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IDENTIFICATION AND CHARACTERIZATION OF COPY NUMBER VARIATIONS (CNVs) IN DENGUE PATIENTS AND ITS IMPACT WITH VASCULAR LEAKAGE

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulation 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

Dengue fever is a mosquito-borne tropical disease caused by dengue virus. When infected by any of the four serotypes of dengue viruses, individuals may be asymptomatic or develop a dengue fever. The latter group can progress into severe form of dengue and the etiology of this is still uncertain. This study aims to elucidate copy number variations (CNVs) of the host genes as the factor causing dengue pathogenesis. Blood samples were collected from hospitalized dengue patients and proceeded to DNA extraction. DNA samples (136) were used for genotyping with Human Genome GeneChip 6.0. CNVs were called using Genotyping Console (GTC) 4.1 and Nexus Copy Number 7.0. Results from both algorithms were merged and stringent CNVs were filtered with three public databases; DGV, HapMap3 and SGVP in search for rare case-specific CNVs. Case-specific underlying genes were used in functional analysis with DAVID and IPA to explicate the dengue related biological processes. Significant genes of interest from both softwares were validated using qPCR. Hundred and thirty-six DNA samples from hospitalized dengue patients in four different hospitals were genotyped. A total number of 50,864 (24,806 gains and 26,059 loss) and 34,257 (31,725 gains and 2,534 loss) CNV events were discovered by GTC and Nexus, respectively. After merging, only 3,052 (2,132 gain and 920 loss) stringent CNVs were left. After filtering with public known databases, of the 385 novel rare CNVs, 257 were specific for cases, and 128 were specific for controls, respectively ($p = 0.388$). Six hundred and eighty genes were found underlying the CNVs specific for cases and were eventually loaded into functional analysis softwares. GO terms from unbiased DAVID analysis such as defense response ($p<0.001$; Benjamini corrected $p = 0.034$), inflammatory response ($p<0.001$; Benjamini corrected $p = 0.04$) and response to cytokine stimulus ($p<0.001$; Benjamini corrected $p = 0.038$). From the biased IPA analysis, the candidate genes were clustered into enrichment with antigen presentation pathway as the most significant ($p <0.001$), followed by IL-12 signaling and production in macrophages ($p <0.001$), and interferon signaling ($p <0.001$). Genes from both significant GO terms and pathways; namely *BCKDHB, CTSB, MR1, TAP2, TNFRS1B* and *CXCR4* were considered for validation using qPCR. The 9 out of 10 similarities between SNP 6.0 genotyping and qPCR supported the reliability of this assay. In conclusion, the rare CNVs may be involved in dengue pathogenesis. The rare CNV, which has been reported in other diseases (neuropsychiatric diseases, congenital heart disease and dilated cardiomyopathy), is reported for the first time in infectious diseases.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Dengue, the most prevalent mosquito-borne viral disease-affecting people in the tropics and sub-tropic regions of the world, is caused by the infection of dengue virus (DENV). The virus exists as four (DENV 1-4) related but antigennically distinct serotypes. All four DENV serotypes have been co-circulated in Malaysia (Abubakar & Shafee, 2002; S. S. Wong, Abd-Jamil, & Abubakar, 2007). In majority of cases, infection by any of these DENV is asymptomatic, but it may also cause dengue fever (DF), a weakening flu-like illness that can lasts for up to two weeks (World Health Organization [WHO], 2009). Some of the cases, however, result in severe dengue that comprises of dengue shock syndrome (DSS), a life threatening manifestation.

Currently, no licensed vaccine or antiviral therapy exists for dengue infection. In Malaysia, year 2014 depicted the highest number of reported dengue cases and death. In fact, it was the highest throughout the history of dengue endemic in Malaysia where a 160% increment of dengue cases was seen from 2013 to 2014. However, the total number of cases may be underreported as the country uses passive surveillance system in capturing the incidence of dengue infection. In addition, there is a reason to be more vigilance as a very high IgG seropositivity (91.6%) was found in 1,000 healthy adults (Muhammad Azami, Salleh, Neoh, Syed Zakaria, & Jamal, 2011). This means, dengue can still be endemic for many more years in Malaysia.

Globally, WHO stated that DF and severe dengue cases have risen from less than a thousand cases in 1955 to almost 100,000 cases in 2007 annually. During that 53 years, the number of countries reporting dengue cases also increased from just a few to more than sixty countries (WHO, 2009). In addition, dengue infection imposed significant health, economic and social burden on the population experiencing endemic outbreak. Intensive care is required for severely ill patients, including intravenous fluids, blood or plasma transfusion and medicines. On the economical