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DNA Methylation Analysis of *AKT1* Promoter and *HTR2A* Exon-I of Malaysian Schizophrenia Multiplex Families with Lower Cognitive Performance

(Analisis Metilasi DNA Promoter *AKT1* dan Exon-I *HTR2A* pada Keluarga Skizofrenia di Malaysia dengan Prestasi Kognitif Rendah)

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ABSTRACT

Dysfunction of cognitive performance in schizophrenia has been associated with aberrant alteration of DNA methylation of several schizophrenia-risk genes. AKT1 *and* HTR2A *are among the candidate genes for schizophrenia. Their expressions were found reduced in schizophrenia patients. Thus, we aimed to study the methylation status of* AKT1 *promoter and* HTR2A *exon-I in Malaysian schizophrenia patients and their affected family members. In this study, each participant was required to perform Trail Making Test (TMT) part A and B to measure their cognitive performance. Genomic DNA extracted from the peripheral blood of 12 Malaysian schizophrenia families and 12 controls families, was subjected to bisulfite conversion. The methylation status of CpG sites of* AKT1 *promoter at Chr14: 104796054 and* HTR2A *exon-I at Chr13: 46896918 were identified using methylation-specific polymerase chain reaction (MSP). Our results showed that schizophrenia patients performed worse in both TMT-A and B ($p<0.0001$) than healthy controls. The patients also displayed significantly ($p=0.023$) high level of methylation in* AKT1 *promoter compared to controls. Meanwhile, no significant difference ($p=0.248$) in methylation status was observed in* HTR2A *exon-I between schizophrenia and control groups. Therefore, methylation of* AKT1 *promoter in peripheral bloods of patients may involve in cognitive impairment and schizophrenia pathology. In addition, we were able to demonstrate the heritability of DNA methylation status across family members.*

Keywords: Cognitive performance; DNA methylation; schizophrenia; Trail-Making Test

ABSTRAK

Disfungsi prestasi kognitif dalam skizofrenia telah dikaitkan dengan perubahan metilasi DNA pada beberapa gen yang berisiko menyebabkan skizofrenia. AKT1 *dan* HTR2A *adalah antara calon gen kepada skizofrenia. Ekspresi gen-gen tersebut didapati berkurangan pada pesakit skizofrenia. Oleh itu, kertas ini bertujuan untuk mengkaji status metilasi promoter* AKT1 *dan exon-I* HTR2A *pada pesakit skizofrenia di Malaysia dan juga ahli keluarga mereka yang menghidap skizofrenia. Dalam kajian ini, setiap peserta dikehendaki melakukan Ujian Membuat Jejak (TMT) bahagian A dan B untuk mengukur prestasi kognitif mereka. DNA genom yang telah diekstrak daripada darah periferal 12 keluarga skizofrenia di Malaysia dan 12 keluarga kawalan sihat, tertakluk kepada penukaran bisulfite. Status metilasi tapak CpG dalam promoter* AKT1 *di Chr14: 104796054 dan dalam exon-I* HTR2A *di Chr13: 46896918 dikaji dengan menggunakan metilasi-khusus tindak balas rantaian polimerase (MSP). Keputusan menunjukkan bahawa pesakit skizofrenia mendapat keputusan yang teruk dalam kedua-dua bahagian TMT-A dan B ($p<0.0001$) berbanding dengan kawalan sihat. Pesakit skizofrenia juga menunjukkan tahap metilasi promoter* AKT1 *yang tinggi secara signifikan berbanding dengan kawalan ($p=0.023$). Sementara itu, tiada perbezaan yang signifikan ($p=0.248$) diperhatikan dalam metilasi status exon-I* HTR2A *antara kumpulan skizofrenia dan kawalan sihat. Kertas ini menunjukkan bahawa metilasi promoter* AKT1 *dalam darah periferal pesakit skizofrenia mungkin terlibat dalam kecacatan kognitif dan patologi skizofrenia. Tambahan pula, kami dapat menunjukkan bahawa kewarisan status metilasi DNA dalam kalangan ahli keluarga.*

Kata kunci: Metilasi DNA; prestasi kognitif; skizofrenia; Ujian Membuat Jejak

Introduction

Schizophrenia pathology involves the interaction between genetic and environmental factors (Gejman et al. 2010). The likelihood for an individual to develop schizophrenia is higher with the present of family history suffering the same psychiatric disorder (Nishioka et al. 2012; Salleh 2004). Impairments in cognitive performance in schizophrenia patients remain stable throughout the course and these affect their quality of life (Bowie & Harvey 2006; Green et al. 2000). Deficit of processing speed has been proposed as the central feature to cognitive impairment in schizophrenia (Dickinson et al. 2007). Processing speed is defined as the time required by an individual to execute different cognitive operations. Several higher cognitive

functions are rely on this domain (Reichenberg & Harvey 2007). Meanwhile, executive functions involve a set of mental processes consists of goal creation, planning, behavioral programming, and effective performance (Lezak 1987). Cognitive flexibility is part of the executive functions referring to the ability to shift back and forth between several tasks (Monsell 1996).

