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Toward the Identification of Peripheral Epigenetic Biomarkers of Schizophrenia

Guidotti A¹, Auta J¹, Davis JM¹, Dong E¹, Gavin DP^{1,2}, Grayson DR¹, Sharma RP^{1,2}, Smith RC³, Tueting P¹, and Zhubi A¹

¹Psychiatric Institute, Department of Psychiatry, School of Medicine, University of Illinois, Chicago

²Jesse Brown Veterans Affairs Medical Center, 820 South Damen Avenue (M/C151), Chicago, IL

³Department of Psychiatry, New York University, School of Medicine, NY, N.Y

Abstract

Schizophrenia (SZ) is a heritable, non-mendelian, neurodevelopmental disorder in which epigenetic dysregulation of the brain genome plays a fundamental role in mediating the clinical manifestations and course of the disease. We recently reported that two enzymes that belong to the dynamic DNA-methylation/demethylation network –DNMT (DNA-methyltransferase) and TET (5-hydroxycytosine translocator)- are abnormally increased in cortico-limbic structures of SZ post-mortem brain suggesting a causal relationship between clinical manifestations of SZ and changes in DNA methylation and in the expression of SZ candidate genes (e.g., brain derived neurotrophic factor [BDNF], glucocorticoid receptor [GCR], glutamic acid decarboxylase67 [GAD67], reelin). Because the clinical manifestations of SZ typically begin with a prodrome followed by a first episode in adolescence with subsequent deterioration, it is obvious that the natural history of this disease cannot be studied only in post-mortem brain. Hence, the focus is currently shifting towards the feasibility of studying epigenetic molecular signatures of SZ in blood cells. Initial studies show a significant enrichment of epigenetic changes in lymphocytes in gene networks directly relevant to psychiatric disorders. Furthermore, the expression of DNA-methylating/demethylating enzymes and SZ candidate genes such as BDNF and GCR are altered in the same direction in both brain and blood lymphocytes.

The coincidence of these changes in lymphocytes and brain supports the hypothesis that common environmental or genetic risk factors are operative in altering the epigenetic components involved in orchestrating transcription of specific genes in brain and peripheral tissues. The identification of DNA-methylation signatures for SZ in peripheral blood cells of subjects with genetic and clinical high risk would clearly have potential for the diagnosis of SZ early in its course and would be invaluable for initiating early intervention and individualized treatment plans.

Keywords

Lymphocytes; DNA methylation; Brain derived nerve growth factor; Glucocorticoid receptor

Corresponding Author: A. Guidotti. Psychiatric Institute, 1601 West Taylor St. University of Illinois at Chicago, Tel: 312-520-1671
aguidotti@psych.uic.edu.

Declaration of Interest

1) Introduction

Schizophrenia (SZ) and related psychiatric disorders are heritable, non-mendelian neurodevelopmental disorders in which multiple gene mutations, polymorphisms, and copy number variants have been implicated in only a small percentage of cases (Cross-Disorder Group of the Psychiatric Genomics Consortium, Lancet, 28 February, 2013). Twin studies, indicate a 40-to 70% concordance rate for the disease strongly suggesting that complex epigenomic-genomic interactions may play a critical role in the emergence of SZ pathophysiology (Dempster et al., 2011; Ptak and Petronis, 2008). In recent years, epigenetic abnormalities (DNA-methylation, chromatin remodeling) have been identified in post-mortem brain of SZ patients (Grayson and Guidotti, 2013; Houston et al., 2013). However, SZ has a natural course, starting with a prodrome, a first episode in adolescence or young adulthood, and later deterioration over adult years. Hence, the natural history of such a complex neuropsychiatric disorder cannot be studied adequately in just post-mortem brain.

To overcome the limitations offered by epigenetic studies in brain, there has been a recent focus on studying epigenetic molecular signatures, such as altered DNA methylation and chromatin remodeling, in peripheral blood cells of SZ and related disorder patients. (Chanet et al., 2011; Chase et al., 2013; Dempster et al., 2011; Gavin et al., 2009; Ikegame et al., 2013; Liu et al., 2013; Melas et al., 2012; Michel et al. 2012; Nishioka et al., 2012; Provencal et al., 2012; Wong et al., 2013). If identified in peripheral blood cells, molecular biomarker signatures for SZ and related psychiatric disorders could be useful in the early detection of the disease. Such biomarkers could even be used for predicting disease course in subjects with high risk for developing SZ and for predicting treatment response at each stage of the illness. Thus, in clinical treatment, biomarkers could be invaluable in monitoring disease progression across time and in individualizing treatment.

