Sheffield Hallam University

Increased DNA methylation in the parvalbumin gene promoter is associated with methamphetamine dependence

VEERASAKUL, Siriluk, WATIKTINKORN, Paritat, THANOI, Samur, DALTON, Caroline <http://orcid.org/0000-0002-1404-873X>, FACHIM, Helene, NUDMAMUD-THANOI, Sutisa and REYNOLDS, Gavin <http://orcid.org/0000-0001-9026-7726>

Available from Sheffield Hallam University Research Archive (SHURA) at:

https://shura.shu.ac.uk/16612/

This document is the Accepted Version [AM]

Citation:

VEERASAKUL, Siriluk, WATIKTINKORN, Paritat, THANOI, Samur, DALTON, Caroline, FACHIM, Helene, NUDMAMUD-THANOI, Sutisa and REYNOLDS, Gavin (2017). Increased DNA methylation in the parvalbumin gene promoter is associated with methamphetamine dependence. Pharmacogenomics, 18 (14), 1317-1322. [Article]

Copyright and re-use policy

See http://shura.shu.ac.uk/information.html

Sheffield Hallam University Research Archive
http://shura.shu.ac.uk

Increased DNA methylation in the parvalbumin gene promoter is associated with methamphetamine dependence

Siriluk Veerasakul [1,2], Paritat Watiktinkorn [3], Samur Thanoi [1, 2], Caroline F. Dalton[4], Helene A. Fachim[4], Sutisa Nudmamud-Thanoi [1,2*] & Gavin P. Reynolds [2, 4]

[1]Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand

[2]Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand

[3]Synphaet Hospital, Bangkok, Thailand

[4]Biomedical Research Centre, Sheffield Hallam University, Sheffield, S1 1WB, United Kingdom

* Author for correspondence: Tel.: +66 55 964 672

Fax: +66 55 964 770

sutisat@nu.ac.th

Abstract

Aim: The parvalbumin (PV)-containing subgroup of GABAergic neurons is particularly affected in schizophrenia and animal models of psychosis, including after METH administration. We investigated whether METH dependence and METH-induced psychosis may involve an effect on DNA methylation of the *PVALB* promoter.

Materials & methods: The methylation of a *PVALB* promoter sequence was determined in 100 METH-dependent and 102 control subjects using pyrosequencing.

Results: A significant increase in *PVALB* methylation was observed in METH dependence and METH-induced psychosis. No significant effect on long interspersed nucleotide element-1 (LINE-1) methylation, a measure of global DNA methylation, was observed.

Conclusions: These results demonstrate a specific association between elevated *PVALB* methylation and METH-induced psychosis. This finding may contribute to the GABAergic deficits associated with METH dependence.

Keywords: Parvalbumin, DNA methylation, Methamphetamine dependence

Introduction

Methamphetamine (METH), an amphetamine derivative, is a potent psychostimulant and highly addictive drug of abuse. Long term use of METH can result in the emergence of psychosis in a substantial proportion of users, the symptoms of which may be indistinguishable from schizophrenia [1,2]. Acutely, METH causes an increase in dopamine release, which is likely to underlie several of its behavioural effects including the development of drug dependence [3]. In the longer term the drug can have neurotoxic effects on several neurotransmitter systems, including that of GABA [4,5]. This has recent support both from pharmacogenetic studies of association between METH psychosis and polymorphisms in the GABA synthetic enzyme glutamate decarboxylase (GAD) genes [6] and from findings, in an animal model of METH abuse, of deficits in, most strongly, the parvalbumin (PV)-containing subtype of GABergic neurons in frontal cortex and hippocampus [7].

Previous studies have provided substantial evidence for a dysfunction of inhibitory GABAergic interneurons in schizophrenia [8-10]. This is primarily reflected in deficits in PV-containing neurons in the frontal cortex [10] and, particularly, the hippocampus [11]. Enduring deficits of PV are also found in several animal models of schizophrenia, including after sub-chronic administration of the psychotogenic drug phencyclidine [12].

