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DETECTION OF DNA METHYLATION OF *DISC1* GENE USING METHYLIGHT TAQMAN® ASSAY

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Introduction

- Genetic functional studies have shown contributions of *DISC1* gene in the pathogenesis of Schizophrenia [1].
- However, the basis of the genetic defect is not yet established [2].
- There has been a shift of emphasis from *DISC1* gene variations to other genetic defects such as copy number and epigenetic [3].
- Epigenetic of *DISC1* has not been well studied [4].

Objective

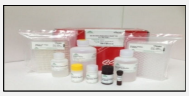
To explore the DNA methylation status of *DISC1* gene in patients with Schizophrenia.

Materials & Methods

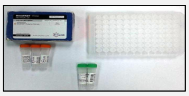
Subjects

- This is a case-control study design.
- Based on data from Carrard et al. (2011) [5], 122 blood samples (1:1 case-control ratio) were needed to give a 95% two-sided confidence level and an 80% power of detection (effect size 0.30).
- 239 Malay subjects (117 Schizophrenia patients; 122 healthy controls) were recruited from the Psychiatry Clinic, Hospital Tengku Ampuan Afzan, Kuantan, Pahang.
- Inclusion criteria:
  1. DSM-IV diagnosis of schizophrenia of at least six months duration.
  2. Symptoms of psychosis not secondary to substance use or neurological disorders.
- Exclusion criteria: Mentally retarded patients and age below 18 years old.
  Biodata and clinical data were gathered from interview and medical records.
- Clinical symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS).
- The study protocol was ethically approved by IIUM Research Ethics Committee (IREC 452) and the study was registered to the National Medical Research Registry of Malaysia (NMRR-10-832-6366).
- The statistical tests used to the variants of the data were: Independent sample t-test, one way-analysis of variance (ANOVA), and bivariate correlation. *p-value* of <0.05 were considered as significant.

Flowchart of DNA Methylation Analysis

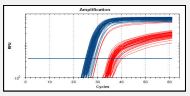


DNA extracted from peripheral blood and bisulfite treated.



Primer *DISC1* was designed and *β–actin* acted as the reference gene [6].

MethyLight Taqman® analysis using CFX96 Real Time PCR Detection System



Percentage of Methylation Ratio (PMR) was calculated

Results

- Successful amplification curve recorded for *DISC1* was limited to 1:729 of DNA serial dilution, and *β-actin* at 1:6501. Standard curve of *DISC1* assay showed 101.2% efficiency with R^2=0.999 (Figure 1) while for *β-actin*, the assay showed 101.8% efficiency with R^2=0.762 (Figure 2). Both assays were sensitive and specific at the DNA methylated region.
- There was no significant differences *p-value*>0.05 in the methylation level of *DISC1* between Schizophrenia patients and healthy controls (Table 1).
- There were no correlation between DNA methylation level of *DISC1* and age (R^2=0.011, p =0.454), age of onset (R^2=0.017, p=0.152), duration of illness (R^2=0.006, p=0.680), and PANSS sub domains (positive symptoms: R^2=0.025, p=0.224; negative symptoms: R^2=0.014, p=0.296; disorganization: R^2=0.019, p=0.851; excitement: R^2=0.005, p=0.242; emotional distress: R^2=0.024, p=0.950) in Schizophrenia.
- There was no significant difference in *DISC1* gene methylation for smoking status and the types of antipsychotic drugs received using independent sample t-test.
- One way-ANOVA was conducted to explore the impact of *DISC1* methylation level in Schizophrenia on BMI status and there was no significant difference for each BMI group.

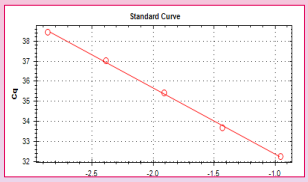


Figure 1: Standard curve of *DISC1*

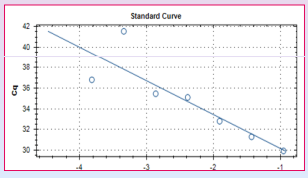


Figure 2: Standard curve of *β-actin*

Table 1: Comparison of PMR value of *DISC1* in between control and Schizophrenia

Gender	Control Mean (SD)	Schizophrenia Mean (SD)	Mean difference (95% CI)	t-statistical (df)	p-value
Male	1.35 (0.05) n=92	1.36 (0.04) n=87	0.005 (-0.02 to 0.01)	-0.772 (177)	0.441
Female	1.36 (0.03) n=30	1.35 (0.08) n=30	0.009 (-0.02 to 0.04)	0.576 (58)	0.567

Independent t-test, $p < 0.05$ is taken as statistically significant at 95% confidence interval.

Conclusions

Study on epigenetic of Schizophrenia, *DISC1* gene is still lacking. Although *DISC1* gene has been one of the positive candidate genes for the pathogenesis of Schizophrenia since 1990 [7], i.e. via gene variations [8], our study suggested that the DNA methylation of *DISC1* gene is most likely not the genetic basic of Schizophrenia.

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