We will review (1) epigenetic alterations found in SZ postmortem brain, (2) epigenetic alterations in peripheral blood cells of SZ patients, and (3) differences and similarities between brain and peripheral blood cells. We will then discuss these findings in the context of future research on SZ.

2) Epigenetic alterations in the brain of SZ patients

a) DNMT-mediated methylation abnormalities of brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (GCR), and GABAergic genes

When the post-mortem brain of SZ and bipolar (BP) disorder patients is compared to that of non-psychiatric subjects, a GABAergic neuropathology is consistently detected in the cortex and hippocampus (Akbarian et al., 1995; Benes et al., 1992; Benes and Beretta, 2001; Fatemi et al., 2000; Guidotti et al., 2000, 2005; 2011; Impagnatiello et al., 1998; Lewis et al., 2005). This GABAergic neuropathology is characterized by a decrease in the expression of glutamic acid decarboxylase67 (GAD67) and reelin that is associated with an over-expression of DNA-methyltransferase 1 (DNMT1) and DNA-methyltransferase 3a (DNMT 3a) in cortical layers I and II of BA9, BA10, and BA17 (Ruzicka et al., 2007; Veldic et al., 2005, 2007; Zhubi et al., 2009). DNMTs, including DNMT1, 3a, 3b, and DNMT3L, belong

to a family of enzymes that catalyze the transfer of a methyl group from the methyl donor S-adenosylmethionine (SAM) to the 5'-carbon of cytosines in the cytosine phosphodiester guanine (CpG) islands of many gene promoters (Moore and Fan, 2013). It has been suggested that the over-expression of DNMT1 and DNMT3a in frontal cortex and hippocampus of SZ patients favors a repressive chromatin conformation that is associated with a downregulation of GABAergic gene expression (Grayson and Guidotti, 2013) and a decreased expression of BDNF (Gavin et al., 2012; Ikegame et al., 2013; Roth et al., 2009, Wong et al., 2010) and GCR (Sinclair et al., 2011, 2012, Zang et al., 2013).

Accumulating evidence suggests a critical role for altered DNA methylation processes in the expression of several SZ candidate genes (Grayson and Guidotti, 2013; Mill et al., 2008). Specifically, atypical methylation of BDNF and GCR promoters has been implicated in the pathogenesis of SZ and other stress-related psychiatric disorders (Dong et al., 2012; Gavin et al., 2012; Grayson and Guidotti, 2013; Wong et al., 2010; Zhang et al., 2013). Levels of BDNF, a neurotrophic factor important for neuronal survival, development, and synaptic plasticity, are reduced in brain and blood cells of patients with psychiatric disorders and the decreased BDNF levels are associated with DNA hypermethylation at specific BDNF promoters (Gavin et al., 2012; Ikegame et al., 2013; Keller et al., 2010; 2011; Mill et al., 2008; Rao et al., 2012; Wong et al., 2010). Similarly, a decrease in GCR expression in cortex and hippocampus of SZ patients has been associated with an increased expression of DNMT and with increased DNA methylation across GCR (NR3C1) promoter regions in response to stress in childhood and adolescence (Labonte et al., 2012; McGowan et al., 2009; Zhang et al., 2013). Increased methylation of alternative splice variants of the exon 1 promoter of the NR3C1 gene could explain the decreased GCR levels observed in hippocampus of SZ and BP disorder patients (McGowan et al., 2009; Sinclair et al., 2011, 2012). These observations reflect the profound influence of environmental epigenetic factors and DNA-methylation of genes active in the pathogenesis of psychiatric disorders.

b) DNA demethylation pathways

Several reports indicate that the methylation status of the promoter proximal to CpG dinucleotides of active genes is in a dynamic balance between DNA methylation and DNA demethylation, and that regulation of both hyper- and hypo-methylation of CpG islands in gene promoters or bodies is under the control of a complex network of methylating and demethylating enzymes. For example, a 5-methylcytosine (5-MC) mark on CpG-rich regions of specific promoters can be oxidized to form 5-hydroxymethylcytosine (5-HMC) by members of the ten-eleven-translocase (TET) family of proteins in the mammalian brain (Bhutani et al., 2011; Guo et al., 2011; Jin et al., 2011; Kriaucionis and Heintz, 2009) (**Fig 1**). Further, it has been proposed that 5-HMC may undergo two successive processing steps: (1) a deamination step catalyzed by the AID/APOBEC family of cytosine deaminases, turning 5-HMC into 5-hydroxymethyluridine (5-HMU); and (2) a "base excision repair" (BER) pathway, in which 5-HMU can be removed by a group of glycosylases (e.g., MBD4 and TDG4), and replaced by unmethylated cytosine (Morgan et al., 2004; Rai et al., 2008; Zhu, 2009). However, to complicate the issue, TET1 and TET3 may play a role in transcription repression independent of catalytic activity by recruiting Polycomb Repressive Complex-2 (PRC2) and SIN3A co-repressor proteins at target genes (Williams et al., 2011).