Changes in GABA-related proteins may be regulated by the epigenetic control of gene expression [13]. DNA methylation of cytosine at CpG sequences can influence transcription factor binding to gene promoter sequences and is one epigenetic factor that can be modified by environmental factors, including drugs, and may well contribute to drug dependence [14]. A previous study has shown an increase in DNA methylation in the *pvalb* gene promoter in mouse hippocampus after manganese-induced neuronal damage [15]. We have hypothesized that effects on DNA methylation of the *PVALB* promoter region might reduce PV expression and relate to the PV deficits in schizophrenia and models of psychosis, and recently reported that hypermethylation at one site of the equivalent *Pvalb* gene promoter sequence was observed in rat prefrontal cortex and hippocampus after phencyclidine administration [16].

The present study aimed to investigate whether there was a change in methylation in the promoter sequence of the *PVALB* gene in DNA isolated from blood samples taken from subjects with METH dependence or METH-dependent psychosis.

Materials & Methods

Subjects

METH dependent subjects were recruited from the Central Correctional Institution for Drug Addicts, the Department of Corrections, Ministry of Justice of Thailand. One hundred male METH dependent subjects (age, 30.13±5.09 years) were diagnosed by a psychiatrist based on clinical assessment according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) [17]. They were divided into two sub-groups, METH without psychosis (n=47) and METH-induced psychosis (n=53). The age of onset of METH use was 19.54±6.51 years, duration of METH use was 9.12±5.32 years and duration of abstinence was 19.20±23.99 months. One hundred and two (age, 28.64±6.28), ethnically-matched healthy male subjects with no history of drug abuse or psychiatric diagnosis were recruited. Written informed consent was obtained from all participating subjects. The study was approved by the Human Ethics Committee of Naresuan University.

Determination of DNA methylation

Genomic DNA was extracted from dried blood on FTA® cards (Whatman, Inc., Florham Park, NJ, USA) using QIAamp DNA Mini Kit (Qiagen, UK). Bisulfite conversion was performed to convert unmethylated cytosine residues to uracil using EpiTect Fast Bisulfite Conversion Kit (Qiagen, UK) according to the manufacturer's protocol.

A sequence containing 4 CpGs in the *PVALB* promoter (GRCh38, Chromosome 22 bases 36817384 - 36817327; Figure 1), overlapping the equivalent sequence previously studied in rat [16], was identified and amplified by PCR (figure 2A) using primers, including a biotinylated reverse primer, as follows: 5'-AGT GGA GAG AGA AAG GGA GTA 3' (forward) and 5'-[btn] AAC ACC AAA AAA AAA ACC ACC TCT AAA AT-3' (reverse) (Eurofins MWG Operon, Ebersberg, Germany). PCR reaction was performed using bisulfite-converted DNA as a PCR template by the

PyroMark PCR kit (Qiagen, UK) in a final volume of 25 µl containing 1x Pyro-Mark PCR Master Mix, 1x CoralLoad Concentrate, 0.2 µM of each primer. Amplification conditions were as follows: 95°C for 15 min, 55 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, finally, 72°C for 10 min. Methylation at the four CpG sites was determined with a PyroMark Q24 pyrosequencer (Qiagen, UK) using 10 µl PCR product and employing a sequencing primer, 5'-TTTAGGTTGGAGTGTAATG-3' (Eurofins MWG Operon, Ebersberg, Germany).

For long interspersed nucleotide element-1 (LINE-1), PCR amplification was performed by PCR (Figure 2B), as follows: 5'-TTT GAG TTA GGT GTG GGA TAT AG -3'(forward) and 5'-[btn] AAA AAA TCA AAA AAT TCC CTT TCC -3' (reverse) (Eurofins MWG Operon, Ebersberg, Germany) using PyroMark PCR kit (Qiagen, UK) according to the manufacturer's protocol. Amplification conditions was initiated by 95°C for 15 min and it was followed by 55 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 30 sec, finally 72°C for 10 min. PyroMark Q24 CpG LINE-1 sequence-based Pyrosequencing® technology was used to quantify mean methylation at three CpG sites in positions 331 to 318 of LINE-1 (GenBank accession number X58075) (Qiagen, UK).

Pyrosequence programme set up and reading were performed using PyroMark Q24 2.0.6.20 software (Qiagen, UK).

