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Evidence of a Sex-Dependent Restrictive Epigenome in Schizophrenia

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Abstract

When compared to women, men have a higher incidence of schizophrenia, with increases in negative and cognitive symptoms, and an overall poorer disease course. Schizophrenia is conceptualized as a disorder of aberrant gene transcription and regulation. Thus, epigenetics, the study of environmentally induced changes in gene regulation, could advance our understanding of the molecular underpinnings of schizophrenia. Peripheral histone methyltransferase (HMT) mRNA levels have been previously shown to be significantly increased in patients with schizophrenia and correlate with symptomology. In this independent study, peripheral lymphocytes were extracted and clinical symptoms were measured on 74 participants, (40 patients with schizophrenia (19 women, 21 men) and 34 healthy individuals (19 women, 15 men)). HMT (G9α, SETDB1 and GLP) mRNA levels and their resulting histone modification H3K9me2 were measured with RT-PCR and ELISA respectively. Plasma estradiol levels were also measured via ELISA and correlated with HMT mRNA. Clinical symptoms were measured utilizing the Positive and Negative Syndrome Scale (PANSS) and the Heinrichs Carpenter Quality of Life Scale (QLS). The results indicate that men with schizophrenia expressed the highest levels of G9α, SETDB1 mRNA and H3K9me2 protein levels. Additionally, higher levels of symptom presentation and an overall poorer quality of life were correlated with higher HMT mRNA and H3K9me2 protein levels in a sex-dependent pattern. These data support the hypothesis of a sex-dependent restrictive

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epigenome contributing towards the etiology of schizophrenia. The histone methyltransferases measured here could be potential future therapeutic targets for small molecule pharmacology.

Keywords

Epigenetics; Schizophrenia; Lymphocyte; Histone Methylation; Sex Differences; Estradiol; H3K9me2

1. Introduction

Schizophrenia is a chronic and debilitating mental disorder that impacts psychological, social, and cognitive processes across the lifespan (American Psychiatric Association, 2000; Saha et al., 2005). While the disease greatly disrupts the quality of life for both men and women, there is convincing evidence that sex modulates the clinical presentation and course of the disease. Schizophrenia is less severe in women, with lower incident levels, and a later age of onset (Leung and Chue, 2000; McGrath et al., 2004). Women also exhibit better premorbid functioning (Allen et al., 2013), less severe negative symptoms, fewer and less frequent acute episodes of psychosis, and a better response to antipsychotic medications compared to men (Halbreich and Kahn, 2003; Leung and Chue, 2000; Riecher-Rossler and Hafner, 2000; Salem and Kring, 1998).

Schizophrenia can be conceptualized as a disorder of aberrant gene regulation, frequently decreases in gene transcription (Torrey et al., 2005). Epigenetics, the study of environmentally induced changes in gene regulation that arise from post-transcriptional modifications to both DNA and DNA interacting proteins (histones), is an opportune avenue to understand the molecular underpinnings of schizophrenia (Wolffe and Hayes, 1999; Sharma et al., 2012). Epigenetic modifications result in protein assemblies that are commonly described as 'permissive' or 'restrictive'. Restrictive assemblies effectively seal the gene promoter from regulation by transcription factors, often by post-translational methylation of the ninth lysine of histone H3 (H3K9me2; Krauss, 2008; Lyons and Lomvardas, 2014). In particular, the formation of H3K9me2 is catalyzed by histone methyltransferases (HMTs), including G9α, GLP, and SETDB1 (Krishnan et al., 2011; Shinkai and Tachibana, 2011). The majority of studies report increases in restrictive chromatin (Chase et al., 2013; Gavin et al., 2008; Gavin et al., 2009b; Sharma et al., 2008) and resulting down-regulation of gene transcription of several candidate genes in patients with schizophrenia (Akbarian et al., 1995; Guidotti et al., 2000; Impagnatiello et al., 1998; Jindal et al., 2010). The H3K9 methyltransferases (HMTs) G9α, GLP, and SETDB1 and the restrictive histone modification H3K9me2 are increased in patients with schizophrenia, and significantly correlated with clinical symptomology (Chase et al., 2013).

