UNIVERSITI PUTRA MALAYSIA

METABOLIC PROFILING OF NEUROSPHERES DERIVED FROM EMBRYONIC CEREBRAL CORTEX Ts1Cje MOUSE MODEL FOR DOWN SYNDROME

ERYSE AMIRA BINTI MOHAMED SETH

FPSK(m) 2021 25

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2019

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

METABOLIC PROFILING OF NEUROSPHERES DERIVED FROM EMBRYONIC CEREBRAL CORTEX OF Ts1Cje MOUSE MODEL FOR DOWN SYNDROME

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September 2019

Chair : Cheah Pike See, PhD
Faculty : Medicine and Health Sciences

Down syndrome (DS) is a genetic disorder caused by triplication of human chromosome 21 (Hsa21) and is the most common cause of intellectual disability. Several studies have revealed proliferation deficits and higher proportion of glial cells compared to neurones in the brains of DS humans and mouse models, which were suggested to contribute to intellectual disability. While a vast majority of previous literature has been focused on the molecular aspects, current knowledge on metabolic dysregulations in neural stem and progenitor cells (NSPCs) derived from embryonic Ts1Cje mice is limited. Ts1Cje mouse model for DS has been indispensable in expanding our knowledge on the molecular and cellular mechanisms of the disorder. In this study, embryonic cerebral cortex of Ts1Cje and wild type (WT) mice were isolated at embryonic day 15.5 and cultured in the form of neurospheres. Biolog Phenotype MicroArray (PM) was employed to obtain metabolic profiles for the embryonic Ts1Cje and WT neurospheres. Four types of PM colourimetric assays pre-coated with 367 biochemical substrates, including oxidizable carbon and nitrogen sources, were utilised. Analysis of Biolog PM data using an established statistical pipeline revealed a significant decrease in utilisation of 17 substrates and a significantly higher utilisation of 6 substrates in the Ts1Cje neurospheres compared to the WT neurospheres. A prominent finding is the significantly decreased utilisation of glucose-6-phosphate (G6P) and α-D-glucose in the Ts1Cje neurospheres compared to WT. L-serine, and dipeptides containing histidine and isoleucine were also utilised significantly lower in Ts1Cje neurospheres, whereas glutamate-containing dipeptides were utilised significantly higher in Ts1Cje neurospheres compared to WT neurospheres. G6P is involved in two energy-producing pathways: pentose phosphate pathway (PPP) and glycolysis. To investigate whether intermediates of G6P metabolism can improve generation of the embryonic Ts1Cje neurospheres, neurospheres were supplemented with 6-phosphogluconic acid (6PG) and fructose-6-phosphate (F6P) and assessed after 6 days *in vitro*. The mean diameter of the embryonic Ts1Cje neurospheres was higher when supplemented with 2.0 mM of 6PG compared to no supplement, suggesting that

supplementation with 6PG may rescue the effects of perturbed PPP in the embryonic Ts1Cje neurospheres. On the other hand, there was no significant difference between the Ts1Cje neurospheres supplemented with and without F6P, indicating that there are possibly no alterations in glycolysis. The enzyme activity of glucose-6-phosphate dehydrogenase (G6PDH), which catalyzes the catabolism of G6P in PPP, was also assessed. The G6PDH activity of Ts1Cje neurospheres was generally lower compared to WT neurospheres, but the difference was not statistically significant. Without supplementation of 6PG, G6PDH activity of the embryonic Ts1Cje neurospheres was found to be lower than that of the WT neurospheres. Meanwhile, increasing concentration of 6PG results in a larger difference in G6PDH activity between the Ts1Cje and WT neurospheres, although the differences were not statistically significant. Taken together, these data suggest that alterations in metabolic pathways, particularly PPP, may contribute to defects observed in NSPCs of embryonic Ts1Cje mice. Investigation on the metabolic properties of Ts1Cje embryonic NSPCs enhances our knowledge on underlying dysregulations in NSPCs during early brain development in DS, and may complement previous genomic, transcriptomic and proteomic studies on Ts1Cje mice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PROFIL METABOLIK NEUROSFERA DIPEROLEHI DARIPADA KORTEKS SEREBRUM EMBRIO MENCIT MODEL SINDROM DOWN Ts1Cje

