Schizophrenia Research 150 (2013) 312–318

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

DNA-methylation gene network dysregulation in peripheral blood lymphocytes of schizophrenia patients☆

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ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 10 July 2013
Accepted 12 July 2013
Available online 12 August 2013

Keywords:
Brain derived neurotrophic factor (BDNF)
DNA demethylation
DNA methyltransferase (DNMT)
Epigenetic
Glucocorticoid receptor (GCortR)
Ten-eleven-translocation hydroxylase (TET)

ABSTRACT

The epigenetic dysregulation of the brain genome associated with the clinical manifestations of schizophrenia (SZ) includes altered DNA promoter methylation of several candidate genes. We and others have reported that two enzymes that belong to the DNA-methylation/demethylation network pathways—DNMT1 (DNA-methyltransferase) and ten-eleven translocator-1(TET1) methylcytosine deoxygenase are abnormally increased in corticolimbic structures of SZ postmortem brain. The objective of this study was to investigate whether the expression of these components of the DNA-methylation–demethylation pathways known to be altered in the brain of SZ patients are also altered in peripheral blood lymphocytes (PBL). The data show that increases in DNMT1 and TET1 and in glucocorticoid receptor (GCortR) and brain derived neurotrophic factor (BDNF) mRNAs in PBL of SZ patients are comparable to those reported in the brain of SZ patients.
The finding that the expressions of DNMT1 and TET1 are increased and SZ candidate genes such as BDNF and GCortR are altered in the same direction in both the brain and PBL together with recent studies showing highly correlated patterns of DNA methylation across the brain and blood, support the hypothesis that a common epigenetic dysregulation may be operative in the brain and peripheral tissues of SZ patients.

1. Introduction

Schizophrenia (SZ) is a heritable genetically heterogeneous neurodevelopmental disorder that affects approximately 1% of the total population. Although multiple gene mutations, polymorphisms, and copy number variants have been implicated in SZ, these currently established genetic causes of SZ account for only a small percentage of all cases (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Twin studies, evaluating the heritability of SZ morbidity, show a significantly less than 100% concordance rate for the disease in monozygotic twins strongly suggesting that complex epigenomic–genomic interactions may play a critical role in the emergence of SZ pathophysiology (Ptak and Petronis, 2008; Dempster et al., 2011).

Recent studies suggest that epigenetic dysregulation of the brain genome that includes altered DNA promoter methylation in corticolimbic brain regions is associated with the neuropathological manifestations of SZ and related psychiatric disorders (Mill et al., 2008; Guidotti et al., 2011; Grayson and Guidotti, 2013; Houston et al., 2013). In neurons, methylation of cytosines in promoters has been traditionally regarded as a highly stable epigenetic mark that ensures gene expression homeostasis and maintains cell phenotype identity (Goll and Bestor, 2005; Ooi and Bestor, 2008). However, recent studies suggest that this epigenetic DNA marking is highly dynamic. In fact, it is now believed that DNA-methylation of active genes is maintained by the opposite action of DNA-methylation (Moore et al., 2013) and an active DNA-demethylation pathway (base excision repair [BER] pathway) (Zhu, 2009). It has been proposed that demethylation pathway involves hydroxylation of 5-methylcytosine (5MC) to 5-hydroxymethylcytosine (5HMC) by a ten-eleven-translocator (TET) methylcytosine deoxygenase and deamination of 5HMC to 5-hydroxymethyluracil (5HMU) by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like family (APOBEC). The resultant 5HMU could then excised by a DNA glycosylase (i.e., methyl binding domain protein 4, [MBD4]) thereby restoring unmethylated cytosine (Guo et al., 2011). Although the existence of this reaction has been proposed, it is still mechanistically speculative (Shen and Zhang, 2013).

It has been suggested that the DNA-methylation/demethylation dynamic is perturbed in neuropsychiatric disorders such as SZ, bipolar disorder (BP), and autism (Dong et al., 2012; Gavin et al., 2012; Mellén et al., 2012; Grayson and Guidotti, 2013). This hypothesis is supported by data indicating that in the brain of psychotic patients there is: 1) increased expression of DNA methyltransferases (DNMT1 and DNMT3a),

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http://dx.doi.org/10.1016/j.schres.2013.07.030

Table 1
Characteristics of patients and controls.

Subject characteristic	Schizophrenic patients (N = 28)	Non-psychotic controls (N = 21)	Comparison test (Patients vs. Controls)
Age (M)	43.6 ± 10.3	32.3 ± 11.3	T = 3.66 df = 47, P = .001
Sex (M/F) (N)	7/21	10/11	X^2 = 3.871, P = .049
Ethnicity (C/AA/H/O)	6/16/6/0	8/7/1/5	FET = 11.135, P = .009
DSM-IV diagnosis (S/SA)	20/8	NA	NA
Outpatient/inpatient	27/1		
PANSS total (M)	62.4 ± 11.64	NA	NA
RBANS total (M)	60.3 ± 13.7	NA	NA
RBANS sum index scores (M)	333.8 ± 12.2	NA	NA
Current antipsychotics (lst generation/2nd generation/combination of lst and second generation)	2/20/6	NA	
Number of current antipsychotics (one/2 or more)	14/14	NA	NA
Concomitant treatment with antidepressant (Yes/No) (N)	7/21		NA
Concomitant treatment with mood stabilizer (Yes/No) (N)	15/13		NA
Cigarettes smoked/day (M, Range)	11.0 ± 13.9 (0–50)	2.3 ± 5.1 (0–16)	T^U = 3.01, df = 36.1, P = .005
Years a cigarette smoker (M)	15.5 ± 16.6[a]	2.7 ± 7.1	T^U = 3.18, df = 25.5, P = .004

