DNA METHYLATION AND COPY NUMBER VARIATION OF THE COMPLEMENT C4A AND CUB AND SUSHI MULTIPLE DOMAINS 1 GENES IN SCHIZOPHRENIA PATIENTS AND HEALTHY CONTROLS

BY

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ABSTRACT

Schizophrenia is a chronic and disabling mental illness with unknown cause and incompletely understood pathogenesis. Evidence from genome-wide association studies (GWAS) and experimental studies had suggested the role of two immune related proteins, the complement C4, coded partly by the *C4A* gene, and the CUB and Sushi Multiple Domains 1 (*CSMD1*). However, there was no available report on the association between schizophrenia and DNA methylation of the *C4A* and *CSMD1* genes. Such study is important because DNA methylation is a modifiable factor that can affect candidate genes' expression and therefore explain the genetic-environment interaction in schizophrenia's pathogenesis. Both genes also have copy number variation (CNV) which can influence gene expression. This study aims to compare the DNA methylation level and the copy number of *C4A* and *CSMD1* genes between schizophrenia patients and healthy controls, and to evaluate their relationship with schizophrenia psychopathology. A total of 183 schizophrenia patients and 212 healthy controls were included in this comparative cross-sectional study. DNA methylation levels and gene copy number were determined from peripheral blood samples using MethyLight^{TM} analysis and droplet digital polymerase chain reaction (ddPCR) respectively. C4 plasma levels was measured using immunoturbidimetry. Psychopathological data of patients were measured using the Positive and Negative Syndrome Scale (PANSS) and the Personal and Social Performance (PSP) scale. Plasma C4 levels were found to be significantly higher in schizophrenia patients compared to controls ($p < 0.001$). While *C4A* DNA methylation levels and copy number were both positively correlated with plasma C4 levels ($p < 0.001$), there was no significant difference in the two variables between patients and controls. The DNA methylation levels of *CSMD1* were significantly lower in schizophrenia patients compared to healthy controls ($p = 0.001$), but its copy number did not differ significantly between the groups. *C4A* deletion and higher *CSMD1* DNA methylation levels were also associated with lesser positive symptom severity ($p = 0.027$). In multivariate analysis, both *CSMD1* DNA methylation levels and plasma C4 levels were significant predictors for schizophrenia. Overall, the results suggested the potential involvement of DNA methylation of *C4A* and *CSMD1* in schizophrenia pathophysiology, particularly in pathways relevant to the positive symptoms. Since DNA methylation may be reversed, this could be a useful target in for the development of new treatment in the future. Further studies are required to identify the underlying mechanism for these findings.

ملخص البحث

الفصام هو مرض عقلي مزمن ومسبب للإعاقة ومجهول السبب ولم يعرف سبب تطوره بشكل كامل. اقترحت الأدلة من دراسات الترابط الجينومي الكامل (GWAS) والدراسات التجريبية دور بروتينين مرتبطين بالمناعة، أولهما عامل جملة المتممة C4، الذي يشفر جزئيًا بواسطة جين *C4A* و ثانيا CUB و (*CSMD1*) Sushi Multiple Domains 1. و لكن لم يوجد هناك تقرير عن العلاقة بين الفصام و مثيلة الحمض النووي لجينين *C4A* و *CSMD1*. الدراسة مثلها مهمة لأن مثيلة الحمض النووي هي عامل قابل للتعديل يمكن أن يؤثر على تعبير الجينات المرشحة وبالتالي يفسر التفاعل بين البيئة والجينات في تطور الفصام. يحتوي كلا الجينين أيضًا على اختلاف في عدد النسخ الذي يمكن أن يؤثر على التعبير الجيني أيضًا. تهدف هذه الدراسة إلى مقارنة مستوى مثيلة الحمض النووي وعدد نسخ الجينين *C4A* و *CSMD1* بين مرضى الفصام والضوابط الصحية ، وتقييم علاقتهم بسيكوباثولوجيا للفصام. تم تضمين ما مجموعه 183 مريضًا بالفصام و 212 من الأصحاء في هذه الدراسة المستعرضة المقارنة. تم تحديد مستويات مثيلة الحمض النووي وعدد نسخ الجين من عينات الدم الطرفية باستخدام تحليل ميثي لايت (MethyLight™) وتفاعل البلمرة المتسلسل الرقمي للقطيرات (ddPCR) على التوالي. تم قياس مستويات C4 في البلازما باستخدام مقياس كدر المناعة. تم قياس البيانات السيكوباثولوجية للمرضى باستخدام مقياس المتلازمة الإيجابية والسلبية (PANSS) ومقياس الأداء الشخصي والاجتماعي (PSP). تم العثور على مستويات البلازما C4 لتكون أعلى بشكل ملحوظ في مرضى الفصام مقارنة بالضوابط ($p < 0.001$). بينما كان كل من مستويات مثيلة الحمض النووي ل*C4A* وعدد نسخه مرتبطين بشكل إيجابي مع مستويات C4 في البلازما ($p < 0.001$)، لم يكن هناك فرق واضح في