Epigenetic mechanisms contribute to various aspects of sex differences in both brain and behavior, particularly during such critical periods as puberty (Morrison et al., 2013). Prepubertal inhibition of DNA methylation or histone deacetylation (both restrictive modifications) result in pubertal failure (Lomniczi et al., 2013; Ojeda et al., 2010), demonstrating the marked contribution of epigenetic mechanisms in the regulation of puberty. Promoter methylation and resulting decreased gene expression of the polycomb

protein EED, known to interact with histone deacetylases, indicates the commencement of puberty through disinhibition of kisspeptin, an upstream signaling ligand for gonadotropin-releasing hormone (GnRH; Lomniczi et al., 2013). Other sexually dimorphic traits emerge as early as postnatal day 1. When compared to females, males have increased levels of both estrogen receptor promoter methylation and histone deacetylase (HDAC) binding (Kurian et al., 2010; Murray et al., 2009), effectively decreasing gene transcription. Additionally, MeCP2, a methylated DNA binding repressive scaffold enzyme, demonstrates a sex and age-specific switch in protein levels, with females exhibiting increased amounts in the amygdala and the hypothalamus up until postnatal day 10, after which males exhibit higher levels (Kurian et al., 2007). Lastly, the bed nucleus of the stria terminalis (BNST) contains more cells, and thus is larger in volume in males when compared to female mice. An injection of valproic acid, a histone deacetylase inhibitor, at postnatal days one and two, significantly reduces both cell count and BNST volume in males, eliminating sex differences (Murray et al., 2009).

The literature has well documented sex differences on the clinical presentation of schizophrenia, and epigenetic mechanisms of both mental illness and sexual dimorphism separately. However, to our knowledge, few studies to date examine the interplay between these areas of research. Given that histone methyltransferase mRNA levels in peripheral lymphocytes are positively correlated with schizophrenia symptomology (Chase et al., 2013), our primary hypothesis is that men with schizophrenia will have higher levels of these HMT mRNA and resulting H3K9me2 levels when compared to control subjects and women with schizophrenia given the higher incidence of these symptoms in men (Leung and Chue, 2000). The result of this independent study replicates earlier findings, indicating increased restrictive epigenetic measures in schizophrenia (Chase et al., 2013). To supplement these primary analyses, we also investigated the relationship of these epigenetic molecules to individual clinical symptoms in men and women separately.

2. Methods

2.1. Patient information

The sample included 74 participants, including 40 patients with schizophrenia (19 women, 21 men) and 34 healthy individuals (19 women, 15 men) recruited from the University of Illinois at Chicago (UIC) medical center. Healthy individuals were group matched to patients on age and sex (see Table 1). Patients with schizophrenia were assessed via SCID interview, DSM-IV-TR criteria and all available information by experienced diagnosticians (MD or PhD). Clinicians established a consensus before final assignment to diagnostic group. All participants were between 21–65 years of age and in good physical health (with no reported infections), and exclusion criteria were: (1) history of neurological disease or head trauma, (2) lifetime history of substance/alcohol dependence or recent substance abuse, (3) pregnancy for women. Exclusionary criteria for control participants also included a major Axis I disorder (assessed by a SCID interview) and a known first-degree familial history of psychosis. All subjects provided written informed consent before participating in any study procedures.