The pathology of schizophrenia and cognitive impairment may be associated with dysfunction of epigenetic programs (Jones et al. 2013; Lee & Huang 2016). Epigenetic processes such as DNA methylation influence the gene expression at transcriptional level (Gibney & Nolan 2010). The binding of methyl group to cytosine residues leads to transcriptional silencing (Gibney & Nolan 2010). DNA methylation are affected by genetics, age, sex, ethnicity, and economic status (Lam et al. 2012). Dysregulation of DNA methylation has been associated with several psychiatric disorders (Connor & Akbarian 2008) and age-related cognitive dysfunction (Chouliaras et al. 2018; Marioni et al. 2015). Genome-wide DNA methylation analysis identified methylated CpG sites at promoters *AKT1*, *NOS1*, *DNMT1,* and *SOX10* (Wockner et al. 2014). Several studies also found that the methylation in *HTR2A* promoter and exon-I was significantly altered in schizophrenia patients (Abdolmaleky et al. 2011; Cheah et al. 2017; Ghadirivasfi et al. 2011). Moreover, hypermethylated promoters was associated with reduced expression of schizophrenia-risk genes (Lee & Huang 2016).

The AKT1, a serine-threonine protein kinase, is required for gamma-Aminobutyric acid (GABA)ergic neuron development and its activity is highly regulated by dopamine type 2 (D_2) receptor (Souza et al. 2011). Dysregulation of AKT1 signaling due to DNA variants is associated with schizophrenia pathogenesis (Bajestan et al. 2006; Ikeda et al. 2004; Schwab et al. 2005; Thiselton et al. 2008). Reduction in AKT1 expression in lymphocytes, hippocampus and frontal cortex of schizophrenia patients (Emamian et al. 2004), downregulation of AKT1 phosphorylation levels in hippocampus as well as impaired hippocampal plasticity and function in mice with selective deletion of AKT1 (Balu et al. 2012), signify the role of AKT1 in modulating hippocampal neuroplasticity and function.

Meanwhile, the *HTR2A*, is the primary cortical serotonergic receptors that linked to schizophrenia pathophysiology through dopaminergic pathway (Eggers 2013). Serotonin regulates myriad processes in central nervous system (González-Maeso et al. 2007). Stress will induce excessive serotonin stimulation in the anterior cingulate cortex (ACC) and dorsolateral frontal lobe through HTR2A, leading to reduce brain plasticity (Eggers 2013). Moreover, an excessive mesolimbic dopamine activity in normal ACC causes positive symptoms (Schwartz et al. 2012). Reduction in the expression of *HTR2A* and declined of HTR2A density in brain of schizophrenia patients, indicate its role in schizophrenia pathology (Dean & Hayes 1996; Garbett et al. 2008; Hurlemann et al. 2008).

As schizophrenia and cognitive performance are associated with DNA methylation (Chouliaras et al. 2018; Lee & Huang 2016), we examine the relationship between cognitive impairments and DNA methylation patterns of *AKT1* promoter and *HTR2A* exon-I in schizophrenia multiplex families.

Materials and Methods

sample collection

This study involved 12 schizophrenia multiplex families (n = 24) and 12 control families (n = 24). The multiplex families were comprised of 12 pro-bands and one of their affected first-degree relatives. All the patients were recruited from Hospital Permai Johor Bahru, Malaysia. They were diagnosed with schizophrenia as they fulfilled the criteria stated in the 4th edition of Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) and interviewed by experienced psychiatrists using the Mini International Neuropsychiatric Interview (MINI). DSM-IV was chosen due to its reliability in filed study (Frances 2013; Vanheule et al. 2014). The patients participated in this study were absence of comorbidity. Meanwhile, healthy controls with psychiatric comorbidity, generalized anxiety disorder, predecessor history of illicit substance abuse, family history of neurodegenerative or psychiatric disorder, history of intellectual retardation, neurological disorder, or any clinical condition that could affect cognitive performance were excluded from this study. The participants are Malaysian citizens, comprising of three ethnic groups, i.e. Malay, Chinese and Indian. They were 18 years or older and were able to understand instructions of neurocognitive assessment. The demographical characteristics of all participants are presented in Table 1. Written consent was obtained for all participants. The study was approved by the University of Malaya Medical Center Medical Ethics Committee Institutional Review Board.

All participants were required to do Trail- Making Test (TMT) part A and B (Reitan 1992). TMT-A was completed by connecting 25 numbers that scattered on a page in ascending order by drawing a line. Meanwhile, for TMT-B, the subjects draw a line to alternately link the sequentially numbers and letters on a page. TMT is a commonly used test to measures processing speed, complex visual scanning, cognitive flexibility and executive functions (Tombaugh 2004). TMT-A is generally being used to measure processing speed (Salthouse 2011) while TMT-B is for cognitive flexibility and executive functions assessment (Mittal et al. 2013). Meanwhile, the TMT derived scores (B: A and B-A/A) may represent a more useful indicator of executive control with relatively no influence of processing speed (Perianez et al. 2007).

