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Brief report

Increased DNA methylation status of the serotonin receptor *5HTR1A* gene promoter in schizophrenia and bipolar disorder

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ABSTRACT

Background: Epigenetic changes may play a role in the etiology of psychotic diseases. It has been demonstrated that the serotonin receptor, 5HTR1A, is implicated in schizophrenia (SCZ) and bipolar disorder (BPD). The aim of this study was to investigate the methylation status of a promoter region of the *5HTR1A* gene in BPD and SCZ patients.
Methods: Our study included 58 BPD and 40 SCZ (DSM-IV criteria) as well as 67 control subjects. DNA was extracted from blood leukocytes and high-resolution melt (HRM) method was used for analysis.
Results: Non-parametric analysis of variance (Kruskal–Wallis) within groups was significant: $H=67.6$; $p<0.0001$. The Mann–Whitney *U*-test showed increased methylation level in both BPD ($Z=-7.4$; $p<0.0001$) and SCZ ($Z=4.2$; $p<0.0001$) compared to controls. No effect either of age or gender by own, was observed. ANCOVA revealed a modest effect of age/gender covariance ($F=3.99$; $p<0.048$).
Limitation: We used a peripheral tissue. The relationship between methylation of blood and brain DNA is not well known. Data need to be replicated in a brain tissue.
Conclusion: We observed increased DNA methylation in the promoter region of the *5HTR1A* gene of SCZ and BPD. This could explain the reported decrease of the receptor expression. The current study supports the growing interest of DNA methylation in psychopathology.
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1. Introduction

A great deal of evidence implicates the serotonin (5HT) system dysfunction in psychiatric diseases, particularly in the two major psychotic diseases, Schizophrenia (SCZ) and Bipolar disorders (BPD) (Lesch, 1998). 5HTR1A is an important subtype of 5HT receptors, widely distributed in the brain, especially in the cortico-limbic regions receiving serotonergic input from the raphe nuclei (Lesch and Gutknecht, 2004). These receptors also serve as somato-dendritic autoreceptors controlling the firing rate of the 5HT neuron (Blier and de Montigny, 1987). Alteration of these receptors has been reported in both BPD and SCZ, mostly (but not always) with decrease in either binding levels of 5HTR1a in the cortex or in 5HTR1A mRNA levels (Gray et al., 2006; Lopez-Figueroa et al., 2004). Genetic studies have also reported association of the *5HTR1A* gene variants in bipolar patients (Kishi et al., 2011). In particular, pharmacogenetic studies reported that one *5HTR1A* gene variant (−1019 C>G), was associated with drug treatment response in both SCZ (Mossner et al., 2009; Reynolds et al., 2006) and BPD (Benedetti et al., 2004). To diversify these studies, another approach for assessing 5HT receptors could be, for example, an epigenetic method of the *5HTR1A* gene, such as DNA methylation of its gene promoter.

DNA methylation is a major epigenetic mechanism which occurs in the context of genome CpG islands by covalently linking CH_3 groups to cytosine molecules, without changing DNA sequence (Gruenbaum et al., 1981). This chemical modification is conserved after cell division and inherited by descendant cells during the successive mitoses (Razin and Riggs, 1980). When present in the gene promoter, this

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covalent modification of DNA can affect gene transcription by altering the accessibility of RNA polymerase and transcription factors (Jaenisch and Bird, 2003). DNA methylation has been offered as an epigenetic explanation for the discordance of monozygote twins for schizophrenia (Petronis et al., 2003). In fact, DNA methylation is implicated in developmental processes such as cell differentiation and thus could contribute to the etiology of neurodevelopmental disorders (Scarano et al., 2005). Embryonic and fetal development is continuously exposed to maternal physiology including drugs and dietary components and some of these are known to affect DNA methylation, leading to recognizable syndromes and subtle deviations in neural development (Singh et al., 2003). It has been widely speculated that epigenetic changes may play a role in the etiology of psychotic illnesses such as schizophrenia (SCZ) and bipolar disorder (BPD) (Abdolmaleky et al., 2004).