At the time of sampling, 58% (n=23) of the patients with schizophrenia were evaluated while hospitalized on the inpatient psychiatric unit and 42% (n=17) were evaluated in the psychiatric outpatient clinic. Prescribed antipsychotic medication was coded on all participants as follows: Haloperidol=2, Fluphenazine=1, Perphenazine=2, Risperidone=18, Quetiapine=5, Ziprasidone=2, Aripiprazole=5, Lurasidone=3 (a total of n=38). Two patients were unmedicated at the time of the blood draw. Due to this heterogeneity, all antipsychotic use was converted to Chlorpromazine (CPZ) units, although several newer medications do not have published CPZ equivalents, including Lurasidone (n=3) (Gardner et al., 2010). There were no significant sex differences in medication use (in CPZ equivalents; $t_{36} = -1.09, p = 0.279$).

2.2. Clinical measures

The Positive and Negative Syndrome Scale (PANSS) (Emmerson et al., 2009; Kay et al., 1987) was administered to all participants to generate measures of positive, negative, and general psychopathology, all summed to create a total score. Additionally, the Heinrichs Carpenter Quality of Life Scale (QLS) (Heinrichs et al., 1984; Song et al., 2011) was administered to all participants to generate measures of overall quality of life, with higher scores reflecting an improved quality of life. The composite scores include: *interpersonal relations* (frequency and capacity of social contact); *instrumental role* (extent of professional functioning and satisfaction), and *intrapsychic foundations* (motivation and feelings of pleasure). The overall total value was a sum of all three composite scores (Heinrichs et al., 1984).

2.3. Lymphocyte isolation and plasma collection

A blood sample was obtained by sterile venipuncture and collected in 0.5M Ethylene-diamine-tetraacetic acid (EDTA), pH 8.0. An aliquot of blood was centrifuged at 2,000RPM at 10°C and plasma was collected into aliquots within one hour of extraction and immediately frozen at -80°C until analysis. Remaining blood sample was then diluted 1:1 with Hanks Balanced Salt Solution (HBSS without calcium; GIBCO 14170-112), and carefully layered over Ficoll-Paque® (Amersham) in a 1:1 ratio, and centrifuged at 1,800RPM for twenty minutes at 10°C. The resulting cream-colored opaque middle interface was collected, diluted with 1:1 HBSS, and pelleted at 2,000RPM for ten minutes at 10°C (Chase et al., 2013; Gavin et al., 2009a; Jayaraman et al., 1999).

2.4. mRNA extraction

Total RNA was isolated using TRIZOL reagent (Life Technologies, Grand Island, NY). Samples were treated with DNase (Amersham 27-0514-03) after extraction. Only total RNA extracts with an OD260/OD280 ratio above 1.96, indicating relatively pure RNA, were processed for RT-PCR; the remainder undergoing re-extraction (Mannhalter et al., 2000).

2.5. Real time RT-PCR quantification

Total RNA was used to prepare cDNA via the Applied Biosystems High Capacity Archive Kit (#4368813). For detection and measurement of expression, Fermentas Maxima SYBR Green/ROX qPCR Master Mix (#K0222) was used. PCR mixtures were run on a Thermo

Scientific PikoReal real-time PCR System. Cycle threshold (CT) value was used for relative quantification, and all values were normalized to three housekeeping genes, GAPDH, TFRC and β -Actin using a geometric mean, and run in triplicate (Vandesompele et al., 2002). Housekeeping genes were selected from a previously validated list (Gavin et al., 2012;) and their stability across diagnostic groups in our lymphocyte sample was further determined using the geNorm algorithm. Primer design parameters and sequences are described in detail elsewhere (Chase and Sharma, 2012).

2.6. H3K9me2 ELISA on acid extracted histones

Histone extraction was performed by acid extraction as published (Shechter et al., 2007), and protein levels quantified by Bradford assay. H3K9me2 (Active Motif #53109) and total H3 protein (Active Motif #53110; for normalization purposes) were measured utilizing their respective ELISA kits, per manufacturer instruction. For both H3K9me2 and total H3 ELISA kits, each sample was run in duplicate. Each plate was loaded with 1) a standard curve utilizing recombinant proteins provided by the manufacturer; 2) an invariant sample from one normal subject, from a single extraction and single thaw and; 3) fixed plate position for each subject across different performed ELISAs. Data analysis was performed in the following sequence: 1) quantification to standard curve measured in ng/ μ l; 2) scaled to plate-control sample and 3) normalized to total histone protein. Intra-assay coefficient of variation (within-plate) was calculated as an average for all individual CVs for the duplicate reading of the plate-control sample and ranges between 2–7% for all modifications; inter-assay (between-plates) for the same plate-control is 2–12%.

