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COPY NUMBER VARIATION OF *FCGR3B* GENE AMONG SEVERE DENGUE PATIENT IN MALAYSIA

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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

Fc Gamma Receptor 3B (FcγRIIIB, encoded by the gene *FCGR3B*) plays a crucial role in immunity triggered by cellular effector and regulatory functions. Copy number variation (CNV) of this gene has been previously reported to affect susceptibility to several autoimmune diseases and chronic inflammatory conditions. However, it remains a challenge to accurately determine the copy number of this gene in different individuals. Thus this study aimed to establish the most robust CNV genotyping assay by comparing the accuracy and efficiency of (i) quantative PCR (qPCR), (ii) Sequenom MassARRAY, and (iii) Paralogue Ratio Test-Restriction Enzyme Digest Variant Ratio (PRT-REDVR). Subsequently the distribution of *FCGR3B* CNV among the dengue patients in Malaysia was characterized and its association with the severity of the disease was determined. A total of 237 samples were recruited from various study hospitals, of which 191 samples were included into further experiments. 120 were clinically diagnosed as severe dengue or warning sign, while 71 were dengue fever (DF). In the comparison of the three CNV genotyping assays, qPCR showed a considerably broader distribution of signal intensity compared to the other assays, potentially introducing error in estimation of copy number. Both Sequenom and PRT-REDVR showed lesser systematic bias, and estimate copy number within the correct range, although PRT-REDVR appears to be more precise and accurate method when genotype *FCGR3B*. Collectively PRT-REDVR was considered to be most appropriate in the study of multiallelic CNV of *FCGR3B*. Multiple independent assays should be considered to accurately genotype the CNV of *FCGR3B*. In the second part of the study, 168 dengue samples (108 case, defined as dengue patients with signs of vascular leakage, and 60 control, defined as dengue patients without vascular leakage) and 52 of healthy samples genotyped with PRT-REDVR were included in the genetic association study. The analysis revealed statistical significance between CNV of *FCGR3B* of control, case and dengue sample against CNV of *FCGR3B* of healthy sample, respectively ($p = 0.012$, $p = 0.007$ and $p = 0.012$). On the other hand, there is no significance association shows between case and control ($p = 0.301$). However, a trend towards the low copy number (CN <2) in case was observed, hence postulating that lower copy number may be attributed with vascular leakage in dengue. Larger number of samples however, is needed to address this postulation.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND

Dengue is a significant threat to the public health worldwide nowadays. The dengue virus is transmitted to humans by infected mosquitoes, mainly by *Aedes aegypti* and *Aedes albopictus.* Dengue virus belongs to the genus of Flavivirus, family Flaviridae (Shekhar, 1992), and consists of four serotypes: DENV1, DENV2, DENV3, and DENV4 (Noisakran and Perng, 2008; Lin et al., 2006). DENV has a single stranded RNA genome approximately 11 kb in length, comprising of three structural protein genes, encoding the Core (C), Membrane (M), and Envelope (E) and seven non-structural (NS) proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (Kurane, 2007).

Any of the four dengue virus serotypes could result in dengue fever (DF), an acute viral infection with typical symptoms of fever, rash, headache, muscle and joint pain. Occasionally, DF may progress to dengue hemorrhagic fever (DHF), a potentially life-threatening illness associated with vascular leakage, hemorrhage and shock (WHO, 1997).

Approximately 50-100 million cases of severe dengue requiring hospitalization have been reported, of which, approximately 500 000 resulted in dengue hemorrhagic fever (DHF) or Dengue Shock Syndrome (DSS), causing more than 20 000 death worldwide (WHO, 2009). Dengue is endemic in more than 100 countries including Southeast Asia, the Caribbean and South Pacific regions, South and Central America; DHF/DSS in more than 60 countries (WHO DengueNet report, 2005).

It has been an important and major public health concern in Malaysia ever since its detection in 1902 (Azami et al., 2011). The incidence rate of dengue in year 2011 was 63.6 per 100 000 populations (Ministry of Health Malaysia, 2011). Dengue is no longer a predominantly urban disease as it has expanded geographically into the rural areas. Azami et al. (2011) also revealed that there was not much difference in the dengue IgG