Recently, studies showing that DNA methylation could be associated with SCZ and BPD, have dramatically raised (Grayson et al., 2006; Guidotti et al., 2000). Increasing number of genes with altered methylation status in psychotic diseases has been reported so far, and this interest is constantly growing (Pidsley and Mill, 2011).

Although epigenetic studies have been mostly conducted on DNA extracted from affected tissues, i.e. tumors or postmortem brain tissues, blood cells have also proven to be good material for epimutation studies (Cui et al., 2003; Weksberg et al., 2002). Following recent studies, DNA from peripheral blood cells may be useful to reveal epigenetic changes resulting from early embryogenesis (Rosa et al., 2008).

Therefore, due to the importance of this receptor in the serotonin neurotransmission, the present study was aimed to explore the methylation status in the *5HTR1A* gene promoter region in both SZP and BPD populations. By studying the two major psychotic disorders, we also searched for a common signature between BPD and SCZ, as both disorders were shown to share a number of genetic and neurobiological features (Craddock et al., 2005).

2. Materials and methods

2.1. Subjects

The study was approved by the Ethics Committee of the Geneva University Hospitals, and all subjects provided written informed consent. The sample consisted of 165 subjects (58% male): 67 controls; 58 BPD and 40 SCZ. Table 1 summarizes the details of demographic and clinical data of the population.

Both BPD and SZP patients were recruited from consecutive admissions to the psychiatric unit of the University Hospitals of Geneva. All patients met the DSM-IV criteria and were descended from at least two generations of Caucasians. For the diagnosis, trained psychiatrists interviewed patients using the French version of the Diagnostic Interview for Genetic Studies (DIGS) developed by the NIMH. The French version has demonstrated high inter-rate and test–retest reliability for the *DSM-IV* Axis-I disorders (Preisig et al., 1999). Included BPD patients have experienced at least one manic episode (BPD-I), while SZP subjects were characterized, for at least 1-month duration, by either psychotic symptoms (i.e. hallucinations, delusions, catatonia behavior etc.), cognitive impairment (i.e. disorganized thoughts, problem of memory etc.) or negative symptoms (i.e. affective flattering, poor social functioning, alogia, etc.). Healthy controls were recruited from blood donors in Geneva, and were screened for psychiatric symptoms, before inclusion in this study.

Table 1
Demographic and clinical features (SCZ: schizophrenic patients; BPD-1: bipolar disorder type-1).

	n =	Mean age ± sd	% of male	% with psychotic symptoms	% with affective symptoms
SCZ	40	32 ± 8	60	100	30
BPD-1	58	42 ± 10	45	63	100
Controls	67	42 ± 12	73	0	0

2.2. Methods

DNA was extracted from peripheral blood leukocytes by using the Nucleon kit (Bioscience Amersham, GE Healthcare, Glatbrugg, CH). After extraction, DNA was bisulfite-modified using the Epigentek Bisulflash Kit according to manufacturer's instructions (Epigentek Group Inc., USA). For analysis, a CpG-rich region including 17 CG sites in the *5HTR1A* promoter region, identified by the *Ensembl* data bank, was amplified. The amplicon is located upstream and includes the ATG-start of the gene. The following primers were designed to screen the 5′- part of the 5HTR1A promoter gene: F 5′-GTTTTTGAACGCGTTGGATT-3′ forward type and 5′-CCCTAAC-CAAAACTAAACACATCC-3′ reverse type.

PCR reaction was carried out with 80 ng of genomic DNA using the Kappa 2 G Robust Hot Start Kit (Kappa Biosystem) in a final volume of 20 µl containing 1x buffer A (Kappa Biosystem, Cape Town, South Africa), 0.02 mM dNTPs, 7.5 µM of each primer, 0.01 mM Hot Start polymerase and 0.04 µM EvaGreen fluorescent intercalating dye (Invitrogen, Eugene, OR, USA). Amplification conditions were as follow: 95 °C for 3 min, 45 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 20 s.