2.7. Estradiol ELISA on coordinated plasma samples

To examine levels of Estradiol 17- β levels in plasma samples from clinical samples, a smaller group of sex and diagnosis matched participants were selected. On this selected group, the Abcam human ELISA kit was utilized per instructions from the manufacturer (Abcam 108667).

2.8. Data analysis

SPSS (version 15.0 for Windows) was used for all statistical analyses. Analysis of all mRNA data was conducted on CT levels of the gene of interest, normalized to the geometric mean of three control genes. Data is presented as mean values $\pm$ standard error. A probability level of $p < 0.05$ was the criterion to achieve statistical significance. All mRNA values were tested for normality in SPSS, and variables not exhibiting a normal distribution were natural log transformed. To examine sex-dependent diagnostic mRNA differences of the various HMTs or H3K9me2, univariate ANOVA analyses were performed with Sex and Diagnostic Group as the independent variables. To examine correlations between peripheral HMT mRNA or H3K9me2 levels and clinical symptomology, Spearman correlations were performed in men and women separately. Correlations were then compared between male and female participants using Fisher's two-tailed r-to z transformation.

3. Results

3.1 Histone methyltransferase diagnosis and sex differences

Three histone methyltransferase genes were examined: G9α, GLP and SETDB1.

Participants with a diagnosis of schizophrenia exhibited increased levels of G9α mRNA in the lymphocyte when compared to controls ($F(1, 74) = 9.04, p = 0.004$). While there were no sex-specific differences in G9α mRNA levels, there was a significant interaction between diagnosis and sex, with male participants with schizophrenia expressing the highest levels of G9α mRNA when compared to women with schizophrenia, and control men and women ($F(1, 74) = 3.96, p = 0.05$; Figure 1a).

Lymphocyte mRNA levels of SETDB1 were also significantly increased in participants with schizophrenia when compared to normal controls ($F(1, 74) = 4.57, p = 0.03$). There were no significant differences found in SETDB1 mRNA between the sexes independently across diagnostic groups. However, further analysis revealed a significant diagnosis by sex interaction, with male patients with schizophrenia again exhibiting the highest amounts of SETDB1 mRNA, a pattern similar to G9α mRNA ($F(1, 74) = 4.1, p = 0.04$; Figure 1b).

Increases in GLP mRNA were seen in lymphocytes from participants with a diagnosis of schizophrenia compared to normal controls ($F(1, 74) = 4.2, p = 0.04$; Figure 1c). There were no independent sex differences or sex by diagnosis interactions seen with GLP mRNA.

3.2 H3K9me2 protein diagnosis and sex differences

Lymphocyte levels of H3K9me2 were also significantly increased in participants with schizophrenia when compared to normal controls ($F(1, 74) = 4.04, p = 0.04$). There were no significant differences found in H3K9me2 levels between the sexes alone. However, further analysis revealed a significant diagnosis by sex interaction, with male patients with schizophrenia exhibiting the highest amounts of H3K9me2 levels in the lymphocyte ($F(1, 74) = 4.85, p = 0.03$; Figure 1d).

3.3 Estradiol plasma correlates

Plasma levels of estradiol were significantly decreased in men compared to women participants ($F(1, 35) = 4.4, p = 0.04$). There were no significant diagnostic differences, or sex by diagnostic interactions in plasma levels of estradiol.