Statistical analysis

The statistical analysis employed SPSS 17.0 (SPSS Inc., USA). The primary analysis was to test for a difference in methylation across the four CpG sites between the METH and control groups. Secondary exploratory analyses were undertaken to determine if any effect was primarily in the METH psychosis group, and to investigate differences at the individual CpG sites. Statistical differences between groups were identified initially by multivariate ANOVA; secondary analyses employed univariate ANOVA. The correlation of DNA methylation with age was measured by Pearson correlation. The methylation results are expressed as % mean±SD.

Results

The percentage methylation across the four CpG sites in the sample groups is shown in Figure 3. A significant difference in *PVALB* methylation between METH and control groups was identified (F=2.944; p=0.022). This reflected increases in methylation in the METH group that reached significance in CpG1 (F=6.740; p=0.010) and CpG2 (F=8.015; p=0.005). The effect remained in the psychosis subgroup (F=2.666; p=0.035) in which the significant effects at CpG1 and CpG2 remained, with a near-significant difference (F= 2.153; p=0.051) emerging for CpG3. No significant difference was apparent between the non-psychotic subgroup and controls (F=2.153; p=0.077), nor was there a significant difference in *PVALB* methylation between METH groups with and without psychosis (F=1.002; p=0.4). A significant correlation between *PVALB* methylation and age was found in the control group but not in the METH subjects (Table 1).

Mean LINE-1 methylation showed no significant difference between the METH and control groups (75.27±6.16 vs controls: 75.04±5.27; t=0.291; p=0.7), nor between the psychosis subgroup and controls (76.35±6.17; t=1.318; p=0.19).

Discussion

These results revealed that DNA methylation of a sequence in the *PVALB* promoter region was significantly increased in METH dependence, with the effect primarily observed in subjects with a METH-induced psychosis. This finding was in the absence of any apparent effect on a global measure of DNA methylation, that of LINE-1. The correlation of methylation with age seen in the control group is an expected finding; a positive correlation with age is a common feature of DNA methylation [18]. The fact that this effect was lost in the METH group suggests that METH dependence has a disruptive effect on the normal control of DNA methylation in which a resultant hypermethylation masks the influence of age.

It seems likely that the PVALB hypermethylation in METH dependence is associated essentially with the emergence of psychosis, although the lack of statistical significance in the difference in methylation between the psychosis and non-psychosis subgroups means that we cannot be confident in drawing this conclusion.

It is not possible to ascribe a causal effect of METH dependence on this specific hypermethylation of *PVALB* in this cross-sectional study. While it is conceivable that individuals with higher levels of *PVALB* promoter methylation may be susceptible to METH-dependent psychosis, studies in animals suggest *PVALB* hypermethylation seems more likely to be a consequence rather than a cause of METH dependence. The observations that hypermethylation in equivalent sequences in the PV gene promoter of mice born to mothers following Mn-induced neurotoxicity [15], and of rats previously undergoing a subchronic regime of phencyclidine administration [16], both indicate that neuro- and psychoactive substances can have specific effects on PV gene methylation. In particular, the latter study and our findings both support an association between PV gene hypermethylation and psychosis, since phencyclidine administration is used as a model for some of the symptoms of schizophrenia.

An increase in *PVALB* methylation in METH dependent subjects may result in a reduction of *PVALB* gene and PV protein expressions. Previous studies showed that DNA hypermethylation is involved in suppressing expression of related genes whereas DNA hypomethylation is often related to gene over-expression [19]. Correlation between DNA hypermethylation of the serotonin transporter (5-HTT) promoter and the depletion in 5-HTT mRNA expression was found in the brain in schizophrenia [20]. An increase in brain-derived neurotrophic factor (BDNF) methylation is reportedly related to reductions in BDNF mRNA [21]. Therefore, it is conceivable that *PVALB* hypermethylation may result in diminished PV protein expression in schizophrenia and METH-induced psychosis. As mentioned above, animal models of both disorders, as well as human schizophrenia, show deficits in PV-expressing neurons, although a direct association between PV gene hypermethylation and diminished PV expression has yet to be demonstrated.