Recent studies have emphasized the important role of the TET enzyme family (TET1, 2, 3) and 5HMC in the epigenetic reprogramming and regulation of tissue-specific gene expression (Ito et al., 2010; Mellen et al., 2012; Tahiliani et al., 2009). Dong et al. (2012) showed an approximately two-fold increase in the levels of TET1 mRNA in the parietal cortex of chronic psychotic patients [SZ or BP] compared to non-psychotic control subjects (**Fig 2**). To examine if the levels of 5-HMC in global DNA are related to the increase in TET levels in tissue, Dong et al. (2012) measured 5-MC and 5-HMC levels relative to genomic DNA in the brain of psychotic patients by immunoblotting using specific 5-MC and 5-HMC antibodies. The results, represented in **Fig 2**, show that while 5-MC levels in global DNA are virtually identical in control and psychotic patients, 5-HMC levels are significantly higher in psychotic patients compared to controls. Taken together, the data suggest that 5-HMC and TET enzymes are positively correlated, reflecting their important roles in epigenetic reprogramming and in the regulation of tissue-specific gene expression in psychosis. These findings support the hypothesis that a generalized alteration of the DNA-demethylation network may be involved in the pathogenesis of SZ (Dong et al., 2012; Gavin et al., 2012; Grayson and Guidotti, 2013).

If these methylation/demethylation related abnormalities are confirmed in future studies, it would provide insight into the epigenetic abnormalities underlying SZ and suggest clues to the underlying mechanisms involved in the development of SZ, especially in the vast majority of subjects who do not appear to have significant genetic abnormalities contributing to risk for the disease.

c) 5-HMC enrichment at the GAD67 and BDNF IX promoters in the brain of psychotic patients

A map of the genome-wide distribution of 5-HMC suggests that 5-HMC is primarily present at CG dinucleotides located in the bodies (particularly exons) of actively expressed genes (Lister et al., 2013). However, 5HMC has also been found in CpG islands located in enhancer and proximal transcriptional start sites of active genes where it may have an important role in transcription (Dong et al., 2012, Lister et al., 2013). Because the majority of the studies on GAD67 and BDNF promoter methylation have focused on establishing enrichment in 5-MC while ignoring the levels of 5-HMC, we studied whether specific promoter regions of GAD67 or BDNF-IX, where mRNA expression is downregulated in the brains of psychiatric subjects, show a change in 5-MC and/or 5-HMC at their CpG rich promoter sites. For these experiments, Gavin et al. 2012, and Dong et al. (2012) used 5MC or 5HMC antibodies the specificity of which was validated by the TET-assisted bisulfite pyrosequencing method (Zhubi et al. 2013). As shown in **Fig 3**, Gavin et al.(2012), and Dong et al. (2012) found significantly higher 5HMC levels for the GAD67 gene at -537 to -415 bp and at -145 to + 21 bp in psychotic patients vs controls. In the same patients these Authors found an increase of 5HMC at the BDNF- IX abcd promoter at -60 + 50 bp in psychotic patients but not within BDNF exon IX at + 1185 to + 1305 bp. 5MC was also increased at BDNF promoter IX at -60 to + 50 bp in psychotic patients. However, there were no differences in 5MC at either the GAD67 promoter site or the BDNF-IX exon site. Interestingly, there was a significant negative correlation between GAD67 and BDNF mRNA level and 5HMC enrichment at these promoters (Dong et al., 2012, Gavin et al.,

2012,). Hence, the 5HMC promoter enrichment may be regarded as an intermediate product of DNA demethylation and also as a repressive epigenetic DNA mark at actively expressed gene promoters on its own right (Dong et al., 2012).

