DEVELOPMENT OF B-
LYMPHOBLASTOID CELL LINE
FROM PERIPHERAL BLOOD
MONONUCLEAR CELL AND THE
CHARACTERIZATION OF FCGR3B
COPY NUMBER VARIATION IN
NEGRITOS AND MALAYS

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of the requirements for the degree of
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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 20th November 2015 to conduct the final examination of Mohd Helmy Bin Yusof on his Master of Medical Science thesis entitled 'Development of B-Lymphoblastoid Cell Line from Peripheral Blood Mononuclear Cell and the Characterization of FCGR3B Copy Number Variation in Negritos and Malays' in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Obtaining a continuous source of normal cells or DNA from a single individual has always been a rate limiting step in biomedical research. The availability of Lymphoblastoid cell lines (LCLs) as a surrogate for replacement of isolated peripheral blood mononuclear cell (PBMC) has substantially accelerated the process of biological investigations. LCLs can be established by in vitro infection of B lymphocytes from peripheral blood with Epstein Barr Virus (EBV) resulting in continuous biomaterials, bearing minor genetic and phenotypic alterations. Fc Gamma Receptor 3B (FcyRIIIB, encoded by the gene FCGR3B) plays a crucial role in immunity triggered by cellular effectors and regulatory functions. Copy number variation (CNV) of this gene has been previously reported to affect susceptibility to several diseases such as autoimmune diseases and chronic inflammatory conditions. Here the first generation of LCL in indigenous populations from Peninsular Malaysia were reported and the method for immortalization B-lymphocytes cell line was establish. First, the most appropriate blood collection tube to collect the peripheral blood was selected. ACD tube was found to be most appropriate to collect Orang Asli blood sample in field trip while CPT tube was used to collect Malay (control) samples. PBMC from Malay sample and Orang Asli samples were isolated by using centrifugation and modified method of CPT (MMC) respectively. The best EBV supernatant to transfsect PBMC was produced from B95-8 marmoset cell line treated by 40ng/ml of tetradecanoyl phorbol acetate (TPA). From 89 PBMC sample isolated including Malay (n=25) and Orang Asli (n=64), a total of 84 samples was successfully transformed into LCL by transfsect with EBV supernatant in a single attempt with a success rate 100% (n= 25) for Malay and 92.2% (n=59) for Orang Asli. Copy number variation (CNV) was characterized by using the Paralogue Ratio Test-Restriction Enzyme Digest Variant Ratio (PRT-REDVR) for FCGR3B. Reliability of using LCLs for Copy number variation (CNV) genotyping were tested by comparing CNV call of primary PBMC with the same sample from LCL where CNV calling was 100%, identical in both PBMC and LCL. A total of 84 of DNA extracted from LCL samples were performed on PRT-REDVR assay. Eighty-two samples (25 Malays, 50 Orang Asli Negrito, and 7 Orang Asli Senoi) were successfully genotyped for FCGR3B CNV. From 50 Orang Asli Negrito samples, 88% of them had two copy numbers (2) of FCGR3B. Meanwhile, there was no low copy number (<2) for Orang Asli Negrito. Then again, 12% of Orang Asli Negrito had high copy number (>2) of FCGR3B. Analysis revealed that no significance difference (p=0.558) for CNV of FCGR3B between Malay and Orang Asli Negrito.
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