.I., McDonald, P., Castellani, C.A., Rajakumar, N., O'Reilly, R., Singh, S.M., 2014. The effects of olanzapine on genome-wide DNA methylation in the hippocampus and cerebellum. Clin. Epigenetics 6, 1.
Mill, J., Tang, T., Kaminsky, Z., Khare, T., Yazdanpanah, S., Bouchard, L., Jia, P., Assadzadeh, A., Flanagan, J., Schumacher, A., Wang, S.C., Petronis, A., 2008. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am. J. Hum. Genet. 82, 696–711.
Osby, U., Westman, J., Hallgren, J., Gissler, M., 2016. Mortality trends in cardiovascular causes in schizophrenia, bipolar and unipolar mood disorder in Sweden 1987–2010. Eur. J. Public Health 26, 867–871.
Ovenden, E.S., McGregor, N.W., Emsley, R.A., Warnich, L., 2018. DNA methylation and antipsychotic treatment mechanisms in

schizophrenia: progress and future directions. Prog. Neuropsychopharmacol. Biol. Psychiatry 81, 38–49.
Pillinger, T., Beck, K., Gobjila, C., Donocik, J.G., Jauhar, S., Howes, O.D., 2017. Impaired glucose homeostasis in first-episode schizophrenia: a systematic review and meta-analysis. JAMA Psychiatry 74 (3), 261–269.
Pillinger, T., D'Ambrosio, E., McCutcheon, R., O, D.H., 2018. Is psychosis a multisystem disorder? a meta-review of central nervous system, immune, cardiometabolic, and endocrine alterations in first-episode psychosis and perspective on potential models. Mol. Psychiatry.
Rahimov, F., King, O.D., Leung, D.G., Bibat, G.M., Emerson Jr., C.P., Kunkel, L.M., Wagner, K.R., 2012. Transcriptional profiling in facioscapulohumeral muscular dystrophy to identify candidate biomarkers. Proc. Natl. Acad. Sci. U.S.A. 109, 16234–16239.
Rimessi, A., Pavan, C., Ioannidi, E., Nigro, F., Morganti, C., Brugnoli, A., Longo, F., Gardin, C., Ferroni, L., Morari, M., Vindigni, V., Zavan, B., Pinton, P., 2017. Protein kinase c beta: a new target therapy to prevent the long-term atypical antipsychotic-induced weight gain. Neuropsychopharmacology, Official publication of the American College of Neuropsychopharmacology 42, 1491–1501.
Seo, M.K., Kim, Y.H., McIntyre, R.S., Mansur, R.B., Lee, Y., Carmona, N.E., Choi, A.J., Kim, G.M., Lee, J.G., Park, S.W., 2018. Effects of antipsychotic drugs on the epigenetic modification of brain-derived neurotrophic factor gene expression in the hippocampi of chronic restraint stress rats. Neural Plast. 2018, 2682037.
Shanely, R.A., Zwetsloot, K.A., Triplett, N.T., Meaney, M.P., Farris, G.E., Nieman, D.C., 2014. Human skeletal muscle biopsy procedures using the modified Bergström technique. J. Vis. Exp. JoVE 10 (91), e51812.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), 22–33 quiz 34-57.
Shi, D.Q., Ali, I., Tang, J., Yang, W.C., 2017. New insights into 5hmC DNA modification: generation, distribution and function. Front. Genet. 8, 100.
Song, C.X., Szulwach, K.E., Dai, Q., Fu, Y., Mao, S.Q., Lin, L., Street, C., Li, Y., Poidevin, M., Wu, H., Gao, J., Liu, P., Li, L., Xu, G.L., Jin, P., He, C., 2013. Genome-wide profiling of 5-formylcytosine reveals its roles in epigenetic priming. Cell 153, 678–691.
Spruijt, C.G., Gnerlich, F., Smits, A.H., Pfaffeneder, T., Jansen, P.W., Bauer, C., Munzel, M., Wagner, M., Muller, M., Khan, F., Eberl, H.C., Mensinga, A., Brinkman, A.B., Lephikov, K., Muller, U., Walter, J., Boelens, R., van Ingen, H., Leonhardt, H., Carell, T., Vermeulen, M., 2013. Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. Cell 152, 1146–1159.
Steele, M.A., Schiestel, C., AlZahal, O., Dionissopoulos, L., Laarman, A.H., Matthews, J.C., McBride, B.W., 2015. The periparturient period is associated with structural and transcriptomic adaptations of rumen papillae in dairy cattle. J. Dairy Sci. 98, 2583–2595.
