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Parvalbumin promoter hypermethylation in postmortem brain in schizophrenia

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Parvalbumin promoter hypermethylation in post-mortem brain in schizophrenia

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Abstract

Deficits of brain parvalbumin (PV) are a consistent finding in schizophrenia and models of psychosis. We investigated whether this is associated with abnormal PV gene (PVALB) methylation in the brain in schizophrenia. Bisulfite pyrosequencing was used to determine cytosine (CpG) methylation in a PVALB promoter sequence. Greater PVALB methylation was found in schizophrenia hippocampus, while no differences were observed in prefrontal cortex. LINE-1 methylation, a measure of global methylation, was also elevated in both regions in schizophrenia, although the PVALB change was independent of this effect. These results provide the first evidence that PVALB promoter methylation is abnormal in schizophrenia and suggest that this epigenetic finding may relate to the reduction of PV expression seen in the disease.

Keywords: Schizophrenia, parvalbumin, DNA methylation, post-mortem brain, LINE-1**.**

1. Introduction

It is now well established that there is a dysfunction of GABAergic systems in the brain in schizophrenia. Early post-mortem studies have shown deficits in interneurons in the neocortex and hippocampus [1] reflected by lower density of hippocampal GABA uptake sites [2]. Subsequent confirmation has come from observations of deficits in the GABAergic marker glutamic acid decarboxylase (GAD)-1 mRNA and GAD-67 protein throughout the cortex [3]. These deficits appear to be selective for subtypes of GABAergic neurons, most notably those containing parvalbumin (PV), immunostaining for which is reduced in frontal cortex and hippocampus in schizophrenia [4,5]. It seems likely that these deficits contribute to the cognitive disturbances in schizophrenia [6], although it is conceivable that hippocampal parvalbumin/GABA deficits may result in dopaminergic hyperfunction [2] and thereby contribute to positive symptoms.

The pathogenic mechanisms underlying the PV deficit in schizophrenia are also unclear, although it has been suggested that the GABAergic cells are intact but hypofunctioning [7]. This is consistent with the fact that the PV deficit in certain animal models of the disease appears to be related to a reversible effect of oxidative stress [8]. We have speculated whether the PV deficit might relate to epigenetic changes that could be induced by such environmental influences. One epigenetic factor is that of DNA methylation occurring at cytosine residues in CpG sequences; within promoter sequences this methylation can have major effects on gene expression [9]. There is some evidence for dynamic effects on methylation of the PV gene (PVALB) promoter sequence associated with manganese-induced neurotoxic damage in the mouse hippocampus [10]; also, we recently found a PVALB hypermethylation in the hippocampus of rats undergoing subchronic phencyclidine administration [11], in which a PV immunostaining and mRNA deficits are well-established [12–16]. Additionally, a specific association between elevated PVALB methylation and methamphetamine (METH)-induced psychosis was reported in METH-dependent subjects compared to controls with no history of drug abuse or psychiatric diagnosis [17].

We hypothesise that changes in methylation of the PVALB promoter might relate to PV deficits in schizophrenia. Thus we have determined the methylation status of several CpG methylation sites within this sequence in frontal cortical and hippocampal tissue taken post-mortem from patients with schizophrenia and control subjects. The results were compared with a global measure of DNA methylation, that of LINE-1.

2. Material and Methods

2.1. Post-mortem human brain tissue

A post-mortem brain tissue sample from 15 schizophrenia subjects and 16 age-matched controls was collected at the University of Nottingham; this sample was previously investigated for glutamatergic and GABAergic markers (e.g. Reynolds et al., 1990). Tissues were taken and stored at -70°C in compliance with the UK Human Tissue Act. Details of the sample subjects are provided in Table 1.