TABLE 1. Demographical data of all subjects

	Scz (n = 24)	HC (n = 24)
	Mean ± SD	Mean ± SD
Age (years)	40.04 ± 9.81	35.91 ± 12.18
Age at onset (years)	29.61 ± 8.60	-
Illness duration (years)	11.56 ± 7.14	-
Education (years)	9.13 ± 2.55	14.10 ± 4.40

HC: healthy controls; n: total number of samples; Scz: schizophrenia; SD: standard deviation

METHYLATION-SPECIFIC POLYMERASE CHAIN REACTION (MSP)

Genomic DNA was extracted from the peripheral blood using QIAmp DNA Blood Mini kit (Qiagen, USA). The methylation of specific single *CpG site* in *AKT1* promoter (Chr14: 104796054) and *HTR2A* exon-1 (Chr13: 46896918) was determined using methylation-specific polymerase chain reaction (PCR) (MSP). Firstly, *AKT1* promoter and *HTR2A* exon-1 regions were identified using ENSEMBL (http://asia.ensembl.org/index.html). Next, based on the desired gene sequences, four sets of primer sequences (Table 2) were designed using MethPrimer (http://wwww.urogene.org/cgi-bin/methprimer/methprimer.cgi) to amplify the two regions. Then, bisulfite conversion was performed using the MethylEasy™ DNA Bisulfite Modification kit (Takara Bio USA, Inc), according to the manufacturer's standard protocol. MSP was performed in a total of 25 µL reaction volume containing 1 µL bisulfite-converted DNA, 0.3 µM of each primer, 12.5 µL of 2X MSP buffer (Episcope® MSP kit, TAKARA Bio Inc, Japan) and 0.6 µL MSP enzyme (Episcope® MSP kit, TAKARA Bio Inc, Japan). The amplification started with 30 s initial denaturation at 95 ºC, followed by 40 cycles of amplification (denaturation at 98 ºC for 5 s, primers' annealing temperature as stated in Table 2 for 1 min and extension at 72 ºC for 1 min) and a final extension at 72 ºC for 7 min. Finally, the PCR products were run on 3% agarose gel and visualized under UV light.

All MSP products were cloned using NEB PCR Cloning kit (New England Biolabs, Inc) and transformed into the NEB 10-beta competent *Escherichia coli* following the manufacturer's instruction. After an overnight incubation at 37 ºC, colonies were screened using colony PCR according to manufacturer's protocol for further confirmation. The PCR products were run on 2 % agarose gel and visualized under UV light. The products of colony PCR were sequenced to verify the results of MSP.

STATISTICAL ANALYSIS

The obtained data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows 14.0 (SPSS Inc., Chicago, IL, USA) with the significant differences at $p<0.05$. The continuous and categorical variables were analyzed by Student's t-test and chi-square/Fisher's exact test, respectively.

RESULTS AND DISCUSSION

COGNITIVE PERFORMANCE

Analysis of the TMT data showed that the patients took significantly ($p<0.001$) longer time to complete TMT-A and B tasks compared to the healthy controls. Besides the TMT direct scores, the patients also obtained significantly ($p<0.05$) higher TMT derived scores (B-A, B-A/A, B:A) than the control group (Table 3). Another study also found that schizophrenia patients performed poorer in both TMT-A and B tasks and the differences in the TMT derived scores between patients and healthy controls were significant (Perianez et al. 2007). This demonstrated that the processing speed, cognitive flexibility and executive function of schizophrenia patients were below average compared with the healthy people.

METHYLATION STATUS OF *AKT1* PROMOTER AND *HTR2A* EXON-I

In total, 8 CpG dinucleotides present in the *AKT1* promoter (Figure 1(a)). However, only one of the CpG dinucleotides was found at the priming sites (Figure 1(b)). These results were further confirmed by sequencing analysis. The majority of the schizophrenia patients and healthy subjects had methylated cytosine residue at this CpG site. However, the detection rate was significantly higher ($p=0.023$) in the patients (Table 4). Nevertheless, sequencing analysis revealed that all of other 7 CpG sites were unmethylated in both patients and healthy control groups (Figure 1(c)).

Our findings were supported by a genome-wide DNA methylation study, which found that *AKT1* was differentially methylated near the promoter region in schizophrenia patients (Wockner et al. 2014). The methylated DNA promoter has been associated with aberrant transcriptional silencing and reduced gene expression (Herman & Baylin 2003; Razin & Cedar 1991). This is thought being regulated by at least two mechanisms. The first one is the methylation of CpG dinucleotides which will prevent the binding of transcription factors to the promoter, thus suppressing the gene transcription (Long et al. 2017).