Oleh

ERYSE AMIRA MOHAMED SETH

September 2019

Pengerusi : Cheah Pike See, PhD
Fakulti : Perubatan dan Sains Kesihatan

Sindrom Down (DS) adalah kecelaruan genetik yang disebabkan oleh triplikasi kromosom manusia 21 (Hsa21) dan merupakan penyebab ketidakupayaan intelektual yang paling biasa. Beberapa kajian telah membuktikan bahawa defisit percambahan dan perkadaran sel glial yang lebih tinggi berbanding dengan neuron di otak manusia DS dan model mencit, yang telah menyumbang kepada ketidakupayaan intelektual. Walaupun beberapa penerbitan sebelum ini telah tertumpu kepada aspek molekul, pengetahuan semasa mengenai ketidakseimbangan metabolik dalam sel stem dan progenitor neural (NSPC) yang diperolehi daripada embrio mencit Ts1Cje adalah terhad. Ts1Cje adalah model mencit DS yang amat diperlukan dalam mengembangkan pengetahuan kita mengenai gangguan mekanisme molekul dan selular yang menyebabkan DS. Dalam kajian ini, korteks serebrum embrio Ts1Cje dan mencit jenis liar (WT) telah diasingkan pada hari embrionik 15.5 dan dikultur dalam bentuk neurosfera. Biolog Phenotype MicroArray (PM) digunakan untuk mendapatkan profil metabolik bagi neurosfera embrio Ts1Cje dan WT. Empat jenis PM ujian kolorimetrik yang telah disalut dengan 367 substrat biokimia, termasuk sumber karbon yang boleh dioksida dan nitrogen, telah digunakan. Analisis data PM Biolog dilakukan menggunakan saluran perangkaan statistik yang telah diasaskan sebelum ini menunjukkan pengurangan ketara penggunaan 17 substrat dan peningkatan ketara dalam penggunaan 6 substrat dalam neurosfera Ts1Cje berbanding neurosfera WT. Pemerhatian yang penting adalah pengurangan dalam glukosa-6-fosfat (G6P) dan α -D-glukosa yang ketara dalam neurosfera Ts1Cje berbanding WT. L-serine, dan dipeptida yang mengandungi histidine dan isoleucine juga digunakan dengan ketara lebih rendah dalam neurosfera Ts1Cje, manakala dipeptida yang mengandungi glutamate lebih banyak digunakan dalam neurosfera Ts1Cje berbanding dengan neurosfera WT. G6P terlibat dalam dua laluan yang menghasilkan tenaga: laluan pentosa fosfat (PPP) dan glikolisis. Untuk menyiasat sama ada perantaraan metabolisme G6P dapat meningkatkan penghasilan neurosfera Ts1Cje, neurosfera telah dibekalkan dengan *6-phosphogluconic acid* (6PG) dan *fructose-6-phosphate* (F6P) dan dikaji selepas 6 hari secara *in vitro*. Diameter purata neurosfera Ts1Cje yang diberikan

suplementasi 2.0 mM 6PG adalah lebih tinggi berbanding dengan kumpulan yang tidak menerima suplemen, menunjukkan bahawa suplementasi dengan 6PG boleh membetulkan ketidakseimbangan PPP dalam neurosfera Ts1Cje. Sebaliknya, saiz neurosfera Ts1Cje yang diberikan suplementasi F6P tidak menunjuk perbezaan yang ketara berbanding dengan kumpulan kawalan, menunjukkan bahawa mekanisme glikolisis memainkan peranan yang minimal dalam penghasilan neurosfera. Seterusnya, aktiviti enzim *glucose-6-phosphate dehydrogenase* (G6PDH), yang memangkinkan katabolisme G6P dalam PPP, dinilai lebih lanjut. Secara amnya, aktiviti G6PDH dalam neurosfera Ts1Cje lebih rendah berbanding neurosfera WT, tetapi perbezaannya tidak ketara. Tanpa suplemen 6PG, aktiviti G6PDH dalam neurosfera Ts1Cje didapati adalah lebih rendah daripada neurosfera WT. Sementara itu, kepekatan 6PG yang semakin meningkat menyebabkan perbezaan aktiviti G6PDH yang lebih besar antara neurosfera Ts1Cje dengan WT, walaupun perbezaannya tidak ketara. Data ini mencadangkan bahawa perubahan dalam laluan metabolik, terutamanya PPP, telah menyumbang kepada kecacatan dalam percambahan NSPC embrio mencit Ts1Cje. Pengkajian sifat metabolik NSPC embrio Ts1Cje dapat meningkatkan pengetahuan tentang ketidakseimbangan dalam NSPC semasa perkembangan otak individu DS dan boleh melengkapkan kajian dari aspek genomik, transkriptomik dan proteomik.