Swathy, B., Banerjee, M., 2017. Understanding epigenetics of schizophrenia in the backdrop of its antipsychotic drug therapy. Epigenomics 9, 721–736.
Swathy, B., Saradalekshmi, K.R., Nair, I.V., Nair, C., Banerjee, M., 2018. Understanding the influence of antipsychotic drugs on global methylation events and its relevance in treatment response. Epigenomics 10, 233–247.
Thiebaud, D., Jacot, E., DeFronzo, R.A., Maeder, E., Jequier, E., Felber, J.P., 1982. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. Diabetes 31, 957–963.
Tse, M.Y., Ashbury, J.E., Zwingerman, N., King, W.D., Taylor, S.A., Pang, S.C., 2011. A refined, rapid and reproducible high resolution melt (HRM)-based method suitable for quantification of global LINE-1 repetitive element methylation. BMC Res. Notes 4, 565.
Vancampfort, D., Correll, C.U., Galling, B., Probst, M., De Hert, M., Ward, P.B., Rosenbaum, S., Gaughran, F., Lally, J., Stubbs, B., 2016. Diabetes mellitus in people with schizophrenia, bipolar disorder and major depressive disorder: a systematic review and large scale meta-analysis. World Psychiatry, Official Journal of the World Psychiatric Association (WPA) 15, 166–174.
Wang, D., Hashimoto, H., Zhang, X., Barwick, B.G., Lonial, S., Boise, L.H., Vertino, P.M., Cheng, X., 2017. MAX is an epigenetic sensor of 5-carboxylcytosine and is altered in multiple myeloma. Nucleic. Acids. Res. 45, 2396–2407.
Weiner, M., Warren, L., Fiedorowicz, J.G., 2011. Cardiovascular morbidity and mortality in bipolar disorder. Ann. Clin. Psychiatry 23, 40–47 Official Journal of the American Academy of Clinical Psychiatrists.
Westman, J., Hällgren, J., Wahlbeck, K., Erlinge, D., Alfredsson, L., Ösby, U., 2013. Cardiovascular mortality in bipolar disorder: a population-based cohort study in Sweden. BMJ Open 3 (4), e002373.
Yatham, L.N., Kennedy, S.H., Parikh, S.V., Schaffer, A., Bond, D.J., Frey, B.N., Sharma, V., Goldstein, B.I., Rej, S., Beaulieu, S., Alda, M., MacQueen, G., Milev, R.V., Ravindran, A., O'Donovan, C., McIntosh, D., Lam, R.W., Vazquez, G., Kapczinski, F., McIntyre, R.S., Kozicky, J., Kanba, S., Lafer, B., Suppes, T., Calabrese, J.R., Vieta, E., Malhi, G., Post, R.M., Berk, M., 2018. Canadian network for mood and anxiety treatments (CANMAT) and international society for bipolar disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder. Bipolar Disord. 20, 97–170.
Yi, Z., Bowen, B.P., Hwang, H., Jenkinson, C.P., Coletta, D.K., Lefort, N., Bajaj, M., Kashyap, S., Berria, R., De Filippis, E.A., Mandarino, L.J., 2008. Global relationship between the proteome and transcriptome of human skeletal muscle. J. Proteome Res. 7, 3230–3241.
Zhang, Y., Liu, Y., Su, Y., You, Y., Ma, Y., Yang, G., Song, Y., Liu, X., Wang, M., Zhang, L., Kou, C., 2017. The metabolic side effects of 12 antipsychotic drugs used for the treatment of schizophrenia on glucose: a network meta-analysis. BMC Psychiatry 17 373–373.
Zheng, D., Kille, P., Feeney, G.P., Cunningham, P., Handy, R.D., Hogstrand, C., 2010. Dynamic transcriptomic profiles of zebrafish gills in response to zinc supplementation. BMC Genom. 11, 553.
Zong, L., Zhou, L., Hou, Y., Zhang, L., Jiang, W., Zhang, W., Wang, L., Luo, X., Wang, S., Deng, C., Peng, Z., Li, S., Hu, J., Zhao, H., Zhao, C., 2017. Genetic and epigenetic regulation on the transcription of GABRB2: genotype-dependent hydroxymethylation and methylation alterations in schizophrenia. J. Psychiatr. Res. 88, 9–17.