UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT FINAL REPORT
Geran Penyelidikan Universiti Laporan Akhir

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A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research: Development of Mutation Screening For the Molecular Diagnosis of Tuberous Sclerosis Complex** *Tajuk Penyelidikan:*
(ii)	**Account Number: 1001/PPSP/812048** *Nombor Akaun:*
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK*
(i)	**Name of Research Leader: Dr. Teguh Haryo Sasongko** *Nama Ketua Penyelidik:*
	Name of Co-Researcher: Prof. Dr. Naradzah Mohd. Yusof, Dr.Salmi Abd. Razak, Prof. Dr. Zabidi Azhar Mohd. Husin, Prof. Dr. Zilfalil Alwi, Prof Dr. Hardyanto Soebono, Prof. Dr. Hisahide Nishio, Dr. Ngu Lock Hock *Nama Penyelidik Bersama:*
(ii)	**School/Institute/Centre/Unit: School of Medical Sciences** *Pusat Pengajian /Institut/Pusat/Unit:*
D.	**Duration of this research:** *Tempoh masa penyelidikan ini:* ***Duration :** 42 months *Tempoh :* **From** : 1 May 2010 *Dari:* **To** : 31 October 2013 *Ke :*

E. **ABSTRACT OF RESEARCH**

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English**. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**.
Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

English

Tuberous sclerosis complex (TSC; OMIM#191100) is an autosomal dominant disorder caused by mutations in either TSC1 (9q34.13) or TSC2 (16p13.3). TSC is characterized by a broad phenotypic spectrum including epilepsy, mental retardation, skin lesions, and tumors in various organs. The broad phenotypic spectrum reflected the development of hamartomas in multiple organs throughout the body and represents difficulties in the diagnosis of the disease. Mutation analysis in TSC patients is useful 1) to confirm a clinical diagnosis of TSC, especially in young patients in whom many clinical features have yet to develop, 2) in families with sporadic cases of TSC, mutation analysis may provide reassurance that the rest of the family members do not carry the mutation. We aimed in this study to develop a protocol for a mutation analysis of TSC1 and TSC2 genes and a database of patients with Tuberous Sclerosis Complex. We employed in this study four different molecular techniques that include denaturing high performance liquid chromatography (dHPLC), DNA Sequencing, multiple ligation-dependent probe amplification (MLPA) and PCR Amplicon Sequencing on MiSeq platform. Using dHPLC and DNA Sequencing we identified 7 nucleotide variations in TSC1 (c.965T>C;pM322T, c. 615 T>C;pS205S, c.1335A>G;p445Glu>Glu, c.1334-55C>G, c.1726T>C;p.L576L and c.210+25A>G) and 2 nucleotide variations in TSC2 (c.2580T>C;p.F860F and c.2639+45G>C) of 10 patients, but none are pathogenic. Using MLPA we identified 3 gross deletions in TSC2 (g.del_ex26-ex31;c.2970-3886del917bp;p.S990R-FsX36, g.del_ex32-41;c.3887-5404del1516bp;p.A1295X and g.del_ex1-ex15) in 3 patients, which are clearly pathogenic. Using PCR Amplicon Sequencing on MiSeq platform we identified 1 variation in TSC1 (c.2071C>T;p.R691X) and 3 variations in TSC2 (c.1361+1G>A, c.4344insC;p.S1448S-FsX1523 and c.3754C>A;p.S1252X) from 5 patients, all of which are pathogenic. Our data suggests that MLPA and PCR Amplicon Sequencing may be more superior as compared to dHLPC and DNA Sequencing in detecting mutations in TSC1 and TSC2 genes. Based on this, for the detection of TSC1 and TSC2 mutations among TSC patients we propose to employ MLPA as first-line molecular diagnostic procedure (10-12% coverage) followed by PCR Amplicon Sequencing as a second-line procedure (80% coverage).

Bahasa Malaysia

Tuberous Sclerosis Complex (TSC ; OMIM # 191100) adalah gangguan *autosomal dominant* yang disebabkan oleh mutasi dalam gene TSC1 (9q34.13) atau TSC2 (16p13.3). TSC mempunyai ciri-ciri spektrum fenotip yang luas termasuk epilepsi , terencat akal , luka-luka kulit, dan tumor dalam organ-organ. Spektrum fenotip luas mencerminkan pembangunan *hamartomas (tumor)* dalam pelbagai organ-organ seluruh badan dan mewakili kesukaran dalam diagnosis penyakit ini. Analisis mutasi pada pesakit TSC berguna 1) untuk mengesahkan diagnosis klinikal TSC, terutamanya pada pesakit muda dimana ciri-ciri klinikal masih banyak yang belum terlihat, 2) dalam keluarga yang mempunyai kes sporadis TSC , analisis mutasi boleh memberi jaminan bahawa seluruh ahli-ahli keluarga tidak membawa mutasi. Kami bertujuan dalam kajian ini untuk membangunkan protokol untuk analisis mutasi TSC1 dan TSC2 gen dan pangkalan data pesakit. Kami menggunkan dalam kajian ini empat teknik molekul yang berbeza, iaitu denaturing high performance liquid chromatography (dHPLC), DNA Sequencing, multiple ligation-dependent probe amplification (MLPA) and PCR Amplicon Sequencing pada platform MiSeq.

	Dengan menggunakan dHPLC dan DNA Sequencing kami telah mengenal pasti 7 variasi nukleotida dalam TSC1 (c.965T > C; pM322T , c 615 T> C; . PS205S , c.1335A > G; p445Glu > Glu , c.1334 - 55C > G , c. 1726T > C; p.L576L dan c.210 +25 A > G) dan 2 variasi nukleotida dalam TSC2 (c.2580T > C; p.F860F dan c.2639 +45 G > C) pada sebanyak 10 pesakit, tetapi tidak ada yang patogenik. Dengan menggunakan MLPA kami telah mengenal pasti 3 delesi luas gen TSC2 (g.del_ex26 - ex31,c.2970 - 3886del917bp,p.S990R - FsX36; g.del_ex32 – 41,c.3887 - 5404del1516bp,p.A1295X dan g.del_ex1-1x15) dalam 3 pesakit , yang jelas patogenik. Menggunakan PCR Amplicon Sequencing pada platform MiSeq kami telah mengenal pasti 1 variasi TSC1 (c.2071C>T;p.R691X) dan 3 variasi TSC2 (c.1361+1G>A, c.4344insC;p.S1448S-FsX1523 and c.3754C>A;p.S1252X) dalam 5 pesakit, kesemuanya patogenik. Data kami mencadangkan bahawa MLPA dan PCR Amplicon Sequencing mungkin lebih hebat berbanding dHLPC dan DNA Sequencing dalam mengesan mutasi dalam gen TSC1 dan TSC2. Berdasarkan ini, untuk mengesan mutasi TSC1 dan TSC2 di kalangan pesakit TSC kami mencadangkan untuk mengambil MLPA sebagai barisan pertama prosedur diagnostik molekul (liputan 10-12 %) diikuti oleh PCR Amplicon Sequencing sebagai prosedur barisan kedua (liputan 80 %).