ACKNOWLEDGEMENTS

This journey of completing my Master's thesis has been a wild roller coaster ride. So many thoughts, memories and emotions are weaving through my mind as I write this acknowledgement. I have learnt so many things along the way, most of which I learnt through making mistakes. As I reach the end of this chapter of my life, the most important lesson that I learnt is how to pick myself up after a huge fall and that everything happens for a reason.

Dr Cheah Pike See, words cannot simply describe how thankful I am for all the effort and time you have dedicated to make sure I complete my Masters degree. It's amazing how sending you that email to express my interest in doing my internship in the Neurobiology and Genetics Group (NBGG) led to a cascade of life-changing events. I truly appreciate your positive attitude and your passion in science, which always encourages and inspires me to strive for more. Thank you for not giving up on me and for sticking around when I am at my worst, for being more than a supervisor who not only guides me through my academic journey but also prepares me for the overwhelming future ahead. You have also given me countless opportunities throughout my time at Universiti Putra Malaysia (UPM), including the opportunity to attend R programming workshop, APSN-ISN Neuroscience School, visit to Universiti Teknologi Petronas for discussion with Dr Eric Tatt Wei Ho, to name a few. I also want to deeply apologise for all the times I put you through any difficulties or did not meet deadlines. Your patience, dedication and compassion have guided me through my personal and academic endeavours. I hope you know how much of a positive impact you have made in my life.

Dr Michael Ling King Hwa, I sincerely appreciate all the time you have spent discussing my research with me and all the opportunities you have given me that have helped enhance my technical skills. You have guided me through so many of my experiments and helped with a lot of troubleshooting. Also, thank you for all the advice and for inspiring me with your passion in research. I hope you continue to inspire and motivate everyone else around you. I would also like to express my gratitude towards my co-supervisors Professor Dr. Cheah Yoke Kqueen and Dr Norshariza Nordin. To Prof. Cheah, thank you for the access to the most important component of my research, the Biolog OmniLog instrument. I am very grateful for your support and guidance throughout my Master's degree. A heartfelt thanks also goes out to Dr. Shariza, for being an amazing mother figure to me and giving me all the support I needed to complete my degree. Thank you for all the times you have shared your experiences and advice with me. Special thanks also goes out to Dr Eric Tatt Wei Ho, who has helped me with the Biolog data analysis by guiding me through the R programming language. He has also introduced me to the world of neuroinformatics through our discussions and I will always be grateful for that experience.

I am also very grateful for my loving husband and my best friend, Hadri Hadi, whose name would have been in a different paragraph in this acknowledgement if not for Allah's plan. A few years ago, I never would have imagined that someday we would build a life together. You have been my source of strength and I will always keep you in my prayers. Thank you for all the times you have accompanied me on MRT rides to find

the right café or area to write my thesis in. Thank you for being so supportive and catering to all my needs. I cannot wait to see what else is in store for us and I'm prepared to face anything as long as you are by my side.

My deepest gratitude goes out to Mama, for showing me what it means to be a mother. The monumental sacrifices you have made for me have made who I am today. Making you happy is what drives me now, as I grow older. Thank you for always pushing me to do my best and giving me the freedom to be who I want to be. I hope you know how much I still need you in my life and that I will always be your baby. To Abah, thank you for always motivating me and encouraging me to dream big. I still do wish I had your work ethics and your confidence. You always find ways to make the most out of everything and I really admire that in you. To my siblings, Nor, Aboy and Esti, thank you for helping raise me and being so supportive of all my decisions, especially the decision to put off getting a job and pursue my Master's degree. I hope we continue to always have each other's backs.