Secondly, the methylated CpG regions will be identified by the methyl-CpG-binding proteins (MeCPs), for an example MeCP1 (Lewis et al. 1992), which later may recruit histone deacetylases to repress gene regulation (Jones & Laird 1999). Several studies have shown a

TABLE 2. Primers for MSP analyses of the target genes

Genes	Primer sequences (5'→ 3')	Ta (ºC)	Fragment sizes (bp)	
AKT1 (Chr14: 104796033-104796133)				
UM	F	GGGTAGTTTGAAAGTTAATTTAAG	57	101
	R	TAACCCTAACAAAAAAAACCCA		
M	F	GGGTAGTTTGAAAGTTAATTTAAG	57	101
	R	TAACCCTAACAAAAAAAACCCG		
HTR2A (Chr13: 46896918-46897029)				
UM	F	AATTAGGAGTTTTTTGGTGTAGAT	56	134
	R	CTAAATTCCTCCCTCCCTATACA		
M	F	AATTAGGAGTTTTTTGGTGTAGAT	56	134
	R	CTAAATTCCTCCCTCCCTATACG		

bp: base pair; F: forward primer; M: methylated; R: reverse primer; Ta: annealing temperature; UM: unmethylated. The underlined letters indicate cytosine at CpG sites

TABLE 3. Comparison of cognitive performance in schizophrenia patients and healthy control subjects

	Scz (n=24) Mean±SD	HC (n=24) Mean±SD	t	df	p
TMT-A	52.82 ± 23.44	28.05 ± 9.74	4.692	45	<0.001
TMT-B	140.61 ± 94.13	51.81 ± 20.79	4.420	45	<0.001
B-A	87.79 ± 82.36	22.76 ± 17.56	3.648	45	0.001
B-A/A	1.73 ± 1.34	0.93 ± 0.62	2.613	45	0.012
B:A	2.73 ± 1.34	1.93 ± 0.62	2.613	45	0.012

df: degree of freedom; HC: healthy control; *n*: sample size; Scz: schizophrenia; SD: standard deviation; *t*: Student's *t*-test.

TABLE 4. DNA methylation statuses of *AKT1*promoter and *HTR2A* exon-1 in the peripheral blood of schizophrenia patients and healthy subjects

Gene	Methylation status	Scz (*n*=24)	HC (*n*=24)	p		
AKT1 (Chr14: 104796054)						0.023[a]
	Unmethylated	1	(4.2%)	8	(33.3%)	
	Methylated	23	(95.8%)	16	(66.7%)	
HTR2A (Chr13: 46896918)						0.248[b]
	Unmethylated	10	(41.7%)	14	(58.3%)	
	Methylated	14	(58.3%)	10	(41.7%)	

HC: healthy control; *n*: sample size; Scz: schizophrenia. [a] Fisher's exact test; [b] Chi-square test

reduction in AKT1 expression in lymphocytes-derived cell lines (Emamian et al. 2004), peripheral blood mononuclear cells (van Beveren et al. 2012) and brain (Thiselton et al. 2008) of schizophrenia patients. Thus, the reduction in *AKT1* expression in schizophrenia patients might be due to the methylation of *AKT1*. Reduced AKT1 activity may lead to the dysregulation of glycogen synthase kinase-3β (GSK3β) signaling and consequently, negatively affecting the neuronal function and synaptic plasticity (Emamian et al. 2004). In addition, the deficiency of AKT1 was reported to be associated with the modulation of GABAergic interneurons and $GABA_A$ receptor expression, leading to the impairment of hippocampus-dependent cognitive functions (i.e. spatial memory) (Chang et al. 2016).

Serotonin, a known neurotransmitter, activates the PI3K/AKT/mTOR/p70S6K phosphorylation signalling through G-protein-coupled receptors (Zamani & Qu 2012). As shown in an *in vivo* study, reduction of AKT1 activity in schizophrenia patients may negatively impact the activation of HTR2A by serotonin via β-arrestin2/phosphoinositide 3-kinase/Src/Akt cascade (Schmid & Bohn 2010). Therefore, methylation of CpG site in *AKT1* promoter may be associated with schizophrenia pathophysiology. As the patients had a higher rate of methylated cytosine residue at CpG sites of the *AKT1* promoter compared to healthy controls, we hypothesized that *AKT1* promoter methylation might be associated with the impairment of cognitive performance show by the

