UNIVERSITI PUTRA MALAYSIA

QUANTIFICATION OF BETA-DEFENSIN COPY NUMBER VARIABLE GENES IN RELATION TO INFLAMMATION IN DIABETIC PATIENTS

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By

MARYAM JAMIELAH BINTI YUSOFF

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

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DEDICATIONS

This thesis is dedicated to you Ibu and Abah for the continuous support and encouragement throughout the ups and downs in my postgraduate journey.

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

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Chairman : Suhaili binti Abu Bakar @ Jamaludin, PhD
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Beta-defensins is one of the predominant antimicrobial peptide which serves as the first line defence against broad spectrum of microbes, including fungi and some viruses. These peptides are encoded by beta-defensins gene; which is variable in copy number. Copy number variations (CNVs) of beta-defensins is present in healthy population. Nevertheless, studies from the past decades have found that beta-defensins gene CNVs also contributed to several inflammatory diseases. Therefore, the objective of this study is to investigate the relationship between copy number variation of beta-defensins with inflammatory condition in type 2 diabetes (T2D). It is hypothesised that copy number variation of beta-defensins contributes to development of T2D through modification of immune response products dosage that exert attacks on host cell that contributed to inflammatory condition in T2D patients. DNA samples from 146 control and 392 T2D individuals were extracted for this study. Beta-defensins copy number quantification was carried out by using paralogue ratio test (PRT107A and HSPD21 primers) and validated by indel polymorphism measurement (5DEL primer) and two microsatellite analysis (EPEV-1 and EPEV-3 primers). Based on the analysis, the copy number variation is more extensive in T2D population ranging between 1 and 12 copies; with copy number 1, 10 and 12 detected in nephropathy group, while in control population the copy number varies between 2 and 8 copies. However, the distribution of copy number are not statistically significant between T2D and control ($p=0.209$) and between those with and without nephropathy among T2D population ($p=0.522$). Despite the result, the white blood cell count between individuals with and without nephropathy from T2D population is significantly different ($p=0.000$). In conclusion, future studies are needed to further explore the true potential of copy number variation of beta-defensins gene towards development of nephropathy in T2D patients.

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

KUANTIFIKASI VARIASI BILANGAN SALINAN GEN BETA-DEFENSINS KE ATAS KERADANGAN DALAM KALANGAN PESAKIT DIABETES

Oleh

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Beta-defensins merupakan salah satu peptida antimikrob yang dominan dan berfungsi sebagai barisan pertama pertahanan terhadap spektrum mikrob yang luas, termasuk kulat dan beberapa virus. Peptida ini dikodkan oleh gen beta-defensins; yang berubah-ubah dalam jumlah salinan. Variasi nombor salinan (CNV) beta-defensin terdapat pada populasi yang sihat. Walaupun begitu, kajian dari dekad yang lalu mendapati bahawa CNV gen beta-defensins juga menyumbang kepada beberapa penyakit radang. Oleh itu, objektif kajian ini adalah untuk mengkaji hubungan di antara variasi bilangan salinan beta-defensin dengan keadaan keradangan pada diabetes jenis 2 (T2D). Dihipotesiskan bahawa variasi bilangan salinan beta-defensin menyumbang kepada perkembangan T2D melalui pengubahsuaian dos produk daripada tindak balas imun yang menimbulkan serangan pada sel hos dan seterusnya membawa kepada keadaan keradangan pada pesakit T2D. Sampel DNA dari 146 kawalan dan 392 individu T2D diekstrak untuk kajian ini. Pengukuran bilangan salinan Beta-defensins dilakukan dengan menggunakan ujian nisbah paralog (primer PRT107A dan HSPD21) dan disahkan oleh pengukuran polimorfisma indel (primer 5DEL) dan dua analisis mikrosatelit (primer EPEV-1 dan EPEV-3). Berdasarkan analisis, variasi bilangan salinan lebih meluas dalam populasi T2D iaitu di antara 1 hingga 12 salinan; dengan salinan nombor 1, 10 dan 12 dikesan dalam kumpulan nefropati, sementara dalam populasi kawalan, nombor salinan berbeza antara 2 dan 8 salinan. Walau bagaimanapun, pengedaran nombor salinan tidak signifikan secara statistik di antara T2D dan kawalan ($p = 0.209$) dan antara mereka dengan dan tanpa nefropati dalam kalangan populasi T2D ($p = 0.522$). Walaupun begitu, jumlah sel darah putih antara individu dengan dan tanpa nefropati dari populasi T2D jauh berbeza ($p = 0.000$). Sebagai kesimpulan, lebih kajian diperlukan pada masa depan untuk meneroka lebih jauh potensi sebenar variasi bilangan salinan gen beta-defensins terhadap perkembangan nefropati pada pesakit T2D.