This study was only undertaken in male Thai subjects, reflecting the limited availability of samples and the sex distribution of METH abuse in the population. This limits the ability to extrapolate to female subjects and, inevitably, other ethnic groups, which needs to be addressed in further work. We recognize that the assessment of blood DNA, rather than DNA deriving from brain tissue, is another substantial but inevitable limitation to our study. However, previous work has demonstrated that hypermethylation of the 5-HTT promoter in saliva of schizophrenia patients paralleled changes in post-mortem brain [20], and others have found concordant changes in methylation of DNA between

blood lymphocytes and brain tissue [22]. Thus differences in DNA methylation in peripheral blood cell may well indicate differences in DNA methylation and its functional consequences in the brain.

Conclusion

This study revealed a significant increase in DNA methylation of the *PVALB* promoter in METH dependence, particularly METH-dependent subjects with psychosis. *PVALB* hypermethylation may well contribute to the reduction of PV protein expression previously reported in an animal model of METH abuse and to GABAergic system deficits in METH dependence. Furthermore, *PVALB* promoter hypermethylation may conceivably contribute to the PV deficits common both to models of psychosis and to human psychotic illness.

Executive summary

Background: Methamphetamine (METH) is a neurotoxin and psychostimulant drug with potent effects on the central nervous system resulting in alterations of neurotransmitter systems such as that of GABA. Parvalbumin (PV)-containing GABAergic interneurons are diminished in schizophrenia and in models of psychosis and drug abuse.

This study was designed to determine whether there is abnormal DNA methylation of the *PVALB* promoter region in subjects with METH dependence and psychosis, as such an effect could contribute to diminished expression of PV.

Results

A significant increase in *PVALB* methylation was found in METH dependence and METH-induced psychosis. This finding was not associated with effects on a global measure of DNA methylation.

Conclusion

Our results show METH dependence and METH-induced psychosis are associated with *PVALB* hypermethylation. This specific increase in *PVALB* methylation may be a consequence of METH dependence and could conceivably contribute to deficits in PV expression that might in turn underlie METH-induced psychosis.

Financial disclosure

This study was supported by the Naresuan University Research Fund for S Nudmamud-Thanoi and S Thanoi. S. Veerasakul was supported by the Royal Golden Jubilee PhD Program. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Acknowledgement

The authors also gratefully acknowledge all patients and volunteers who participated in this study as well as the Central Correctional Institution for Drug Addicts, Bangkok, Thailand. We thank The Biomolecular Sciences Research Centre, Faculty of Health and Wellbeing, Sheffield Hallam University, UK for facility support. Special thanks to Dr. Umarat Srisawat for her technical support.

Ethical conduct of research

The experimental protocols in this study were approved by the Naresuan University Institutional Review Board and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Papers of special note have been highlighted as: * of interest

1 Srisurapanont M, Arunpongpaisal S, Wada K, Marsden J, Ali R, Kongsakon R. Comparisons of methamphetamine psychotic and schizophrenic symptoms: a differential item functioning analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. 35(4), 959-964 (2011).

2 Glasner-Edwards S, Mooney LJ. Methamphetamine psychosis: epidemiology and management. *CNS. Drugs.* 28(12), 1115-1126 (2014).

3 Volkow N, Li TK. The neuroscience of addiction. *Nat. Neurosci*. 8, 1429-1430 (2005).

4 Armstrong BD, Noguchi KK. The neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine on serotonin, dopamine, and GABA-ergic terminals: an in-vitro autoradiographic study in rats. *Neurotoxicology*. 25(6), 905-914 (2004).

5 Jiao D, Liu Y, Li X, Liu J, Zhao M. The role of the GABA system in amphetamine-type stimulant use disorders. *Front. Cell. Neurosci*. 9, 162 (2015).

*** This review describes the crucial role of the GABAergic system in the development of stimulant drug use disorder.**

6 Veerasakul S, Watiktinkorn P, Thanoi S, Reynolds GP, Nudmamud-Thanoi S. Association of polymorphisms in *GAD1* and *GAD2* genes with methamphetamine dependence. *Pharmacogenomics*. In Press Accepted Manuscript (2016).

7 Veerasakul S, Thanoi S, Reynolds GP, Nudmamud-Thanoi S. Effect of methamphetamine exposure on expression of calcium binding proteins in rat frontal cortex and hippocampus. *Neurotox. Res*. 30(3), 427-433 (2016).