Methylation status was identified by high-resolution melt (HRM) assay on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia). This technique was proven to be accurate, rapid and sensitive (Wojdacz et al., 2008). Immediately following PCR cycling, the HRM was set from 68 °C to 90 °C, with the temperature rising by 0.2 °C per second. All samples were tested in duplicate. With this assay, the percent of methylation of samples was determined by HRM profile. Commercial methylated and unmethylated DNA standards (Chemicon, Temecula, CA) were used for quantification of unknown samples.

2.3. Statistics

The results are expressed in percentages of methylation. Power was calculated using Rollin Brant's Sample Size Calculator available at http://www.stat.ubc.ca~rollin/stats/ssize/. Assuming the sizes of the samples and their value's distribution, the study had 99% power to detect a significance of 0.001 at α level in three groups, *i.e.*, BPD, SCZ and combined cases. The PASW-18 statistical software

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(former SPSS) was used for statistical analyses. Non parametric statistics were computed: Kruskal–Wallis for analysis for variance between groups, followed by appropriate pairwise Mann–Whitney *U*-tests. We used Spearman correlation test for the effect of age on methylation status, and ANCOVA test for the covariance effect of age and gender. Significance was set at $p<0.05$.

3. Results

Results expressed in percentage of methylation (mean values ± standard deviation) are displayed in Fig. 1. Kruskal–Wallis yields $H=67.6$; $p<0.0001$. Mann–Whitney analysis indicated significant increases in methylation percentages between each diagnostic group *versus* controls: BPD vs controls ($Z=-7.4$; $p<0.0001$); SCZ vs controls ($Z=-4.2$; $p<0.0001$) and all combined cases vs controls ($Z=-7.1$; $p<0.0001$). There was also a significant difference between SZP vs BPD ($Z=-4.2$; $p<0.0001$). Effects of age and gender on methylation status were tested. For age, non parametric Spearman correlation analysis yielded $Z=0.036$; $p=0.98$; for gender, analysis of variance gave $F=2.2$; $p<0.13$. ANCOVA for gender and age gave a modest effect their covariance of $F=3.99$; $p<0.048$. Effects of symptoms were also tested. SCZ subjects were split into affective and non-affective psychoses and mean values were 5.4 ± 2% and 5.5 ± 2%, respectively: *U*-test, not significant. BPD subjects were split into psychotic and non-psychotics and their respective mean values were 8.4 ± 2% and 7.5 ± 3%, respectively: *U*-test, not significant.

4. Discussion

The aim of this study was to assess the methylation status of *5HTR1A* promoter region in SCZ and BPD subjects compared with healthy controls. The study observed significant increase in DNA methylation status of SCZ and BPD patients, compared to healthy controls. This is the first time that such information on a major gene in psychiatry, namely the *5HTR1A*, is reported in these two major psychotic disorders. As expressed above, previous studies have indeed reported changes in 5HT1A receptors levels, particularly a decrease in mRNA expression was reported in these disorders (Lopez-Figueroa et al., 2004; Gray et al., 2006). Consistent with these studies, our observation shows an increase in gene methylation status in both SZP and BPD. Consequently, this increase could result in decreased expression of 5HT1A receptors, as previously suggested (Jaenisch and Bird, 2003; Meltzer et al., 2003). The gene area that we have assessed is a promoter region spanning the initiation site for gene transcription. As a result, increase in methylation could affect the gene transcription by hindering the interaction of the gene and transcription factors or RNA polymerase II.