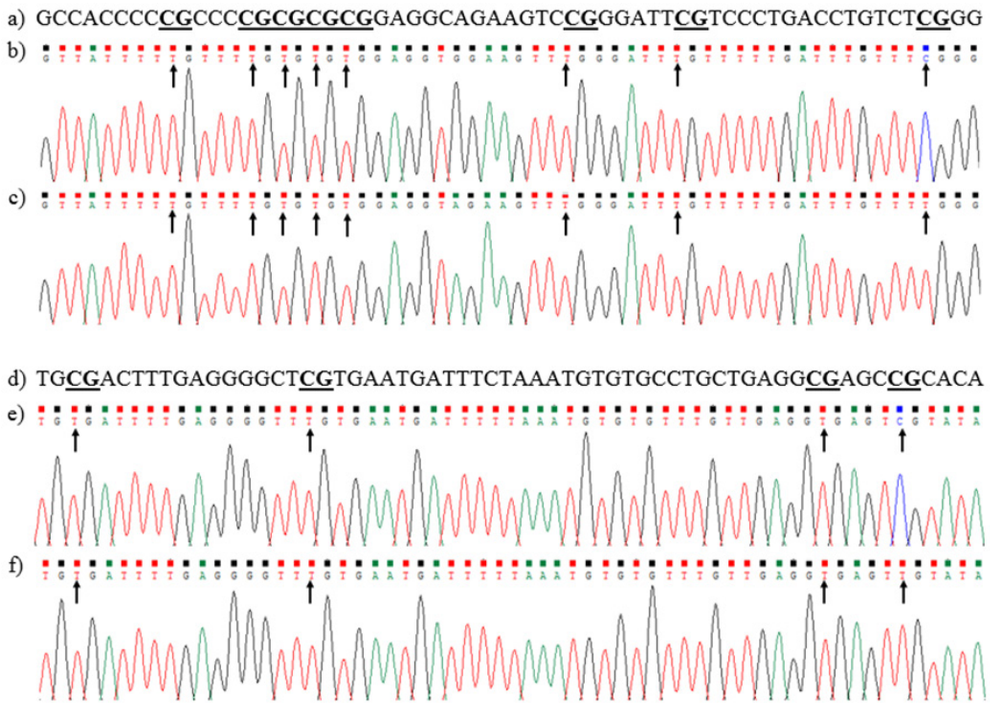

FIGURE 1. Sequence of MSP products. The sequence of (a) AKT1 promoter retrieved from ENSEMBL, (b) methylated CpG site in AKT1 at Chr14: 104796054, and (c) unmethylated CpG site in AKT1 at Chr14: 104796054. The sequence of (d) HTR2A exon-I retrieved from ENSEMBL, (e) methylated CpG site in HTR2A at Chr13: 46896918, and (f) unmethylated CpG site in HTR2A at Chr13: 46896918. The underlined dinucleotides are CpG sites and the arrow shows the cytosine residue after being treated with bisulfite

patients. This could be observed in the current study, where schizophrenia patients with impaired cognitive performance showed methylated CpG at *AKT1* promoter.

For *HTR2A* exon-I, only one CpG dinucleotide of the amplified *HTR2A* could be identified at the priming sites (Figure 1(e)). Although majority of the patients had methylated CpG, the difference was not significant (p=0.248) compared to healthy controls (Table 4). This finding is consistent with the results obtained by Cheah et al. (2017) for this CpG site. Their study using post-mortem frontal cortex brain tissue demonstrated that there was no significant difference in the methylation status at this CpG site in schizophrenia and healthy subjects (Cheah et al. 2017). The cloning and sequencing analysis also showed that the other three CpG dinucleotides present in the first-exonic region of *HTR2A* were unmethylated in both schizophrenia patients and healthy controls (Figure 1(f)). This demonstrated that these CpG sites might not be associated with schizophrenia pathology and reduced cognitive performance in the current study. However, the dysregulation of *HTR2A* expression in schizophrenia patients might be caused by other factors such as promoter-region-hypermethylation or genetic variants. An inverse correlation between *HTR2A* expression and its promoter methylation level was observed by Abdolmaleky et al. (2011). Besides that, several studies found a significant association of single nucleotide polymorphisms (SNPs) rs6311 and rs6313 in schizophrenia patients (Gu et al. 2013; Sujitha et al. 2014; Tee et al. 2010; Williams et al. 1996). The SNP rs6311 that located in the promoter region of *HTR2A* is proposed to cause reduced promoter activity and consequently may affect the gene transcription (Myers et al. 2007). Meanwhile, SNP rs6313 in the first exonic region of *HTR2A* may lead to reduce mRNA levels and thus, deficient protein synthesis (Myers et al. 2007). Deregulation of *HTR2A* expression caused by promoter hypermethylation and genetic polymorphisms may lead to impaired cognitive performance in schizophrenia. This is because, as activation of HTR2A in the prefrontal cortex was able to enhance the GABAergic neuron activity (Abi-Saab et al. 1999; Shen & Andrade 1998), hence deregulation of *HTR2A* expression may affect working memory functioning regulated by these neurons (Volk & Lewis 2005). However, the TMTs used in the current study did not assess working memory of the subjects. This may explain the possibility of no association between *HTR2A* exon-I methylation and patients' impaired processing speed, cognitive flexibility and executive functions.

The methylation patterns of *AKT1* promoter as well as *HTR2A* exon-I were compared among family members. Majority of schizophrenia family members shared similar DNA methylation status in *AKT1* promoter and *HTR2A* exon-I (Table 5). Another study also showed that the methylation status of *HTR2A* at first exonic region was similar between the patients and their first-degree relatives (Ghadirivasfi et al. 2011). Moreover, unaffected family

TABLE 5. Number of families with family members with the same methylation status

Gene	Methylation statuses	Scz (*n* =12)		HC (*n*=12)	
AKT1 (Chr14: 104796054)					
	Unmethylated	0	(0.0%)	3	(25.0%)
	Methylated	11	(91.7%)	7	(58.3%)
	Mixed	1	(8.3%)	2	(16.7%)
HTR2A (Chr13: 46896918)					
	Unmethylated	4	(33.3%)	4	(33.3%)
	Methylated	6	(50.0%)	2	(16.7%)
	Mixed	2	(16.7%)	6	(50.0%)

HC: healthy control; *n*: family number; Scz: schizophrenia

members of schizophrenia patients showed a predisposition for the same aberrant alteration of DNA methylation, which might be due to shared genetic or environmental factors (Abdolmaleky et al. 2015). This proved that DNA methylation is heritable.