Spearman correlations demonstrated a significant relationship between the various HMT mRNA levels and circulating blood plasma estradiol levels. GLP mRNA was positively correlated ($\rho = 0.42, p = 0.012$; Figure 2a), while G9α was negatively correlated ($\rho = -0.42, p = 0.010$; Figure 2b), with plasma estradiol. These results were not dependent of diagnosis or sex.

3.4 Clinical correlates with biological measures

3.4.1 Positive and Negative Syndrome Scale (PANSS)—A Spearman correlation indicated that lymphocyte levels of G9α ($\rho = 0.47, p = 0.008$; Figure 3a) and SETDB1 ($\rho = 0.366, p = 0.04$; Figure 3b) mRNA were positively correlated with the PANSS overall total

subscale only in male subjects. Fisher's *r*-to-*z* transformation indicated there was a significant difference between the two correlation coefficients for G9α ($p = 0.03$), but no significant difference between the two correlation coefficients for SETDB1.

In contrast, GLP levels were positively correlated with PANSS overall totals in women only ($\rho = 0.393$, $p = 0.031$; Figure 3c). Fisher's *r*-to-*z* transformation did not indicate a significant difference between the two correlation coefficients.

A Spearman correlation indicated that lymphocyte H3K9me2 levels were positively correlated with the PANSS overall total subscale only in male subjects ($\rho = 0.48$, $p = 0.006$; Figure 3d). Fisher's *r*-to-*z* transformation indicated there was a significant difference between the two correlation coefficients for H3K9me2 ($p = 0.005$).

Histone methyltransferase mRNA and H3K9me2 levels were also significantly correlated with other PANSS subscales in a sex-specific manner, which are presented in text form Supplemental table 1a.

3.4.2 Heinrichs Carpenter Quality of Life (QLS)—Spearman correlations demonstrated significant sex-specific relationships with various HMT mRNA levels. Increases in mRNA levels of G9α were correlated with decreases in the overall total of the Heinrichs Carpenter Quality of Life Scale (QLS) in men only ($\rho = -0.46$, $p = 0.004$; Figure 4a). Fisher's *r*-to-*z* transformation indicated a significant difference between the two correlation coefficients ($p = 0.04$).

Spearman correlations also indicated a sex-specific negative relationship between SETDB1 mRNA and QLS overall totals in men only ($\rho = -0.39$, $p = 0.02$; Figure 4b). Fisher's *r*-to-*z* transformation also indicated a significant difference between the two correlation coefficients ($p = 0.03$). In contrast, GLP did not show any sex-dependent relationships with total QLS scores (Figure 4c).

Increases in H3K9me2 protein levels were correlated with decreases in the overall total of the Heinrichs Carpenter Quality of Life Scale (QLS) in men only ($\rho = -0.402$, $p = 0.03$; Figure 4d). Fisher's *r*-to-*z* transformation indicated there was not a significant difference between the two correlation coefficients.

Various subscales of the QLS were also correlated with histone methyltransferase mRNA and H3K9me2 levels, and are presented in supplemental table 1b.

4. Discussion

We report an independent replication of our previous publication demonstrating increases in HMT mRNA and H3K9me2 protein levels in lymphocytes extracted from patients with schizophrenia (Chase et al., 2013). In this independent sample, we were able to extend these previous findings and demonstrate that the increases in a restrictive epigenome seen in schizophrenia are sex dependent. Specifically, G9α, SETDB1, and H3K9me2 were significantly increased in lymphocytes from men with schizophrenia. Levels of plasma estradiol were also correlated with levels of HMT mRNA. Further, increases in G9α and

SETDB1 mRNA and H3K9me2 protein levels demonstrated male-specific correlations with both increased symptom presentation, as measured by the PANSS, and a worse quality of life, as measured by the QLS.