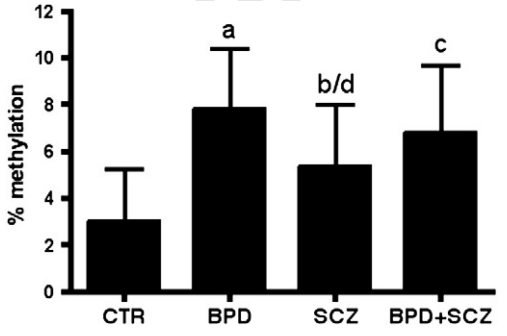

Fig. 1. Graph of the mean values (± sd) for percentage (%) methylation in the different groups. Kruskal–Wallis yields $H=67.6$; $p<0.0001$; Mann–Whitney *U*-tests: a) BPD vs controls: $Z=-7.4$, $p<0.0001$; b) SCZ vs controls: $Z=-4.2$, $p<0.0001$; c) BPD+SCZ vs controls: $Z=-7.1$, $p<0.0001$; and d) SCZ vs BPD: $Z=-4.2$, $p<0.0001$.

Interestingly, the two diagnostic categories show an increase of methylation percentage in this region, albeit a small advantage for the BPD. This suggests that this epigenetic process affects both SCZ and BPD categories of psychotics. The possibility that gene variations and expression are shared between these two major psychoses is currently debated, thanks to data from molecular genetics (Craddock et al., 2009). Actually, the process could overlap a wide spectrum of psychiatric diseases, including major depression disorder (MDD). Recently, increased methylation of the promoter region of the 5HT1A receptor gene in the frontal cortex of MDD subjects was reported (Albert et al., 2008). This increased methylation was interpreted as being indicative of decreased expression of the prefrontal cortex 5HT1A receptor by the authors. There is, however, dispute on the decrease of the 5HT1a receptors density, especially in schizophrenia and some authors have reported increase or no change in protein levels (Tauscher et al., 2002; Cruz et al., 2004). According to Gray et al., these discrepancies are probably due to heterogeneities in cohorts of schizophrenia subjects, and methodological variations (Gray et al., 2006). In our study, there was no effect either of the age, or of the gender.

This study has used lymphocyte DNA, instead of brain tissue, as would be expected for brain diseases. However, several lines of evidence suggest that blood cells can be successively used for epigenetic studies, either for schizophrenia (Tsujita et al., 1998) or for bipolar disorder (Kuratomi et al., 2008). The latter authors used blood leukocytes to study the differential methylation of X-chromosome in bipolar disorder and lymphoblastoid cell lines were also used to demonstrate aberrant DNA methylation associated with bipolar disorder twins (Kuratomi et al., 2008). In an early study, blood cells were used to identify epigenetic difference between a pair of monozygotes twins discordant for SCZ (Tsujita et al., 1998). From then, a number of laboratories have used blood cells either for global DNA methylation or site-specific DNA methylation studies in psychotic illness (Bromberg et al., 2008). As previously stated, it was argued that blood leukocytes may be useful to reveal epigenetic changes resulting from early embryogenesis, even highlighting inherited epigenetic variation (Rosa et al., 2008). However, before drawing firm conclusion, studies are warranted to correlate DNA methylation data from specific brain regions and blood sources. Therefore, these data should be regarded as a preliminary study, which should be replicated on brain tissue. Besides this, our study has other limitations. The population used was heterogeneous in sample size and in their affective and psychotic symptoms, although we did not observe any difference either between affective and non-affective schizophrenia, or between male

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and female subjects. Moreover, the gene region selected for this study was not highly methylated. Therefore, it is possible that all these factors could impact on false positive observations. However, owing to the growing interest of DNA methylation in psychiatric diseases, these findings remain interesting and innovative, as they contribute to proving the involvement of the epigenome in the psychopathology of the two ill conditions.

In conclusion, this study showed increased levels of DNA methylation of the *5HTR1A* gene in both SCZ and BPD compared to control subjects. Increased methylation status could lead to a lower level of 5HT1a receptors expression previously reported in these diseases and to an altered serotoninergic system. Interestingly, both SCZ and BPD were similarly affected, which is consistent with the partial overlap model of these two major psychotic disorders.

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Conflict of interest

All authors declare that they have no conflict of interest.

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