Several limitations should be considered in evaluating these findings. Firstly, this study was conducted with a very small sample size. The results may not reflect the overall Malaysian schizophrenia population. Secondly, there was limited information on patients' social-demographic factors such as medication histories and their occupation. Antipsychotic medication was suggested as one of the factors that could affect the gene methylation patterns. For an example, drugs such as valproate are believed to decrease the methylation of *HTR2A* exon-1 and lead to increase in gene expression (Abdolmaleky et al. 2015). Thus, taking into consideration of these limitations, further study should be done in a large sample size and the effect of antipsychotic medications on the methylation of *AKT1* promoter as well as *HTR2A* exon-I should be investigated.

Conclusion

In conclusion, our study suggested that the methylation profiles on the studied CpG site of *AKT1* promoter may associate with schizophrenia psychopathology and cognitive impairment. Moreover, inheritance of the altered methylation was observed within the majority of schizophrenia families. Our findings warrant further study involving the association of methylation of *AKT1* promoter with its gene expression as well as their correlation with cognitive performance in schizophrenia patients.

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References

Abdolmaleky, H.M., Pajouhanfar, S., Faghankhani, M., Joghataei, M.T., Mostafavi, A. & Thiagalingam, S. 2015. Antipsychotic drugs attenuate aberrant DNA methylation of DTNBP1 (Dysbindin) promoter in saliva and post-mortem brain of patients with schizophrenia and psychotic bipolar disorder. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 168(8): 687-696.

Abdolmaleky, H.M., Yaqubi, S., Papageorgis, P., Lambert, A.W., Ozturk, S., Sivaraman, V. & Thiagalingam, S. 2011. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. *Schizophrenia Research* 129(2-3): 183-190.

Abi-Saab, W.M., Bubser, M., Roth, R.H. & Deutch, A.Y. 1999. 5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology* 20(1): 92-96.

Bajestan, S.N., Sabouri, A.H., Nakamura, M., Takashima, H., Keikhaee, M.R., Behdani, F., Fayyazi, M.R., Sargolzaee, M.R., Bajestan, M.N., Sabouri, Z., Khayami, E., Haghighi, S., Hashemi, S.B., Eiraku, N., Tufani, H., Najmabadi, H., Arimura, K., Sano, A. & Osame, M. 2006. Association of AKT1 haplotype with the risk of schizophrenia in Iranian population. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics* 141B(4): 383-386.

Balu, D.T., Carlson, G.C., Talbot, K., Kazi, H., Hill-Smith, T.E., Easton, R.M., Birnbaum, M.J. & Lucki, I. 2012. AKT1 deficiency in schizophrenia and impairment of hippocampal plasticity and function. *Hippocampus* 22(2): 230-240.

Bowie, C.R. & Harvey, P.D. 2006. Cognitive deficits and functional outcome in schizophrenia. *Neuropsychiatric Disease and Treatment* 2(4): 531-536.

Chang, C.Y., Chen, Y.W., Wang, T.W. & Lai, W.S. 2016. Akting up in the GABA hypothesis of schizophrenia: AKT1 deficiency modulates GABAergic functions and hippocampus-dependent functions. *Scientific Reports* 6: 33095.

Cheah, S.Y., Lawford, B.R., Young, R.M., Morris, C.P. & Voisey, J. 2017. mRNA expression and DNA methylation analysis of serotonin receptor 2A (HTR2A) in the human schizophrenic brain. *Genes* 8(1): 1-11.

Chouliaras, L., Pishva, E., Haapakoski, R., Zsoldos, E., Mahmood, A., Filippini, N., Burrage, J., Mill, J., Kivimäki, M., Lunnon, K. & Ebmeier, K.P. 2018. Peripheral DNA methylation, cognitive decline and brain aging: Pilot findings from the Whitehall II imaging study. *Epigenomics* 10(5): 585-595.

Connor, C.M. & Akbarian, S. 2008. DNA methylation changes in schizophrenia and bipolar disorder. *Epigenetics* 3(2): 55-58.

Dean, B. & Hayes, W. 1996. Decreased frontal cortical serotonin(2A) receptors in schizophrenia. *Schizophrenia Research* 21(3): 133-139.

Dickinson, D., Ramsey, M.E. & Gold, J.M. 2007. Overlooking the obvious: A meta-analytic comparison of digit symbol coding tasks and other cognitive measures in schizophrenia. *Archives of General Psychiatry* 64(5): 532-542.

Eggers, A.E. 2013. A serotonin hypothesis of schizophrenia. *Medical Hypotheses* 80(6): 791-794.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M. & Gogos, J.A. 2004. Convergent evidence for impaired AKT1-GSK3 β signaling in schizophrenia. *Nature Genetics* 36(2): 131-137.

Frances, A. 2013. Saving normal: An insider's revolt against out-of-control psychiatric diagnosis, DSM-5, big pharma and the medicalization of ordinary life. *Psychotherapy in Australia* 19(3): 14-18.

Garbett, K., Gal-Chis, R., Gaszner, G., Lewis, D.A. & Mirnics, K. 2008. Transcriptome alterations in the prefrontal cortex of subjects with schizophrenia who committed suicide. *Neuropsychopharmacologia Hungarica* 10(1): 9-14.