3) Epigenetic alterations in peripheral blood cells of SZ patients

a) Evidence for altered DNA methylation

In SZ post-mortem brain, DNA methylation alterations in the promoters of reelin, GAD, COMT (catechol methyl transferase), BDNF, GCR and other genes have been reported. (Abdolmaleky et al., 2005, 2006; Dong et al., 2012; Gavin et al., 2012; Grayson and Guidotti, 2013; Iwamoto et al., 2005; Mill et al., 2009; Zhang et al., 2013).

Although DNA methylation profiles are considered to be different from that of brain, investigations of epigenetic alterations in peripheral tissue samples obtained from SZ patients have revealed both altered global and gene specific DNA methylation profiles associated with the disease. It has been reported that promoters in peripheral blood cells are globally hypomethylated in SZ patients (Melas et al., 2012; Nishioka et al., 2012; Shimabukuro et al., 2007), but other studies fail to report global promoter methylation differences between SZ patients and controls in peripheral blood cells (Bromberg et al., 2008; Dempster et al., 2011). Gene specific genome-wide DNA methylation studies have indicated hypermethylation of genes such as HTR1A (Ghadirivasfi et al., 2011), S-COMT (Melas et al., 2012) and BDNF promoter 1 (Ikegame et al., 2013) and hypomethylation of the HTR1E, COMTD1 (Nishioka et al., 2012), and MB-COMT (Nohesara et al., 2012) genes in peripheral blood cells of SZ patients.

A number of top-ranked differentially methylated genes, previously implicated in SZ or BP disorder, have been identified in the peripheral blood cells of affected monozygotic twins discordant for SZ or BP disorder. The differentially methylated genes in SZ discordant monozygotic twins include ST6GALNAC1 (a member of the sialyltransferase family of molecules, involved in protein glycosylation), GPR24 (G protein-coupled receptor-24), CTNNA2 (alpha catenin gene-2), as well as other genes known to influence embryonic and nervous system development and cell signaling that have been previously identified as susceptibility genes for psychosis (Dempster et al., 2011). In a recent study (Liu et al., 2013), sixteen CpG sites with hyper- or hypomethylation were identified in the blood of SZ patients. Eleven of these CpG sites significantly correlated with the “reality distortion symptoms” of SZ. Pathway analyses showed that the most significant biological function of the differentially methylated genes -CD224, LAX1, TXK, PRF1, CD7, MPG, and MPO genes - was direct activation of inflammatory cellular responses.

The emergence of inconsistency in the results of different DNA methylation studies may be partly attributed to the fact that steady-state DNA methylation is a relatively fast dynamic process maintained by an equilibrium between DNA methylation and demethylation processes (Grayson and Guidotti, 2013). DNA methylation/demethylation balance has been shown to be affected by the interplay between genetic and environmental factors and has been shown to differ under various conditions including age (Bocklandt et al., 2011;

Christensen et al., 2009; Liu et al., 2013), gender (Liu et al., 2010), race, diet, and lifestyle (Cordero et al., 2013; Lim et al., 2012).

In a recent study, Matrisciano et al. (2013) reported that DNMT1 and DNMT 3a expression in the GABAergic interneurons of mouse frontal cortex and hippocampus is age dependent and is about 10 fold higher at birth than at adulthood (60-90 days of age). Furthermore in this study it was found that DNMT1 and DNMT3a expression in the frontal cortex and hippocampus of offspring born from stressed mothers (PRS mice) was approximately two fold higher at PND 1, 7, 14, and 60, compared to controls. Similar results were obtained for the expression of TET. Importantly, in this mouse study it was demonstrated that the stress-induced increase of brain DNMT expression was associated with a decrease in reelin and GAD67 expression and a concomitant increase binding of DNMT and an enrichment in 5MC and 5HMC at specific CpG –rich regions of reelin and GAD67 promoters.

The impact of nicotine and alcohol abuse on DNA methylation has been specifically studied, and both global changes and changes in specific genes have been reported in various tissues (Ouko et al., 2009; Philibert et al., 2012; Satta et al., 2008). In support of the hypothesis that DNA methylation and its promoting and removing factors contribute to the epigenetic gene expression abnormalities in alcoholism, in a recent study , we find not only a marginal decrease of DNMT1 but also a significant increase of TET1 mRNA expression (Guidotti et al. 2013, Dong et al. 2012, Gavin et al. 2012) as well as increased 5HMC at GAD67 and BDNF promoters in the prefrontal cortex and inferior parietal lobule of psychotic patients who were also chronic alcoholics compared to psychotic patients without an history of alcohol abuse.