هذين المتغيرين بين المرضى والضوابط. كانت مستويات مثيلة الحمض النووي لـ *CSMD1* أقل بشكل ملحوظ في مرضى الفصام مقارنة بالضوابط الصحية ($p = 0.001$) ، لكن عدد نسخه لم يختلف بشكل واضح بين المجموعتين. وأيضًا، ارتبط حذف *C4A* وأعلى مستويات مثيلة الحمض النووي ل*CSMD1* مع أقل حدة أعراض إيجابية ($p = 0.027$). في التحليل متعدد المتغيرات ، كان كل من مستويات مثيلة الحمض النووي ل*CSMD1* ومستويات C4 في البلازما تنبئًا مهمًا للفصام. بشكل عام ، اقترحت النتائج المشاركة المحتملة لميثيل الحمض النووي لـ *C4A* و *CSMD1* في الفسيولوجيا المرضية للفصام، لا سيما في المسارات ذات الصلة بالأعراض الإيجابية. نظرًا لأنه قد يتم عكس مثيلة الحمض النووي ، يكون هذا مفيدًا في التفكير في تطوير علاج جديد في المستقبل. هناك حاجة إلى مزيد من الدراسات لتحديد الآلية الكامنة وراء هذه النتائج.

APPROVAL PAGE

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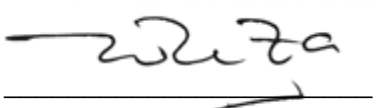

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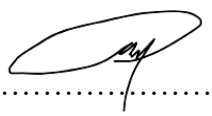

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I dedicated this thesis to all mental illness sufferers. May there be light at the end of your tunnels.

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LIST OF ABBREVIATIONS / SYMBOLS

5-HT2A	Serotonin 2A
ACTB	Beta actin (gene)
α7nAChR	α7 nicotinic acethyl choline Receptor
BDNF	Brain-derived neurotrophic factor (gene)
C4	Complement 4
C4A	Complement 4A (protein), a C4 isomer
C4A	Complement 4A (gene)
C4a	A subunit of complement C4 protein
C4B	Complement 4B (protein), a C4 isomer
C4B	Complement 4B (gene)
C4b	A subunit of complement C4 protein
cDNA	Complementary DNA
CGI	Clinical Global Impression scale
CNS	Central nervous system
CNV	Copy number variation
COMT	Catechol-O-methyltransferase (gene)
COX-2	Cyclooxygenase-2
CpG	Cytosine-phosphate-guanine
CSF	Cerebrospinal fluid
CSMD1	CUB and sushi multiple domains 1 (protein)
CSMD1	CUB and sushi multiple domains 1 (human gene)
Csmd1	CUB and sushi multiple domains 1 (mouse gene)
CYP21	Steroid 21-hydroxylase (gene)
D1	Dopamine 1 (receptor)
D2	Dopamine 2 (receptor)
DAF	Decay accelerating factor
ddPCR	Droplet digital polymerase chain reaction
DISC1	Disrupted in schizophrenia 1 (gene)
DMP	Differentially methylated position
DNA	Deoxyribonucleic acid
DTNBP1	Dystrobrevin binding protein 1 (gene)
ECT	Electroconvulsive therapy
EPS	Extrapyramidal symptoms
EWAS	Epigenome-wide association study
FGA	First-generation antipsychotic
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (gene)