Gejman, P.V., Sanders, A.R. & Duan, J. 2010. The role of genetics in the etiology of schizophrenia. *Psychiatric Clinics of North America* 33(1): 35-66.

Ghadirivasfi, M., Nohesara, S., Ahmad Khaniha, H.R., Eskandari, M.R., Mostafavi, S., Thiagalingam, S. & Abdolmaleky, H.M. 2011. Hypomethylation of the serotonin receptor type-2A Gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. *American Journal of Medical Genetics Part B* 156B(5): 536-545.

Gibney, E.R. & Nolan, C.M. 2010. Epigenetics and gene expression. *Heredity* 105(1): 4-13.

González-Maeso, J., Weisstaub, N.V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealfon, S.C. & Gingrich, J.A. 2007. Hallucinogens recruit specific cortical 5-HT2A receptor-mediated signaling pathways to affect behavior. *Neuron* 53(3): 439-452.

Green, M., Kern, R.S., Braff, D.L. & Mintz, J. 2000. Neurocognitive deficits and functional outcome in schizophrenia: Are we measuring the 'right stuff'? *Schizophrenia Bulletin* 26(1): 119-136.

Gu, L., Long, J., Yan, Y., Chen, Q., Pan, R., Xie, X., Mao, X., Hu, X., Wei, B. & Su, L. 2013. HTR2A-1438A/G polymorphism influences the risk of schizophrenia but not bipolar disorder or major depressive disorder: A meta-analysis. *Journal of Neuroscience Research* 91(5): 623-633.

Herman, J.G. & Baylin, S.B. 2003. Gene silencing in cancer in association with promoter hypermethylation. *New England Journal of Medicine* 349(21): 2042-2054.

Hurlemann, R., Matusch, A., Kuhn, K.U., Berning, J., Elmenhorst, D., Winz, O., Kolsch, H., Zilles, K., Wagner, M., Maier, W. & Bauer, A. 2008. 5-HT2A receptor density is decreased in the at-risk mental state. *Psychopharmacology* 195(4): 579-590.

Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., Inada, T. & Ozaki, N. 2004. Association of AKT1 with schizophrenia confirmed in a Japanese population. *Biological Psychiatry* 56(9): 698-700.

Jones, M.J., Farré, P., McEwen, L.M., Macisaac, J.L., Watt, K., Neumann, S.M., Emberly, E., Cynader, M.S., Virji-Babul, N. & Kobor, M.S. 2013. Distinct DNA methylation patterns of cognitive impairment and Trisomy 21 in down syndrome. *BMC Medical Genomics* 6: 58.

Jones, P.A. & Laird, P.W. 1999. Cancer epigenetics comes of age. *Nature Genetics* 21(2): 163-167.

Lam, L.L., Emberly, E., Fraser, H.B., Neumann, S.M., Chen, E., Miller, G.E. & Kobor, M.S. 2012. Factors underlying variable DNA methylation in a human community cohort. *Proceedings of the National Academy of Sciences* 109(2): 17253-17560.

Lee, S. & Huang, K. 2016. Epigenetic profiling of human brain differential DNA methylation networks in schizophrenia. *BMC Medical Genomics* 9(3): 217-228.

Lewis, J.D., Meehan, R.R., Henzel, W.J., Maurer-Fogy, I., Jeppesen, P., Klein, F. & Bird, A. 1992. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* 69(6): 905-914.

Lezak, M.D. 1987. Assessment for rehabilitation planning. In *Neuropsychological Rehabilitation*, edited by Meier, M.J., Benton, A.L. & Diller, L. New York: Guilford Press. pp. 41-58.

Long, M.D., Smiraglia, D.J. & Campbell, M.J. 2017. The genomic impact of DNA CpG methylation on gene expression: Relationships in prostate cancer. *Biomolecules* 7(1): 1-20.

Marioni, R.E., Shah, S., McRae, A.F., Ritchie, S.J., Muniz-Terrera, G., Harris, S.E., Gibson, J., Redmond, P., Cox, S.R., Pattie, A., Corley, J., Taylor, A., Murphy, L. Starr, J.M., Horvath, S., Visscher, P.M., Wray, N.R. & Deary, I.J. 2015. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth cohort 1936. *International Journal of Epidemiology* 44(4): 1388-1396.

Mittal, P.K., Mehta, S., Solanki, R.K. & Swami, M.K. 2013. A comparative study of cognitive flexibility among first episode and multi-episode young schizophrenia patients. *German Journal of Psychiatry* 16(4): 130-136.

Monsell, S. 1996. Control of mental processes. In *Unsolved Mysteries of the Mind: Tutorial Essays in Cognition*, edited by Bruce, V. Oxford, England: Erlbaum (UK): Taylor & Francis. pp. 93-148.

Myers, R.L., Airey, D.C., Manier, D.H., Shelton, R.C. & Sanders-Bush, E. 2007. Polymorphisms in the regulatory region of the human serotonin 5-HT2A receptor gene (HTR2A) influence gene expression. *Biol. Psychiatry* 61(12): 167-173.

Nishioka, M., Bundo, M., Kasai, K. & Iwamoto, K. 2012. DNA methylation in schizophrenia: Progress and challenges of epigenetic studies. *Genome Medicine* 4(12): 1-13.