2.2. DNA extraction, Bisulphite Conversion and Pyrosequencing

Genomic DNA from human samples was extracted from PFC and hippocampus, using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA), and was bisulphite-modified to convert unmethylated cytosine residues to uracil using the EpiTec Fast DNA Bisulphite Kit (Qiagen) with a calculated mean conversion of 99%. We identified an equivalent DNA sequence to that chosen previously in an animal study [11], in the 5' regions of the human PVALB gene and developed a pyrosequencing method for determination of methylation at each CpG sites within this sequence following bisulphite reaction. The sequence was amplified by PCR using primers, including a biotinylated reverse primer, as follows: 5'-AGTGGAGAGAGAAAGGGAGTA-3' (forward) and 5'-[btn]AACACCAAAAAAAAACCACCTCTAAAATT-3' (reverse) (Eurofins MWG Operon).

PyroMark Q24 CpG LINE-1 sequence-based pyrosequencing was used to quantify methylation at four CpG sites in positions 331 to 318 of LINE-1 (GenBank accession number X58075) (Qiagen).

PCR reactions, amplification conditions and the methylation profile were carried out according to our previous study [11]. The sequencing primer used for PVALB studies was as follows: 5'- ATTAGTTAAGGTTTTTAGATTTGA -3' (Eurofins MWG Operon). Pyrosequence setup and data reading were conducted by PyroMark Q24 2.0.6.20 software (UK). Samples underwent PCR and pyrosequencing in duplicate; any inconsistencies between samples were resolved following further repetition.

2.3. Statistical Analysis

Data obtained from the pyrosequencing were compared by unpaired t test and were considered significantly relevant when $p \leq 0.05$. All the analysis was done using SPSS 20.0 (IBM Corp: Armonk, NY, USA). Variance analysis was used to evaluate possible associations of age and sex of the patients with the methylation levels found.

3. Results

A series of samples of both frontal cortex and hippocampus from 16 control subjects and 15 schizophrenia subjects (Table 1) successfully underwent bisulphite conversion, PCR and pyrosequencing to determine methylation in the LINE-1 and PVALB sequences. All samples demonstrated single PCR bands with no evidence of DNA degradation. A significant effect of diagnostic category on PVALB methylation was found in the hippocampus (F=3.465; p=0.021) but not in the frontal cortex (F=0.715; p=0.591). Figure 1 shows that the effect in the hippocampus reflected increases in methylation in schizophrenia at CpG2 (F=8.250; p=0.008) and CpG4 (F=12.195; p=0.002).

The mean methylation of LINE-1 was highly significantly increased in both frontal cortex (t=2.995; p=0.006) and hippocampus (t=2.786; p=0.009) in schizophrenia (Figure 2). Including the respective LINE-1 methylation results as a covariate in the PVALB analyses above, there were no qualitative differences in the statistical results: methylation at CpG2 and CpG4 in the hippocampus remained significantly elevated in schizophrenia.

Age was significantly different between the two groups but showed no significant correlation with any methylation measure; including it as a covariate also had no substantial influence on the results of the analyses above; differences in LINE-1 methylation remained significant as did hippocampal PVALB methylation at CpG2 and CpG4.

4. Discussion

The major findings from our study indicate a specific increase in PVALB promoter methylation in the hippocampus in schizophrenia which is independent of increases in a measure of global methylation in the brain.

To the best of our knowledge, this is the first study reporting hypermethylation in PVALB promoter in schizophrenia patients; these data are supported by previously identified evidence suggesting that hyperfunctional DNA methylation may be responsible for deficiencies in GABAergic neurotransmission [18,19]. As DNA promoter hypermethylation can contribute to reduced gene expression, we suggest that the well-established reduction in PV expression in the brain in schizophrenia may be related to increased methylation of CpG sites within the gene promoter region. The deficit in PV expression is much greater in the hippocampus [5] than in the cortex [4], which may relate to the fact that a statistically significant hypermethylation was only observed in the hippocampus. We have reported elevated methylation of an equivalent sequence in the PVALB promoter of the hippocampus of rats which have undergone a sub-chronic phencyclidine (PCP) regime, modelling some symptoms of schizophrenia [11] and also find an elevation of CpG 2 methylation in blood-derived DNA in subjects with methamphetamine-induced psychosis [17].