Antipsychotics may also alter DNA methylation/demethylation dynamics. For example, it has been reported that haloperidol can reduce the global DNA hypomethylation detected in leucocytes of patients with SZ (Melaset al., 2012). Furthermore, global or specific DNA methylation levels may be regulated by the bio-availability of the methyl donor SAM (Tremolizzo et al., 2005) the biosynthesis of which is altered in the brain of SZ patients (Guidotti et al., 2007). An important complexity relates to the discovery that 5MC can be converted into 5HMC by the action of TET enzymes. This methylation mark may represent up to 40% of the methylated DNA in the promoters of active genes and 5HMC cannot be differentiated biochemically from 5MC after bisulfite conversion (Yu et al., 2012).

Given that DNA-methylation/demethylation process is a relative fast dynamic event influenced by several environmental factors, it is not surprising that DNA-methylation patterns in peripheral cells differ among studies due to inevitable differences and limitations in the experimental design. For example the study of Dempster et al. (2011) was limited to only 22 pair of twins and it is plausible that many of the changes identified occur downstream of the disease, resulting from exposure to, perhaps, antipsychotic medications. Some major limitations were also identified in the DNA-methylation studies conducted in leukocytes of SZ patients by Melas et al. (2012). In fact due to the anonymity of the control specimens, the authors were unable to associate their global DNA-methylation status with the characteristics known for the patients, and to control for potential confounding variables. Finally, Liu et al. (2013) were able to correlate altered methylation patterns of inflammatory

response genes in whole blood with symptoms of SZ. One main limitation of the study was that methylation values derived from whole blood presents a mixture of various leukocyte subtypes and as reported by Reinius et al. (2012) methylation differs between leukocyte subtypes, and methylation patterns in whole blood can be different from those detected in specific cell types. Hence, although DNA-methylation signatures in peripheral lymphocytes show promise of serving as important SZ biomarkers in the future, it still remains unclear whether the observed DNA methylation changes represent the cause or the consequence of the disease.

b) DNA methylation pathways altered in the brain of psychotic patients are also altered in peripheral blood lymphocytes

To avoid the limitations encountered in measuring DNA methylation in peripheral blood cells, an important objective of the molecular epigenetic studies in lymphocytes of psychotic patients is to investigate whether the expression of the different components of the DNA methylation/demethylation pathways known to be altered in the brain are also altered in peripheral blood lymphocytes of SZ patients. We previously reported that the lymphocytes of SZ patients show overexpression of DNMT1 and DNMT3a (Zhubi et al., 2009). In a new cohort of SZ patients Auta et al., (2013) confirmed that DNMT1 mRNA expression is increased by approximately 40% in lymphocytes of psychotic patients (**Fig 4**). Also increase by approximately 60% were the levels of TET1,2,3 mRNA.

To elucidate whether the increase of DNMT1 and TET1 expression in peripheral blood lymphocytes of SZ patients correlates with alteration in the expression of epigenetic candidate targets, Auta et al (2013) focused on two genes whose expression, in brain, is under methylation/demethylation control and that in brain play an important role in neurodevelopment, neuroplasticity, and immune function and are also highly expressed in peripheral blood lymphocytes (Ikegame et al., 2013; Michel et al., 2012; Zhang et al., 2013). These genes are GCR and BDNF. Auta et al. (2013) chose to study these genes rather than GAD67 or reelin because the latter are expressed at very low levels in peripheral blood lymphocytes. As indicated above, GCR mRNA expression in brain is under epigenetic control including DNA methylation and hydroxymethylation (Zhang et al., 2013). Auta et al. (2013) chose to study BDNF-IX mRNA expression because the expression of BDNF- IX-abcd (the homolog of human BDNF-IX in mouse, Wong et al., 2010), is regulated by cytosine promoter methylation/demethylation (Gavin et al., 2012; Ikegame et al., 2013; Ma et al., 2009). Hence, in addition to the expression of DNA methylating/demethylating enzymes, we assessed the expression of GCR and BDNF as biomarkers of ongoing epigenetic regulation in lymphocytes of SZ patients.

In the same peripheral blood lymphocyte samples of SZ patients that show an increase of DNMT1 and TET1 expression, Auta et al. (2013) found that the levels of GCR mRNA were decreased by approximately 50% when compared to controls (**Fig4**).