To my labmates in Medical Genetics Laboratory (MGL) and NBGG, Angeline, Arcana, Amin, Asween, Avin, Asraa, Ahmed, Kak Adila, Afiqah, Kak Ayu, Chai Ling, Elson, Han Chung, Hamizun, Kak Izzati, Kak Linda, Kak Marlini, Melody, Nabilah, Niu Jin, Omar, Rusheni, Rozita, Shirley, Suleiman, Shahidee, Usman, thank you for showing me what teamwork is about. I will eternally be thankful for the opportunity to work with a group of intelligent and talented individuals. You have all made my time in the lab much more bearable and I hope that we always stay in touch.

Finally, I would like to acknowledge Ministry of Higher Education Malaysia (MOHE) for funding my Master's research through the Fundamental Research Grant Scheme and also Universiti Putra Malaysia for awarding me the Graduate Research Fellowship (GRF).

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Cheah Pike See, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Cheah Yoke Kqueen, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Norshariza Nordin, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name and Matric No.: Eryse Amira Binti Mohamed Seth (GS47594)

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6-AN	6-aminonicotinamide
6PGL	6-phosphogluconolactonase
6PG	6-phosphogluconic acid
6PGDH	6-phosphogluconate dehydrogenase
AD	Alzheimer's diseases
ALL	Acute lymphoid leukaemia
AML	Acute myeloid leukaemia
AMPK	AMP-activated protein kinase
APP	Amyloid precursor protein
aRG	Apical radial glia
AVSD	Atrioventricular septal defect
ASD	Autism spectrum disorder
ATP	Adenosine triphosphate
BLBP	Brain lipid binding protein
bRG	Basal radial glia
BSA	Bovine serum albumin
CHD	Congenital heart disease
Cho	Choline
CNS	Central nervous system
Cr	Creatinine
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
DIV	Days *in vitro*
DS	Down syndrome
E	Embryonic day
EDTA	Ethylenediaminetetraacetic acid
F6P	Fructose-6-phosphate
FBS	Foetal bovine serum
FDG	Deoxy-2[(18)F]fluoro-D-glucose
G1P	Glucose-1-phosphate
gDNA	Genomic Deoxyribonucleic acid
G6P	Glucose-6-phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
GABA	γ-aminobutyric acid
GC	Gas chromatography
GD	Gestational day
GFAP	Glial fibrillary acid protein
GLAST	Astrocyte-specific glutamate transporter
GMP	Granulocyte-macrophage progenitor
Grik1	Glutamine receptor ionotropic kainite 1
hESC	Human embryonic stem cell
^{1}HMRS	Proton magnetic resonance spectroscopy
HPLC	High performance liquid chromatography
HSA	Human chromosome
IDO	Indoleamine 2,3-deoxygenase
IFN-γ	Interferon-γ

INM	Interkinetic nuclear migration
IPC	Intermediate progenitor cell
ISVZ	Inner subventricular zone
IQ	Intellectual quotient
kDA	Kilodalton
KYN	Kynurenine
LTP	Long term potentiation
mI	Myoinositol
MMU	Mouse chromosome
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MWM	Morris water maze
NAA	N-acetylaspartate
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NEC	Neuroepithelial cell
Neo	Neomycin
NMDA	N-Methyl-D-aspartic acid
NMR	Nuclear magnetic resonance
NPC	Neural progenitor cell
NSC	Neural stem cell
NSPC	Neural stem and progenitor cell
oRGC	Outer radial glial cell
OSVZ	Outer subventricular zone
P	Postnatal day
Pax6	Paired box 6
PCR	Polymerase chain reaction
PDH	Pyruvate dehydrogenase
PET	Positron emission topography
PBS	Phosphate buffered saline
PGI	Phosphoglucose isomerase
PM	Phenotype MicroArray
PPP	Pentose phosphate pathway
R5P	Ribose-5-phosphate
RGC	Radial glial cell
RNA	Ribonucleic acid
Ru5P	Ribulose-5-phosphate
SDS	Sodium dodecyl sulphate
SHMT	Serine hydroxymethyltransferase
Sod1	Superoxide dismutase 1
Stat1	Signal transducer and activator of transcription 1
SVZ	Subventricular zone
Tbr2	T-domain transcription factor
TCA	Tricarboxylic acid
VZ	Ventricular zone
VSD	Ventricular septal defect
WT	Wild type
Znf295	Zinc finger protein 295