8 Guidotti A, Auta J, Davis JM *et al*. Decrease in reelin and glutamic acid decarboxylase$_{67}$ (GAD_{67}) expression in schizophrenia and bipolar disorder. *Arch. Gen. Psychiatry*. 57(11), 1061-1069 (2000).

9 Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology*. 25(1), 1–27 (2001).

10 Beasley CL, Zhang ZJ, Patten I, Reynolds GP. Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol. Psychiatry*. 52(7), 708-715 (2002).

11 Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophr. Res*. 55(1-2), 1-10 (2002).

12 Abdul-Monim Z, Neill JC, Reynolds GP. Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. *J. Psychopharmacol*. 21(2), 198–205 (2007).

13 Grayson DR, Guidotti A. The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. *Neurosychopharmacology*. 38(1), 138-166 (2013).

*** This review describes the critical function of DNA methylation in GABAergic system-related gene expression in psychiatric diseases.**

14 Renthal W, Nestler EJ. Epigenetic mechanisms in drug addiction. *Trends. Mol. Med*. 14(8), 341-350 (2008).

*** A valuable review highlighting the potential importance of epigenetic modification in drug addiction.**

15 Wang L, Shiraki A, Itahashi M *et al*. Aberration in epigenetic gene regulation in hippocampal neurogenesis by developmental exposure to manganese chloride in mice. *Toxicol. Sci*. 136(1), 154-165 (2013).

* **This paper first reported an increase in PV methylation in the brain after exposure to a neurotoxin.**

16 Fachim HA, Srisawat U, Dalton CF *et al.* Subchronic administration of phencyclidine produces hypermethylation in the parvalbumin gene promoter in rat brain. *Epigenomics*. 8(9), 1179-1183 (2016).

* **A recent study identifying hypermethylation in the PV gene in a model of psychosis associated with deficits in PV expression.**

17 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (4th ed.)*. American Psychiatric Association, Washington, DC, 181 (1994).

18 Jung M, Pfeifer GP. Aging and DNA methylation. *BMC. Biol.* 13, 7 (2015).

19 Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biol.* 15(2), R37 (2014).

20 Abdolmaleky HM, Nohesara S, Ghadirivasfi M *et al*. DNA hypermethylation of serotonin transporter gene promoter in drug naïve patients with schizophrenia. *Schizophr. Res*. 152(2-3), 373-380 (2014).

21 Keller S, Sarchiapone M, Zarrilli F *et al*. Increases *BDNF* promoter methylation in the Wernicke area of suicide subjects. *Arch. Gen. Psychiatry*. 67(3), 258-267 (2010).

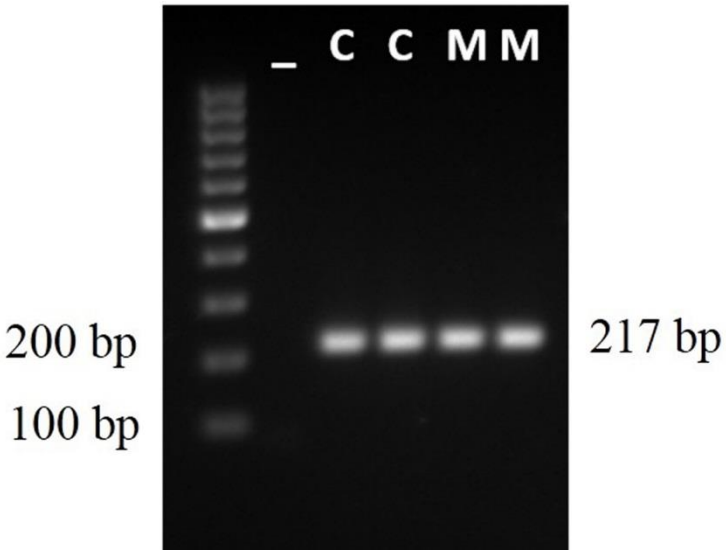
22 Masliah E, Dumaop W, Galasko D, Desplats P. Distinctive patterns of DNA methylation associated with Parkinson disease: identification of concordant epigenetic changes in brain and peripheral blood leukocytes. *Epigenetics.* 8(10), 1030–1038 (2013).