The methylation status of histone tail residues are subject to dynamic modifications through the activities of histone methyltransferases (HMTs). G9α is the predominant methyltransferase of H3K9 (Tachibana et al., 2002), and its closely related protein, G9α-like-protein (GLP), with which it forms a functional dimer (Dillon et al., 2005; Volkel and Angrand, 2007). G9α and GLP can form homomeric or heteromeric complexes (Tachibana et al., 2005), although they do not substitute for each other, and their function is not redundant (Shinkai and Tachibana, 2011). SETDB1 specifically di- and tri-methylates H3K9 (Harte et al., 1999), and acts in a progressive manner, binding to a previously di-methylated residue and remaining bound until tri-methylation occurs, effectively repressing gene transcription (Wang et al., 2003; Zee et al., 2010).

H3K9me2 levels are the functional consequence of the catalytic activity of the histone methyltransferases examined here. These findings build on our previous demonstrations of increased H3K9me2 levels both peripherally and centrally in patients with schizophrenia (Chase et al., 2013; Gavin et al., 2009b). Our hypothesis is that a restrictive epigenome contributes to the etiology of schizophrenia, a process that could originate at the initial inactivation of candidate genes, but is then capable of spreading to healthily regulated genomic regions (Sharma et al., 2012). H3K9me2 can act as a scaffold, allowing for the accumulation of additional restrictive enzymes, effectively spreading a heterochromatic message across 'healthy genomic territory.' This hyper-restrictive insult then will impede the gene expression modifying effects of medication, leading to treatment nonresponse (Sharma et al., 2012).

While there were no diagnostic differences found in estradiol 17-β plasma levels in this cohort, there was, as expected, a sex difference. G9α mRNA levels have previously been demonstrated to be correlated with increased negative symptoms in patients with schizophrenia (Chase et al., 2013), an effect further informed by this paper to be sex-dependent. Negative symptoms are more prevalent in men with schizophrenia, when compared to women (Leung and Chue, 2000). In women, symptomology follows the menstrual cycle, with an improvement of symptomology during the high estrogen phase and worsening of symptoms premenstrually (Bergemann et al., 2007; Rubin et al., 2010). Menopause also marks a significant increase of both symptom onset and exacerbation (Hafner, 2003). Together, these results propose a protective effect of oestrogen in serious mental illness. Several studies have indicated that short-term estrogen co-therapy in participants with schizophrenia improves clinical symptoms (Kulkarni et al., 2001; Kulkarni et al., 2012), with estrogen supplementation in post-menopausal women significantly reducing negative symptoms (Lindamer et al., 2001). Research examining the interplay between estrogens and histone methyltransferases is limited, but the N-terminal region of G9α can act as a co-regulator with the estrogen receptor (ERα), resulting in altered gene transcription (Purcell et al., 2011).

One limitation of this paper is the lack of systematic collection and normalization of menstruation data or hormonal contraceptive use in woman participants. Given the effects of estrogen cycling on symptomology, this variable needs to be rigorously examined in future research. Another limitation was the significantly increased number of African Americans in comparison to other races in our sample, although we saw no significant differences in the variables measured and race. Additionally, the data examined in this study is subject to the problem of multiple testing. If we consider our primary hypothesis as a sex by diagnosis interaction of the epigenetic markers G9a, GLP and SETDB1, then application of the Bonferroni correction will retain the difference in G9a expression as significant, while the differences in GLP and SETDB1 will approach significance at the new cutoff. However, if we include all tests conducted on clinical subscales examined as exploratory variables, and items that are generally obtained in symptom inventories and presented here for completeness, then clearly we would need a much larger sample to verify these results. Finally, more causative relationships could potentially be established in these systems when examining woman participants pre- and post-menopause, given the increase of both symptom severity and onset.