The PVALB promoter region selected spans many transcription factor (TF) binding sites including those for paired box domain gene 5 (PAX5) and cyclic AMP-responsive element (CREB). At CpG2 there is a recognition site for PAX5, which has an important role in regulate the mid-hindbrain organisation during neurodevelopment [20–22], while at CpG4 is spanned by the binding site for CREB which possesses intrinsic histone acetyltransferase activity [23,24] important for gene regulation. It has been demonstrated that methylation can block this binding [25]. Additionally, genome-wide association studies have demonstrated that this TF is associated with schizophrenia [26] and interestingly, increases in DNA methylation of the CREB binding protein gene following clozapine treatment were significantly correlated with clinical improvements in treatment-resistant schizophrenia [23].

Our results reveal hypermethylation in LINE-1 in brain tissue of schizophrenia patients compared to controls. These repetitive elements play an important role in gene expression and may be involved in the regulation of diverse biological processes, including DNA damage and repair, inflammation, immune function, embryogenesis, cell differentiation, cell response to external stimuli and hormonal responses [27], so epigenetic dysfunction in these elements in the brain might be involved in neurodegenerative and psychiatric diseases [28].

The increase in LINE-1 methylation indicates that there may be a global elevation in brain DNA methylation in schizophrenia. It has been reported that in the brain in schizophrenia there is an upregulation of DNA-methyltransferases (DNMT) [29,30], so a LINE-1 hypermethylation found in our study could well be a consequence of this DNMT upregulation. Abnormalities in LINE-1 methylation are seen in association with early life trauma in schizophrenia [31] and in PTSD [32], although such studies inevitably rely on blood-derived DNA.

However, we found that the increase in PVALB methylation was unrelated to the change in LINE-1 methylation, and thus it would appear that the finding in PVALB is an independent effect, perhaps selective to this gene and potentially related to the specific deficit in PV in the brain in schizophrenia. It was not possible to determine PV expression in the samples used in the current study, and thus a direct assessment of the correlation between DNA methylation and gene expression could not be performed.

This study has some further limitations; the sample size was not large and, as it is a post-mortem study, the patients were inevitably mostly elderly. There are other variables associated with post-mortem studies that are difficult to control; these include the post-mortem interval, although modelling this with rat brains at room temperature over 96 hours has demonstrated no effect on DNA methylation [33]. Furthermore, the patients were not drug free and, as it is known that antipsychotic drug administration may have effects on gene methylation [34], we cannot distinguish relationships with disease from effects of drug treatment.

5. Conclusions and Future Perspectives

This is the first evidence for an elevation of DNA methylation in the promoter sequence of PVALB in schizophrenia, consistent with recent findings in both drug-induced psychosis and in an animal model of the disease. This epigenetic effect may underlie the PV deficits seen in both the disease and the PCP model. The PVALB hypermethylation occurs in conjunction with, but independent of, increases in a measure of global DNA methylation in the brain in schizophrenia. Much more needs to be investigated in order to determine the effects of PVALB promoter methylation in schizophrenia and related diseases and animal models. It would be important to determine if the hypermethylation seen in these specific CpG sites is directly related to decreased PV expression in the disease.

Executive Summary

- A deficit of parvalbumin (PV) expression in GABAergic neurons of the hippocampus and frontal cortex is a feature common to schizophrenia.
- Increased methylation of the promoter region of the PV gene (PVALB) is associated with methamphetamine psychosis.
- Equivalent sequence in rat brain DNA also shows increased methylation in the phencyclidine model of schizophrenia.
- We found greater PVALB promoter DNA methylation in hippocampus of post-mortem schizophrenia patients compared to control subjects.
- This increase in methylation is specific to a site within a transcription factor binding sequence.
- We found hypermethylation in LINE-1 in hippocampus and prefrontal cortex of schizophrenia post-mortem brains.
- The changes in PVALB methylation were independent of those in LINE-1.
- This hypermethylation may, through effects on transcription, contribute to the enduring reduction in PV in schizophrenia.

Ethical Conduct of Research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Figure 1.