CHAPTER 1

INTRODUCTION

1.1 Background

Down syndrome (DS), or Trisomy 21, is a genetic disorder that results from the triplication of human chromosome 21 (HSA21), which leads to multiple phenotypes with varying complexity. The frequency of DS worldwide is approximately 1 in 1000 live births (Busciglio et al., 2013), whereas in Malaysia, the prevalence of DS is approximately 1 in 660 live births (Kiwanis Down Syndrome Foundation, KDSF). Clinical manifestations that are prominent in individuals with DS include intellectual disability, craniofacial abnormalities and muscle weakness (hypotonia). Some individuals with DS are also affected by other medical complications such as congenital heart defects, early-onset Alzheimer's disease, vision and hearing disorders (Asim et al., 2015; Roper and Reeves, 2006, Weijerman and de Winter, 2010). Furthermore, children with DS are also at a higher risk of developing both acute myeloid leukaemia and acute lymphoblastic leukaemia (Rabin and Whitlock, 2009).

In recent years, the development of various mouse models for DS has been indispensable in enhancing our knowledge on the molecular mechanisms and ensuing complex phenotypes observed in DS. The partial synteny between HSA21 and mouse chromosome 16 (*Mus musculus* 16, MMU16), chromosome 10 (MMU10) and chromosome 17 (MMU17) have been an impetus for the generation of mouse models with DS (Rachidi and Lopes, 2010). Developed in 1998 by Sago and colleagues, the Ts1Cje mouse model carries ~80 genes of MMU16 that are syntenic to HSA21. This partially trisomic mouse model was reported to display DS-associated behavioural deficits and neuropathologies including hippocampus-related learning and memory impairment (Sago et al., 1998), as well as reduced cerebellar volume (Olson et al., 2004; Laffaire et al., 2009).

Neural stem and progenitor cells (NSPCs) is the collective term for a population of stem cells that can self-renew and also progenitor cells that are more committed to either the neuronal or glial lineage (Noctor et al., 2007). The process of transition from NSPCs to neurones and glial cells are known as neurogenesis and gliogenesis, respectively. Both these processes can occur during prenatal, postnatal and adult stages in the normal mouse brain (Urbán & Guillemot, 2014). Studies using an *in vitro* method, known as the neurosphere culture, have contributed to the existing knowledge on proliferative capacity and cell fate determination of NPCs (Reynolds and Weiss, 1992; Qian et al., 2000). Neurospheres are free-floating spheres that are composed of a heterogeneous population and can differentiate into neuronal or glial cells (Bez et al., 2003). They can be generated in the presence of growth factors epidermal growth factor (EGF) and foetal growth factor (FGF).

Emerging evidence has demonstrated impaired NSPC proliferation and neurogenesis in Ts1Cje mice. Hewitt and colleagues reported a reduced proportion of neurones and an increased proportion of astrocytes derived from adult Ts1Cje neurospheres (Hewitt et al., 2010). This neurogenic-to-gliogenic shift was also found in embryonic Ts1Cje mice. A higher proportion of cells positive for the immunohistochemical marker for glial cells, glial fibrillary acidic protein (GFAP) was observed when neurospheres from the embryonic neocortex of Ts1Cje mice were allowed to differentiate (Moldrich et al., 2009). Also, the same study reported a decreased rate of proliferation in embryonic NSPCs derived from Ts1Cje mice compared to control. To increase our understanding of perturbed mechanisms that occur in cortical development of Ts1Cje mice, the focus of this present study will be on embryonic NSPCs in Ts1Cje mice.

The emerging field of metabolomics involves the investigation of metabolites within cells, tissues or organisms and their role in biochemical processes. It is a complex, yet an integrative approach to understanding the interplay between genes, proteins and the environment. Metabolic studies on DS brain have shown that dysregulated level of metabolites and impaired glucose metabolism in the brain of individuals with DS are correlated with the progression of cognitive dysfunction in DS (Hsia et al., 1971; Labudova et al.,, 1999; Simo et al., 2004; Smigielska-Kuzia & Sobaniec, 2007). However, studies on the metabolic properties of embryonic NSPCs in mouse models, particularly Ts1Cje mice, are still limited. Biolog Phenotype MicroArray (PM) is a technology that provides cellular analysis of multiple physiological traits simultaneously, using a 96-well microplate pre-coated with different known carbon sources, L-amino acids and dipeptides (Bochner et al., 2011). Metabolic profiling using the PM technology has been useful for identification of biomarkers associated with Autism spectrum disorder (ASD) (Boccuto et al., 2013) and discovery of increased amino acid metabolism in prostate cancer cells as an effect of androgen signalling (Putluri et al., 2011). Therefore, utilizing this approach in the present study would enable the identification of altered metabolic pathways underlying the defective proliferation and neurogenesis observed in embryonic Ts1Cje mice.