The peripheral blood lymphocyte levels of BDNF-IXabcd mRNA were also decreased by 32%, but the difference was of borderline statistical significance ($p=0.05$). However, BDNF-IX mRNA levels showed a statistically significant negative correlation with DNMT1 mRNA levels in SZ patients (**Fig 5**). These data are consistent with the report that the BDNF

promoter 1 is hypermethylated (Ikegame et al., 2013) and that BDNF proteins (Favalli et al., 2012) are decreased in peripheral blood cells of SZ patients. It is important to note that age, sex, ethnic composition, modest cigarette smoking, or antipsychotic medications were not confounding factors influencing the significant differences between SZ and control samples in this study (Auta et al., 2013).

Taken together the data suggest that the epigenetic abnormalities found in SZ post-mortem brain, such as 1) increased expression of DNMT1 and TET1, the enzymes that methylate/hydroxymethylate promoter cytosines, and 2) decreased GCR and BDNF mRNAs, whose gene promoters are responsive to DNA-methylation/demethylation processes, (Dong et al., 2012; Gavin et al., 2012; Grayson and Guidotti, 2013; Labonte et al., 2012; Lewis et al., 2005; Ma et al., 2009; McGowan et al., 2009; Provencal et al., 2012; Roth et al., 2009; Ruzicka et al., 2007; Sinclair et al., 2011, 2012; Veldic et al., 2005, 2007; Zhang et al., 2013) are also found in the peripheral blood lymphocytes of SZ patients.

4) Pathophysiological consequences of the increased expression of DNMT and TET in the peripheral blood lymphocytes and brain of SZ patients

In agreement with the brain studies, our lymphocyte data suggest that the increased expression of DNMT in SZ patients may be responsible for the decreased expression of BDNF (see also **Fig 5**) and perhaps of GCR by eliciting increased promoter methylation (Auta et al., 2013). The consequences of the increased expression of TET in the lymphocytes of SZ patients are at the present time more difficult to interpret. Dong et al. (2012) found that an increase of TET1 in brain of SZ patients associates with increased conversion of 5-MC into 5-HMC that in turn functions as a rate-limiting DNA-marking step that facilitates repression of transcriptionally active genes. An alternative explanation for the epigenetic role of the increased expression of TET in SZ is that TET may concur with DNMT1 in inducing transcriptional repression by directly acting at GCR or BDNF promoters as a component of a repressor protein complex that includes DNMT, HDAC, MeCP2, MBD3-NURD, and SIN3A (Williams et al., 2011). A role for TET1 and 5-HMC in transcriptional repression in the brain of SZ patients has been recently suggested (Dong et al., 2012). However, this conclusion contrasts with a model in which increased levels of 5-HMC at specific gene body regions (Mellen et al., 2012) facilitates transcription through its effects on chromatin remodeling by favoring an open chromatin conformation [euchromatin]. We conclude that our data confirm brain/lymphocyte homology in the alteration of DNMT (Zhubi et al., 2009) and extend this homology to TET1, suggesting that common environmental or genetic risk factors may be operative in altering the epigenetic components involved in orchestrating transcription of specific genes (i.e., BDNF and GCR) in both lymphocytes and terminally differentiated neurons in cortex (e.g., GABAergic and glutamatergic neurons).

Several lines of evidence support the brain/lymphocyte homology hypothesis: Davies et al. (2012), using an unbiased methylome-wide approach, found that distinct patterns of DNA-methylation across human post-mortem brain and blood were highly correlated. Differentially methylated DNA regions of genes related to neurodevelopment and neuronal differentiation, including BDNF, showed high correlations between blood and brain in the

same individual. Additionally, the broad impact of maternal rearing on DNA-methylation in both the brain and T-cells of Rhesus Macaque (Provencal et al., 2012) supports the hypothesis that the response to aversive environmental insults is system and genome-wide and persists in adulthood. Taken together, correlations between methylated genomic regions and alterations in DNMT1, TET1, GCR, and BDNF expression in blood and brain of psychiatric patients, support the concept that peripheral blood cells can be used to address questions about epigenetic modifications in inaccessible tissues such as the brain.

That the increase of TET in peripheral blood lymphocytes of SZ patients correlates positively with increased endocrine/metabolic measurements (i.e., increased morning ACTH levels and increased triglyceride levels) (Auta et al., 2013) further supports the concept that a common altered epigenetic mechanism may be operative in brain and peripheral tissues in SZ.