Figures and Figure legends

5'G**CG**CACCTCCTG**CG**TGTCCTTGA**CG**GCAGGTGGACTTTCCAGACCCAGCTGGTGA**CG**C 3’

Figure 1. The DNA sequence in the promoter region of the *PVALB* gene studied. The four methylated CpG sites are shown in bold**.**

A



B

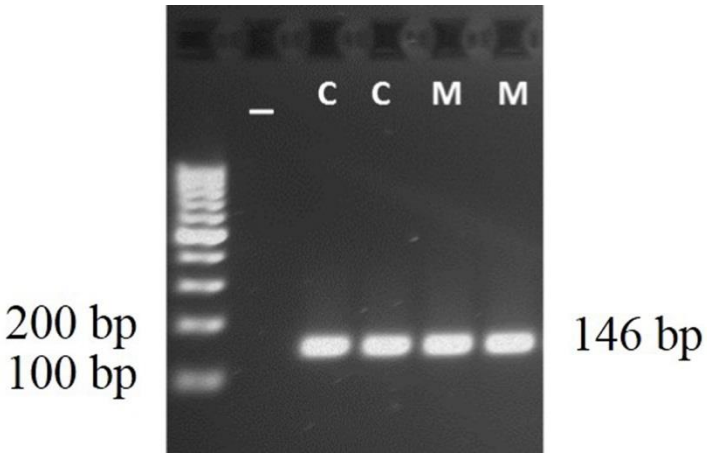


Figure 2. Gel electrophoresis of *PVALB* (A) and LINE-1 (B) PCR products from bisulfite DNA template. –: negative control, C: from control group, M: from METH-dependent group.

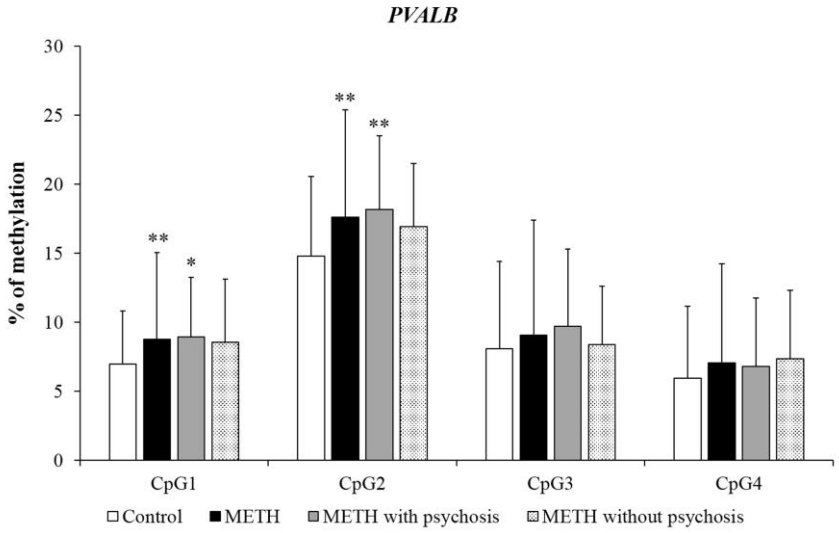


Figure 2. Percentage methylation of four consecutive CpG sites in the *PVALB* promoter sequence of controls, METH-dependent subjects and the subgroups of psychotic or non-psychotic METH-dependent subjects. Columns and bars represent mean values and standard deviations respectively. *p ≤0.05, **p≤0.01 vs control.

Table

Table 1. Correlation of parvalbumin methylation and age in case and control subjects

Groups	Parvalbumin methylation sites				
	CpG1	CpG2	CpG3	CpG4	mean
METH (n=100)					
rho, p	-0.01, 0.91	0.07, 0.49	0.03, 0.79	0.16, 0.10	0.03, 0.73
Control (n=102)					
rho, p	0.37**, 0.000**	0.22**, 0.03**	0.21**, 0.03**	0.16, 0.12	0.33**, 0.001**

Values in bold type show statistical significance after Pearson correlation test.