Much of the current literature has been emphasised on the molecular mechanisms underlying the disruptions in NSPC proliferation and neurogenesis observed in the Ts1Cje mouse model. However, our knowledge on the metabolic alterations in embryonic NSPCs derived from Ts1Cje mice is still limited. Indeed, recent findings indicate a correlation between metabolic dysfunction and some neurological disorders (Cai et al., 2012). Studying from a metabolic perspective may provide more insight into the abnormalities in NSPCs and abnormal brain development in Ts1Cje mice. By investigating the dysregulated metabolic pathways in NSPCs, this study aims to contribute to the understanding of mechanisms underlying the defective proliferation and neurogenesis observed in Ts1Cje mice.

1.2 Problem statement

Down syndrome (DS), the most common genetic cause of intellectual disability, was found to be associated with a disruption in proliferation and differentiation of NSPCs. Previous studies have also provided some evidence for metabolic properties in DS

human samples, giving clues to the possible correlation between metabolic disruption and cognitive abnormalities in DS. However, the metabolic properties of NSPCs derived from embryonic Ts1Cje mice have not been fully assessed, and metabolic alterations that could potentially lead to defective NSPCs in embryonic Ts1Cje mice has yet to be elucidated.

1.3 Hypotheses

This study hypothesises that:

1. Ts1Cje embryonic neurospheres have a distinct energy metabolism compared to that of WT embryonic neurospheres.
2. Abnormalities in NSPCs derived from embryonic Ts1Cje mice are associated with dysregulations in energy-producing metabolic pathways.

1.4 Objectives

1.4.1 General objective

This study aims to investigate the metabolic properties of neural stem and progenitor cells (NSPCs) derived from embryonic Ts1Cje mouse model for Down syndrome.

1.4.2 Specific objectives

1. To investigate energy metabolism in neurospheres generated from embryonic day (E) 15.5 cerebral cortex of embryonic Ts1Cje and wild type (WT) mice using Biolog Phenotype MicroArray.
2. To determine the effects of 6-phosphogluconic acid (6PG) and fructose-6-phosphate (F6P) supplementation on neurospheres generated from embryonic Ts1Cje mice.
3. To assess the enzyme activity levels of glucose-6-phosphate dehydrogenase (G6PDH) with and without 6PG supplementation in NSPCs derived from embryonic Ts1Cje mice.

1.5 Significance of the study

The findings from this study aim to provide more insight into the metabolic alterations that may contribute to defective proliferation and differentiation of NSPCs from embryonic Ts1Cje mice. This may be important for future researchers in their effort to enhance the current knowledge on cognitive dysfunction in DS. The findings of the study may also be a fundamental step in the discovery of therapeutic strategies using the nutritional approach to regulate proliferation and differentiation of NSPCs during early brain development in DS.

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BIODATA OF STUDENT

Eryse Amira Mohamed Seth completed her International Baccalaureate Diploma in International School of Kuala Lumpur, Malaysia in 2011. She then went on to pursue her undergraduate degree in Bachelor of Science (Biomedical Sciences) at Durham University, United Kingdom. She first discovered her interest in neuroscience when she completed her final year dissertation on neuroinflammation in schizophrenia. Following her graduation in 2014, she worked as a Medical Laboratory Technologist at Ramsay Sime Darby Medical Centre Subang Jaya.

She held on to her interest in neuroscience research and later decided to pursue a Master's degree at Universiti Putra Malaysia. She first joined the Neurobiology and Genetics Group (NBGG) laboratory as an intern in 2016, after which she started her Master's degree in Neuroscience under the supervision of Associate Professor Dr. Cheah Pike See. During her time as a Master's student at UPM, she was awarded travel grants such as the APSN-ISN Neuroscience School Travel Award and also the IBRO-APRC Neuroscience Associate School Travel Award. She was also granted the MyNEURO2017 Education Grant Award by Malaysian Society for Neurosciences (MSN) to attend the MyNEURO2017 conference and received the Graduate Research Fellowship (GRF) from UPM to help finance her Master's education. She has published one journal article for her Master's research.