GO	Gene ontology
GWAS	Genome-wide association study
HERV-K	Human endogenous retrovirus K
IDO	Indoleamine 2,3-deoxygenase
IFN	Interferon
IL	Interleukin
IQR	Interquartile range
KMO	Kynurenine monooxygenase
KYNA	Kynurenic acid
MAC	Membrane attack complex
MAM	Methylazoxymethanol acetate
MASP1	MBL-associated serine protease 1
MASP2	MBL-associated serine protease 2
MBL	Mannose-binding lectin
Mbp	Million base pairs
MHC	Major histocompatibility complex
MIA	Maternal immune activation
miRNA	MicroRNA
NMDA	N-methyl-D-aspartate
NRG1	Neuregulin 1 (gene)
NRXN1	Neurexin 1 (gene)
PAMP	Pathogen-associated molecular pattern
PANSS	Positive and negative syndrome scale
PFC	Prefrontal cortex
PMR	Percentage of methylated reference
Poly I:C	Polyinosinic:polycytidylic acid
PRR	Pattern recognition receptor
qPCR	Quantitative polymerase chain reaction
QT interval	The interval between Q and T peaks on an echocardiogram
RELN	Reelin (gene)
RM	Ringgit Malaysia
RNA	Ribonucleic acid
RP	Serine/threonine kinase 19 gene (old name)
r_s	Spearman correlation coefficient
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SGA	Second-generation antipsychotic
sRNA	Small RNA
SNP	Single nucleotide polymorphism
STK19	Serine/threonine kinase 19 (gene)
TDO	Tryptophan 2,3-deoxygenase
TGF-β	Transforming growth factor β

TMS	Transcranial magnetic stimulation
TNF-α	Tumour necrosis factor α
TNX	Tenascin-X (gene)

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Schizophrenia is a chronic and incapacitating mental illness, with 0.64% of the world population being at risk of developing at some point in their life (Moreno-Kustner, Martin, & Pastor, 2018). In 2019, there were estimated over 23.6 million people living with schizophrenia, or around 0.31% of the world population (Global Burden of Disease 2019 Mental Disorders Collaborators, 2022). Despite the relatively low prevalence, schizophrenia causes significant disease burden. It contributes to 12.7 million years (1.5% of total) of life lived with disability worldwide. Economy-wise, developed countries lost between 0.02% to 1.65% of their gross domestic product to schizophrenia (Chong et al., 2016). Schizophrenia onset is during late teens to early adulthood, costing patients their productive years and their futures. In Malaysia, schizophrenia was estimated to cost around RM 428 million in economic burden in year 2015. This figure included all treatment related cost, as well as costs due to absenteeism and unemployment (Teoh et al., 2017). Schizophrenia patients also have lower life expectancy than the general population (Hjorthoj, Sturup, McGrath, & Nordentoft, 2017). This could partly be because they are at higher risk of co-morbidities, substance abuse, violence, and suicide (Subramaniam et al., 2021).

Schizophrenia has complex clinical presentations and high heterogeneity among patients. There is a wide spectrum of signs and symptoms, usually grouped into the positive, negative, and cognitive domains. The contrasting nature of positive and negative symptoms suggests the involvement of different pathologies. Despite better effectiveness and side effect profiles in the newer generations of antipsychotics, the currently available agents do not have significant impact in treating the negative and cognitive symptoms such as lack of motivation, reduced emotional range, memory problems and deficit in executive functions (Haddad & Correll, 2018). While not as prominent as the positive symptoms, the negative and cognitive symptoms are equally as disruptive and disabling (Carbon & Correll, 2014). Therefore, it is important to