NA=not applicable, M = Mean ± S.D. N = Number of Subjects; Ethnicity C = Caucasian, AA = Black or African American, H = Hispanic, O = Other; Diagnosis S = Schizophrenia, SA = Schizoaffective. Outpatient = Outpatients who lived in community residences or partial hospitalization ward attached to the Inpatient hospital campus site. PANSS = Positive and Negative Symptom Scale. RBANS = Repeatable Battery for Assessment of Neuropsychological Status in Schizophrenia. [a]For years of smoking we had data on only 20 of the 28 schizophrenic patients. T = *T*-test, equal variances, T^U = *t*-test unequal variances of patients and controls, with unequal variance calculated df. X^2 = chi-square statistic. FET = Fisher's exact test.

the enzymes that methylate promoter cytosines of SZ candidate genes including reelin (Zhubi et al., 2009; Grayson and Guidotti, 2013), brain derived neurotrophic factor (BDNF) (Gavin et al., 2012), and glucocorticoid receptor (GCortR) (Zhang et al., 2013), and 2) increased expression of TET-1 which is strongly associates with increased levels of 5HMC at glutamic acid decarboxylase 67 (GAD67) and BDNF specific promoter regions (Dong et al., 2012; Gavin et al., 2012). Thus, TET appears to function as a rate-limiting enzyme that facilitates DNA demethylation (Guo et al., 2011; Dong et al., 2012). Abnormalities of these markers in the brain of psychotic patients may favor the appearance of a repressive chromatin conformation at promoter regions responsible for the expression of GABAergic or glutamatergic genes and suggest an important role for DNA methylation/demethylation processes in the pathophysiology of psychosis (Dong et al., 2012; Gavin et al., 2012).

Abnormalities in some of the methylation network components present in the brain of SZ patients are also present in their lymphocytes. As in brain GABAergic neurons (Ruzicka et al., 2007; Kadriu et al., 2012), the lymphocytes of SZ patients have higher levels of DNMT1 and DNMT3a mRNA expression than non-psychotic controls (Zhubi et al., 2009). However, it is virtually unknown whether the alterations of the DNA-demethylating network components found in the brain of SZ patients (Guidotti et al., 2011; Dong et al., 2012; Grayson and Guidotti, 2013) are also present in their lymphocytes.

The objective of the current study is to investigate whether the expression of DNMT1 and TET1 known to be altered in discrete corticolimbic structures of SZ patients are also altered in peripheral blood lymphocytes (PBL). Peripheral biomarker studies may help to reveal whether changes in DNA-methylation/demethylation enzymes correlate with altered DNA-methylation marking of target genes such as glucocorticoid receptor (Sinclair et al., 2011, 2012; Zhang et al., 2013) and BDNF (Lewis et al., 2005; Ma et al., 2009; Roth et al., 2009; Wong et al., 2010; Gavin et al., 2012) whose alternate spliced transcripts are epigenetically downregulated in the brain of SZ patients.

2. Materials and methods

2.1. Subjects

Subjects were 28 patients in the outpatient or inpatient units research clinic of the Nathan Kline Institute (NKI). They had a diagnoses of DSM-IV schizophrenia or schizoaffective disorder (SZ) determined from chart review and research interview. Additional anthropomorphic, fasting, glucose–lipid metabolic, and hormonal measures (cortisol, ACTH) were collected. Non-psychotic controls (NPC) were recruited from the outpatient research volunteer program and from NKI and hospital staff (N = 21). NPC did not have psychiatric symptoms or a diagnosis of psychosis, bipolar disorder, or major depressive disorder. Subjects with brain trauma or symptomatic major neurological illness were excluded. All subjects signed informed consent for protocols approved by the IRB of NKI. The demographic and other characteristics of the subjects are summarized in Table 1.

2.2. Lymphocyte collection

Peripheral blood lymphocytes (PBL) were isolated from a 30–40 ml blood sample with the Ficoll–Paque Plus method using the Amersham Kit (Biosciences 2001–2006). Anticoagulant-treated diluted blood (15 ml blood plus 10 ml phosphate buffered saline [PBS]) was layered on the Ficoll–Paque PLUS (FPP) solution and centrifuged for 30 min at 400 ×*g* at 18–20 °C. Differential cell sedimentation time during centrifugation resulted in the formation of stratified layers containing different cell types. The bottom layer contains erythrocytes that have been aggregated by Ficoll and therefore sedimented completely through the FPP. The layer immediately above that of erythrocytes contains mostly granulocytes that attain sufficient density to migrate through the FPP layer at the osmotic pressure of the FPP. Because of their lower density, blood lymphocytes are detected at the interface between the plasma and the FPP with other slowly sedimenting particles (platelets and monocytes). The lymphocytes are then recovered from the interface and subjected to short washing steps with a balanced salt solution to remove residual platelets, FPP solution, and plasma. Typical purified lymphocyte preparations contain 95 ± 5% mononucleated lymphocytes, 3 ± 2% granulocytes and approximately 0.5% platelets.

2.3. RNA extraction

Total RNA from lymphocytes was isolated using the TRIzol reagent (Life Technologies 15596-026; Life Technologies Corporation, USA; Mannhalter et al., 2000) and further purified using the Qiagen RNeasy Kit (Qiagen, Valencia, CA, USA).

2.4. Real-time polymerase chain reaction (PCR) quantification

Total RNA was converted to cDNA using the Applied Biosystems (USA) High Capacity Archive Kit (4368813). Relative quantitative real-time polymerase chain reaction (qPCR) was performed with the Applied Biosynthesis Real-Time PCR system using Fermentas Maxima SYBR Green/ROX qPCR Master Mix (K0222; Fermentas International