Taken together, the results of this paper contribute some knowledge of sex differences in the epigenomic profile of schizophrenia, and how these differences correlate to symptom presentation and disease etiology. Once biologically influenced symptomology is characterized, methods for utilization of small molecule modifiers to reduce this hyper-restrictive epigenomic state can be identified as another source of therapeutic potential (Sharma, 2005; Sharma et al., 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Peripheral samples were used for all study procedures, including lymphocyte mRNA and H3K9me2 levels and blood plasma estradiol levels.
- Male patients with schizophrenia expressed the highest levels of G9α and SETDB1 mRNA and H3K9me2 protein when compared to both normal controls and females with schizophrenia.
- Histone methyltransferase mRNA levels correlated with plasma estradiol levels
- Worse symptom presentation and overall quality of life positively correlated with HMT mRNA and H3K9me2 protein levels in a sex-dependent pattern.
- The results suggest that a sex-dependent restrictive epigenome may underlie the etiology of schizophrenia.

Sex and Diagnosis Differences in Biological Measures

Figure 1.

Sex and diagnosis differences in both histone methyltransferase mRNA and H3K9me2 ELISA levels. a&b) G9α and SETDB1 mRNA levels were significantly increased in participants with schizophrenia when compared to normal controls, with schizophrenic men expressing the statistically highest levels. c) GLP mRNA expression in lymphocytes was significantly increased in participants with schizophrenia when compared to normal controls. d) H3K9me2 levels were significantly increased in participants with schizophrenia when compared to normal controls. Specifically, men with schizophrenia expressed the highest levels of H3K9me2 in the lymphocyte. * $p<0.05$, ** $p<0.01$

Estradiol Plasma Correlates with Histone Methyltransferase mRNA

Figure 2.

Lymphocyte HMT mRNA and plasma estradiol correlations. a) GLP mRNA was positively correlated with plasma estradiol 17- β levels. b) G9 α mRNA was negatively correlated with plasma estradiol 17- β levels. Spearman rho values, sample size and significance levels are all listed on individual graphs.

PANSS Clinical Correlates with Biological Measures

Figure 3.

Sex differences between both histone methyltransferase mRNA and H3K9me2 levels and clinical symptomology as measured by the Positive and Negative Syndrome Scale (PANSS). a&b) G9 α and SETDB1 mRNA levels were higher in male participants with increased symptom presentation. This effect was not seen in women. c) Increases in GLP mRNA were correlated with increased symptomology in women, not men. d) H3K9me2 levels were significantly increased in male participants with increases in symptom presentation, an effect not seen in women. Spearman rho values, sample size and significance levels are all listed on individual graphs.

Quality of Life Correlates with Biological Measures

Figure 4.

Sex differences between both histone methyltransferase mRNA and H3K9me2 levels and quality of life, as measured by the Heinrichs Carpenter Quality of Life Scale (QLS). a&b) G9α and SETDB1 mRNA levels were increased in male participants with worse quality of life, an effect not seen in women. c) GLP mRNA and the QLS were not significantly correlated with either sex. d) H3K9me2 levels were increased in male participants with worse quality of life, an effect not seen in women. Spearman rho values, sample size and significance levels are all listed on individual graphs.

Table 1
Demographics Comparing Healthy Controls and Schizophrenia Cases by Sex in PBMC Samples

Means and standard deviations of participant demographics comparing healthy controls and patients with schizophrenia in PBMC samples. Statistical differences between the various parameters were determined by a Student's t-test, and indicated where performed.

Demographic	Healthy Controls		Patients with Schizophrenia	
	Men	Women	Men	Women
	15	19	21	19
Age (Mean $\pm$ SD)	33.9 $\pm$ 9.65	35.1 $\pm$ 11.03	35.4 $\pm$ 12.18	40.1 $\pm$ 13.51
	F _{1,74} =0.43; p=0.516			
Race (%)				
Caucasian, non-Hispanic	7 (47)	4 (21)	0 (0)	2 (11)
Black, non-Hispanic	5 (33)	8 (42)	17 (81)	16 (84)
Asian or other Pacific Islander	2 (13)	4 (21)	0 (0)	0 (0)
Hispanic	1 (7)	3 (16)	4 (19)	1 (5)
	F _{1,74} =5.18; p=0.026*			