LIST OF PUBLICATIONS

Accepted

Eryse Amira Seth, Han-Chung Lee, Hadri Hadi Yusof, Norshariza Nordin, Yoke Kqueen Cheah, Eric Tatt Wei Ho, King-Hwa Ling, Pike-See Cheah. Phenotype microarrays reveal metabolic dysregulations of neurospheres derived from embryonic Ts1Cje mouse model of Down syndrome. 2020. *PLoS ONE.* https://doi.org/10.1371/journal.pone.0236826

Han-Chung Lee, Hadri Hadi Yusof, Melody Pui-Yee Leong, Shahidee Zainal Abidin, **Eryse Amira Seth**, Chelsee A. Hewitt, Sharmili Vidyadaran, Norshariza Nordin, Hamish S. Scott, Pike-See Cheah, King-Hwa Ling. Gene and protein expression profiles of JAK-STAT signalling pathway in the developing brain of the Ts1Cje down syndrome mouse model. 2019. *International Journal of Neuroscience.* doi: 10.1080/00207454.2019.1580280.

Hadri Hadi Yusof, Han-Chung Lee, **Eryse Amira Seth**, Xiangzhong Wu, Chelsee A. Hewitt, Hamish S. Scott, Pike-See Cheah, Yue-Ming Li, De-Ming Chua, King-Hwa Ling. Expression profiling of notch signalling pathway and gamma-secretase activity in the brain of Ts1Cje mouse model of Down syndrome. 2019. *Journal of Molecular Neuroscience.* doi: 0.1007/s12031-019-01275-2.

Proceedings

Seth EA, Lee HC, Yusof HH, Ling KW and Cheah PS (2016). Metabolic profiling of neurospheres derived from E15.5 cerebral cortex of Ts1Cje mouse model for Down syndrome. Frontiers in Cellular Neuroscience Conference Abstract: 14th Meeting of the Asian-Pacific Society for Neurochemistry. doi: 10.3389/conf.fncel.2016.36.00184

Cheah PS, Tan NJ, Vidyadaran S, Nordin N, Baharuddin MN, Lim CL, **Seth EA,** Ling KH. (2016) Alzheimer's Brain Changes. In: Nature's Yield and Wonders of Art (NYAWA)16: Brain. University Putra Malaysia, Serdang, Selangor, pp. 32-35.

Awards

Award Recipient for MyNeuro2017 Education Grant (NATIONAL). Malaysian Society for Neurosciences, Istana Hotel, Kuala Lumpur, Malaysia, 11th – 13thAugust 2017.

Award Recipient for IBRO/APRC Neuroscience Associate School 2017 (INTERNATIONAL). Singapore Neuroscience Association, National University of Singapore (NUS), Singapore, 3rd – 7th July 2017.

Award Recipient for APSN-ISN Neuroscience School 2016 (INTERNATIONAL).
Asian–Pacific Society for Neurochemistry and International Society for Neurochemistry, Faculty of Medicine and Health Sciences, UPM, Serdang, Selangor, 22nd -26th August 2016.

Poster Presentations

Dysregulations in energy metabolism of embryonic neurospheres derived from Ts1Cje mouse model for Down syndrome. Mini Symposium for Phenotypic MicroArray, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Selangor, Malaysia, 6th – 7th September 2017.

Dysregulations in metabolic activity of neurospheres derived from embryonic Ts1Cje mouse model for Down syndrome. MyNeuro2017, Istana Hotel, Kuala Lumpur, Malaysia, 12th – 13th August 2017.

Metabolic characterization of embryonic neural stem and progenitor cells of Ts1Cje mouse model for Down syndrome, Singapore Neuroscience Association (SNA) Symposium, National University of Singapore (NUS), 5th July 2015.

Metabolic profiling of neurospheres derived from E15.5 cerebral cortex of Ts1Cje mouse model for Down syndrome. APSN 2016, Istana Hotel, Kuala Lumpur, Malaysia, 27th – 30th August 2016.

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