Perianez, J.A., Rios-Lago, M., Rodriguez-Sanchez, J.M., Adrover-Roig, D., Sanchez-Cubillo, I., Crespo-Facorro, B., Quemada, J.I. & Barcelo, F. 2007. Trail making test in traumatic brain injury, schizophrenia, and normal ageing: Sample comparisons and normative data. *Archives of Clinical Neuropsychology* 22(4): 433-447.

Razin, A. & Cedar, H. 1991. DNA methylation and gene expression. *Microbiological Reviews* 55(3): 451-458.

Reichenberg, A. & Harvey, P.D. 2007. Neuropsychological impairments in schizophrenia: Integration of performance-based and brain imaging findings. *Psychological Bulletin* 133(5): 833-858.

Reitan, R.M. 1992. *Trail Making Test: Manual for Administration and Scoring*. Tucson, Arizona: Reitan Neuropsychology Laboratory.

Salleh, M.R. 2004. The genetics of schizophrenia. *The Malaysian Journal of Medical Sciences: MJMS* 11(2): 3-11.

Salthouse, T.A. 2011. What cognitive abilities are involved in trail-Making performance? *Intelligence* 39(4): 222-232.

Schmid, C.L. & Bohn, L.M. 2010. Serotonin, but not N-Methyltryptamines, activates the serotonin 2A receptor *via* a β-Arrestin2/Src/Akt signaling complex *in vivo*. *Journal of Neuroscience* 30(40): 13513-13524.

Schwab, S.G., Hoefgen, B., Hanses, C., Hassenbach, M.B., Albus, M., Lerer, B., Trixler, M., Maier. & Wildenauer, D.B. 2005. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. *Biological Psychiatry* 58(6): 446-450.

Schwartz, T.L., Sachdeva, S. & Stahl, S.M. 2012. Glutamate neurocircuitry: Theoretical underpinnings in schizophrenia. *Frontiers in Pharmacology* 3: 195-205.

Shen, R.Y. & Andrade, R. 1998. 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. *Journal of Pharmacology and Experimental Therapeutics* 285(2): 805-812.

Souza, B.R., Romano-Silva, M.A. & Tropepe, V. 2011. Dopamine D2 receptor activity modulates Akt signaling and alters GABAergic neuron development and motor behavior in Zebrafish larvae. *The Journal of Neuroscience* 31(14): 5512-5525.

Sujitha, S.P., Nair, A., Banerjee, M., Lakshmanan, S., Harshavaradhan, S., Gunasekaran, S. & Gopinathan, A. 2014. 5-Hydroxytryptamine (Serotonin) 2A receptor gene polymorphism is associated with schizophrenia. *Indian Journal of Medical Research* 140(6): 736-743.

Tee, S.F., Chow, T.J., Tang, P.Y. & Loh, H.C. 2010. Linkage of schizophrenia with TPH2 and 5-HTR2A gene polymorphisms in the Malay population. *Genetics and Molecular Research* 9(3): 1274-1278.

Thiselton, D.L., Vladimirov, V.I., Kuo, P.H., McClay, J., Wormley, B., Fanous, A., O'Neill, F.A., Walsh, D., Van den Oord, E.J.C.G., Kendler, K.S. & Riley, B.P. 2008. AKT1 is associated with schizophrenia across multiple symptom dimensions in the Irish study of high density schizophrenia families. *Biological Psychiatry* 63(5): 449-457.

Tombaugh, T.N. 2004. Trail making test A and B: Normative data stratified by age and education. *Archives of Clinical Neuropsychology* 19(2): 203-214.

van Beveren, N.J.M., Buitendijk, G.H.S., Swagemakers, S., Krab, L.C., Röder, C., de Haan, L., van der Spek, P. & Elgersma, Y. 2012. Marked reduction of AKT1 expression and deregulation of AKT1-associated pathways in peripheral blood mononuclear cells of schizophrenia patients. *PLoS ONE* 7(2): e32618.

Vanheule, S., Desmet, M., Meganck, R., Inslegers, R., Willemsen, J., De Schryver, M. & Devisch, I. 2014. Reliability in psychiatric diagnosis with the DSM: Old wine in new barrels. *Psychotherapy and Psychosomatics* 83(5): 313-314.

Volk, D.W. & Lewis, D.A. 2005. GABA targets for the treatment of cognitive dysfunction in schizophrenia. *Current Neuropharmacology* 3(1): 45-62.

Williams, J., Spurlock, G., McGuffin, P., Mallet, J., Nothen, M.M., Gill, M., Aschauer, H., Nylander, P.O., Macciardi, F. & Owen, M.J. 1996. Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. *The Lancet* 347(9011): 1294-1296.

Wockner, L.F., Noble, E.P., Lawford, B.R., Young, R., Morris, C.P.C., Whitehall, V.L.J. & Voisey, J. 2014. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Translational Psychiatry* 4: e339.

Zamani, A. & Qu, Z. 2012. Serotonin activates angiogenic phosphorylation signaling in human endothelial cells. *FEBS Letters* 586(16): 2360-2365.

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