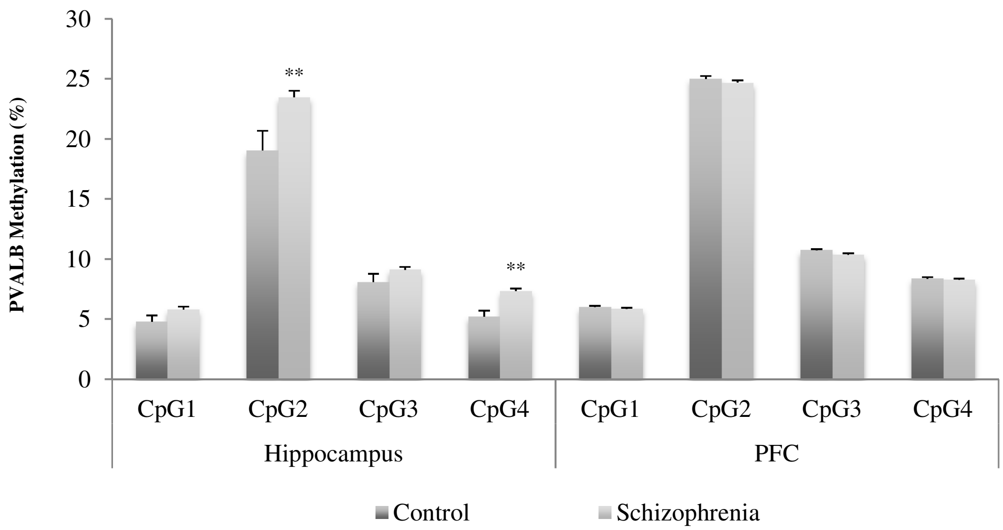

Figure 1. Percentage methylation PVALB in the hippocampus and prefrontal cortex (PFC) in post mortem brains in schizophrenia and controls of Nottingham Series. Values are expressed as the mean ± SEM. (Student's t test, n = 15 schizophrenia and n= 16 controls). **p<0.01.

Figure 2.

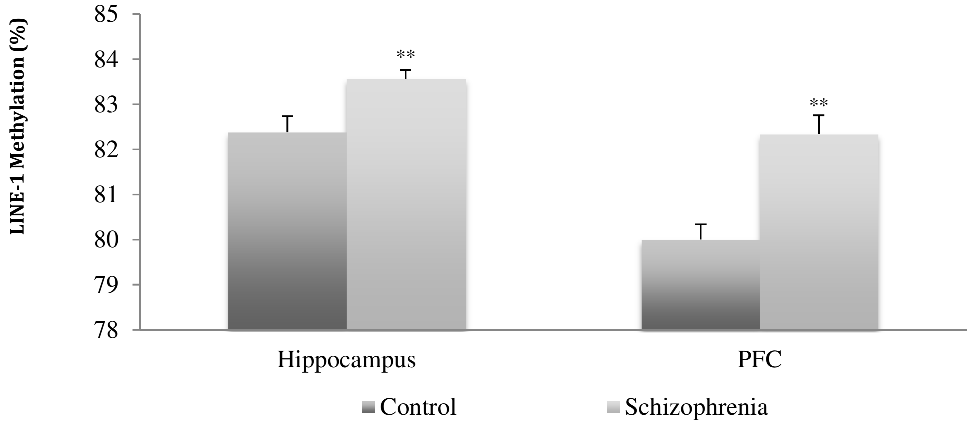

Figure 2. LINE-1 global methylation in the hippocampus and prefrontal cortex (PFC) in post mortem brains in schizophrenia and controls of Nottingham Series. Values are expressed as the mean ± SEM. (Student's t test, n = 15 schizophrenia and n= 16 controls). **$p<0.01$.

Table 1. Description of demographic data of schizophrenia patients and controls

	Control (n = 16)	Schizophrenia (n = 15)	P value
Age (Mean ±SD)	67.25 ± 12.73	52.60 ± 18.18	0.016
Men (%)	11 (68%)	11 (73%)	
PM Hrs (Mean ±SD)	27.68 ± 11.42	27.08 ± 14.05	0.906

*PM Hrs: *Post-mortem* interval of collection of the samples in hours