5) The potential significance and limitations of epigenetic peripheral blood lymphocyte biomarkers in treatment/clinical research aimed at SZ

The research community has, in recent years, shown limited enthusiasm for studies of epigenetic gene dysregulation in peripheral plasma or blood cells as biomarkers of psychosis or other psychiatric illness. The hesitation is due, in part, to the fact that epigenetic changes relevant to behavior (such as promoter methylation of genes relating specifically to synaptic function and proteins detected in brain regions relevant to behavior) would not be expected to be found in peripheral blood lymphocytes. Although the overall mechanisms of epigenetic regulation may be similar in lymphocytes and brain, it is likely that the genes relating to synaptic function in brain and to behavior may not be expressed peripherally or may not be the target of DNA-methylating/demethylating enzymes in lymphocytes.

Although the expression of DNMT1 and TET1 (Auta et al. 2013) and histone covalent modifications (Fraga et al., 2005) in peripheral blood cells show stable differences among individuals across several weeks, we are aware that, unlike neurons, peripheral blood lymphocytes have a relatively short half-life and epigenetic modifications associated with the psychopathology in these cells may have a limited probability of being maintained intact during each cell division. Recently It has been reported that there are important variations in DNA methylation profiles among different populations of peripheral blood mononuclear cells (i.e., T, NK, B cells, and monocytes) (Reinius et al., 2012). Differences in methylation profiles among different mononuclear cell subtypes and differences in the expression of DNA-methylating/demethylating enzymes could explain the challenges encountered in correlating changes in DNA-methylating/demethylating enzymes in whole peripheral blood lymphocytes with illness manifestations in SZ patients (Auta et al., 2013).

Nevertheless, findings of recent studies that have compared brain and peripheral blood cells (Davies et al., 2012; Provencal et al., 2012) and the findings of our study on DNA-methylation/demethylation pathways in brain and lymphocytes (Grayson and Guidotti 2013) suggest that the overall mechanism of epigenetic regulation may be similar in lymphocytes and brain in the sense that parallel alterations associated with SZ are present in both lymphocytes and brain. The similarities in altered epigenetic components in the lymphocytes

and brains of SZ patients potentially link changes in gene promoter methylation in lymphocytes to a wide variety of brain pathologies including the decreased number of synaptic dendritic spines found in brains of SZ patients (Lewis et al., 2005). It is possible that those patients with decreased spines, decreased grey matter (neuropil), and enlarged ventricles constitute a subtype of SZ patients with global epigenetic pathology (brain and lymphocytes), rather than tissue specific DNA-methylation abnormalities.

Lymphocyte epigenetic studies, if combined with studies involving PET, functional MRI or spectroscopic imaging, psychophysiology, and cognitive function, have the potential for promoting a better understanding of the link between brain and lymphocyte epigenetic processes for future clinical research in SZ.

6) Do epigenetic lymphocyte biomarkers have prognostic value in genetically high risk subjects and in subjects exhibiting the prodrome for SZ?

The first episode of SZ typically occurs during puberty or young adulthood and it is often preceded by a prodrome. Consequently, patients at risk for SZ might be identified by the presence of a prodrome (persistent cognitive deficits, negative symptoms, and attenuated positive symptoms) or by family genetic history (see review by Correll et al., 2010). However, only a small percentage of subjects with prodromal syndrome transition to DSM-IV diagnosed psychosis or SZ although a recent study suggests that subjects who do not meet conversion criteria still have decreased cognitive and social functioning as well as some attenuated positive symptoms (Addington et al., 2011). Research on biological risk factors associated with prodromal syndrome or conversion to psychoses has concentrated on structural and functional (fMRI) brain imaging (Correll et al., 2010), and there have been only a few studies investigating other biological markers of this syndrome. One recent study reported similar changes in stress related brain dopamine response in both SZ patients and high risk prodromal subjects (Mizrahi et al., 2012).

Epigenetic changes associated with SZ may be present at birth because many epigenetic changes occur *in utero* (Grayson and Guidotti, 2013). However, SZ has a fluctuating course with further episodes of psychosis and deterioration after the first episode. DNA-methylation /demethylation of candidate SZ genes is likely to continue postnatally and these changes may be reflected in the prodromal syndrome and may be associated with progressive decreases in cortical grey matter and deteriorating cognitive function typical of the long term course of SZ. Since there is an association between longer duration of untreated psychosis and greater psychopathology and poorer functional outcome (Guo et al., 2010; Perkins et al, 2005; Ziermans et al., 2011), the identification of epigenetic biomarkers of SZ in peripheral blood cells could be invaluable in clinical decisions relating to prophylactic treatment leading to prevention of the prodromal syndrome or the onset of the first episode or of the onset of an impending relapse. The long term deterioration typically observed in the course of SZ might therefore be avoided if the targeted intervention is successful.

7) Implications for Drug Development

There is evidence that clozapine, the most efficacious of the antipsychotics, may influence brain epigenetic processes by increasing histone 3 acetylation levels and by activating promoter demethylation of SZ candidate genes (Guidotti et al., 2011). Similarly, sulpride, which has the structure of the benzamides (histone deacetylase inhibitors), may also facilitate demethylation (Guidotti et al. 2011). In addition, the structurally related drug amisulpride is the second most efficacious antipsychotic (Leucht, et al., 2013). Thus it is possible that DNA methylation/demethylation pathways and chromatin remodeling processes may be useful targets for developing new drugs aimed at preventing or limiting the impact of psychosis.

8) CONCLUSION

If abnormalities in potential epigenetic biomarkers identified in brain can be confirmed in lymphocytes of subjects at high risk for SZ or in individuals with a prodromal syndrome before they progress to first episode SZ, some of the underlying biochemical developmental pathology leading to SZ may be uncovered objectively in living subjects. Identifying biomarkers specific for different diagnostic disease subtypes would have profound implications for prognosis and for targeting patients for more intensive and early intervention by identifying subjects who would be more likely to benefit from specialized treatment in clinical trials. In the future, this research could point the way to individualized new pharmacological treatments for the disease or for arresting the development of SZ and related psychosis in vulnerable individuals.

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Fig 1.

Schematic representation of putative DNA methylation/demethylation pathways. TET: ten-eleven translocation protein; DNMT: DNA-methyltransferase; AID/APOBEC: activation-induced deaminase/apolipoprotein B RNA editing catalytic component; TDG: thymine DNA glycosylase ; BER: Base excision repair; C: cytosine; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine; 5hmU: 5-hydroxymethyluracil.

Fig 2.

Plots of TET1 mRNA and 5-hydroxymethylcytosine (5-hmc) levels in the inferior cortico-parietal lobule of 11 Control (CTR) subjects and 19 Psychotic (PSY) patients (10 SZ and 9 BP). Figure obtained from the data originally reported by Dong et al. (2012). TET mRNA levels were measured with quantitative real time RT-PCR using beta-actin as internal standard. 5hmc levels on genomic DNA were measured by immune-dot-blot analyses.

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The horizontal lines represent median values. (Dong et al 2012).

Fig 3.

Increased 5-hydroxymethylcytosine (5hmC) levels at GAD67 and BDNF Promoters in Psychotic Patients (PSY). Figure obtained from the data originally reported by Gavin et al. (2012), and by Dong et al. (2012).

Binding of 5hmC and 5mC to GAD67 and BDNF IXabcd promoters was measured by immunoprecipitation using specific 5hmC and 5mC antibodies with previously published procedures (Gavin, et al. 2012). We find significantly increased 5hmC in PSY compared with CTR at both regions of the GAD67 promoter examined and a region of the BDNF IXabcd gene around the putative transcription start site (-60 to +50), but not at a location within the BDNF IXd exon (+1185 to +1305). We also find increased 5mC at the -60 to +50 region of the BDNF IXabcd gene, but not at either of the GAD67 locations examined, nor at the +1185 to +1305 site within the BDNF IXd exon. * $P < 0.05$ vs. CTR. Reprinted with permission under the Creative Commons license. For the original report see Dong et al., 2012; doi:10.1038/npp.2011.221 and Gavin et al., 2012 ; doi 10.1038/npp.2011.221.

Fig 4.

DNMT1, TET1, MBD4, APOBEC3A, GCR, BDNF mRNA levels in lymphocytes of schizophrenia patients. mRNA levels were measured with quantitative real time RT-PCR using beta-actin as internal standard.

The cohort is composed of 28 schizophrenia patients and 22 control subjects. * $P < 0.05$ vs. control subjects. Potential confounding variables (age, gender, ethnicity, cigarette smoking, medications) were not a significant factor for any of the mRNA measured.

Modified from the original as reported in Auta et al. (2013); <http://dx.doi.org/10.1016/j.schres.2013.07.030>.

Fig 5.

Pearson's correlation analysis between DNMT1 and BDNF IX mRNA levels in lymphocytes of 17 SZ patients.

mRNA levels were measured with quantitative real time RT-PCR using beta-actin as internal standard.

The original data are reported in Auta et al. (2013); <http://dx.doi.org/10.1016/j.schres.2013.07.030>.