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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:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

AMP	Antimicrobial peptide
ANCA	Antineutrophil cytoplasmic antibody
ANOVA	Analysis of Variance
APP	Amyloid beta (A4) precursor protein
array-CGH	Array-comparative genomic hybridization
BAC	Bacterial artificial chromosome
C4	Complement component C4
CCL3L1	Chemokine (C-C) motif ligand 3 like 1
CCR2	C-C chemokine receptor type 2
CNVs	Copy number variations
CRP	C-reactive protein
DEFB	Beta-defensin
DNA	Deoxyribonucleic acid
EBV	Epstein Barr virus
ECACC	European Collection of Cell Cultures
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor
GSK3β	Glycogen synthase kinase 3 beta
GWAS	Genome-wide association study
hBD	Human beta-defensin
HIV	Human immunodeficiency virus
HRC	Human random control
IDF	International Diabetes Federation

IL-1	Interleukin-1
IL-6	Interleukin-6
Indel	Insertion/deletion
MAPH	Multiplex amplifiable probe hybridization
MBL	Mannose-binding lectin
ML	Maximum likelihood
MLPA	Multiplex ligation-dependent probe amplification
mRNA	Messenger RNA
NCBI	National Center for Biotechnology Information
NHMS	National Health and Morbidity Survey
PCA	Principal Component Analysis
NMRR	National Medical Research Register
PCR	Polymerase chain reaction
PLP1	Proteolipid protein 1
PRR	pattern recognition receptors
PRSS1	Protease, Serine 1
PRT	Paralogue ratio test
QMPSF	Quantitative multiplex PCR of short fluorescent fragment
q-PCR	Real-time PCR
rBD	Rat beta-defensin
REDVR	Restriction enzyme digest variant ratio
RFLP	Restriction fragment length polymorphism
RIA	Radioimmunoassay
SLE	Systemic lupus erythematosus
SNCA	Synuclein, alpha (non A4 component of amyloid)

SNP	Single nucleotide polymorphism
SPAG11	Sperm-associated antigen 11
STR	Short tandem repeat
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TNF-α	Tumour necrosis factor-α
TSPAN8	Tetraspanin 8
UCSC	University of California, Santa Cruz
UGT2B17	UDP-glucuronosyltransferase 2B17
VNTRs	Variable number of tandem repeats
WBC	White blood cells
WTCCC	Wellcome Trust Case Control Consortium

CHAPTER 1

INTRODUCTION

1.1 Background of study

Over the past decade, scientists have started to appreciate the contribution of structural genetic variations to human diseases. One of the most extensively studied structural variations is Copy Number Variation (CNV). Since then, increasing number of loci containing functional genes has been described to be variable in copy number (Iafrate et al., 2004; Sebat et al., 2004; Tuzun et al., 2005; McCarroll and Altshuler, 2007). This genetic variation affects the humans through dosage-dependent manner, thus increasing or decreasing the susceptibility towards certain diseases (Wain et al., 2009). Chromosome band 8p23.1 contains immune-related, copy number variable gene known as beta-defensins which encode important antimicrobial peptide of the body (Schutte et al., 2002). This predominant peptide is known for its ability to kill various pathogens including some fungi and viruses by forming pores on the membrane, acknowledging its important role in innate immune defence (Ganz, 2003). Moreover, beta-defensins also engage to the adaptive immune system through its cytokine-like properties, attracting cells of the immune system towards infection or inflammation site (Rohrl et al., 2010). Dysregulation of body's beta-defensin peptides increased susceptibility to a number of diseases such as human immunodeficiency virus (HIV) infection, psoriasis, systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA)-associated small vasculitis (Hollox et al., 2008a; Hardwick et al., 2012; Zhou et al., 2012). Recent in vivo study in diabetic rats found low rodent beta-defensin-1 (rBD)-1 level in the kidney compared to the controls, indicating an inflammatory reaction (Froy et al., 2007; Barnea et al., 2008). This finding is parallel to the nature of its human orthologue known as hBD-1 (human beta-defensin-1); encoded by *DEFB1* gene that is constitutively expressed in the kidney. Although *DEFB1* is map outside the beta-defensins repeat unit (Hollox et al., 2008d), it gives a hint of how significant an inflammatory effect can be resulted in relation to this antimicrobial peptide. Thus, a more profound consequence is speculated if association exist with other beta-defensins of variable repeats through alteration in product dosage. Nevertheless, the reports concerning this locus are still lacking. Hence, further investigation of the effect of beta-defensins copy number variable gene to the development of T2D is crucial to elucidate the role played by copy number variations in complex metabolic disease. Therefore, in this study, copy number of beta-defensins is quantified by using Paralogue Ratio Test (PRT) which is a comparative PCR technique. Validation of the results was carried out by using insertion-deletion (indel) polymorphism measurement and microsatellite analysis. In most diagnostic laboratory, white blood cell (WBC) count which acts as a parameter was utilised in this study to assess the inflammatory status of the patients. Thus, the relationship between this biological marker and beta-defensins is investigated from the genetics point of view.

1.2 Problem statement

The contribution of copy number variation towards diseases mostly involves genes that mediate the immune system; thus, predisposing humans to either infectious or inflammatory diseases, with respect to the gene dosage (Wain et al., 2009). Beta-defensins gene has an extensive variation and this variation has been demonstrated in healthy individuals from other populations (Hollox et al., 2003; Hollox, 2008d). Research in distinguishing between non-threatening and disease-causing variants involving copy number variation is still in the early stages (Zhang et al., 2009). The hurdles faced in understanding copy number variations increases when it involves complex diseases, as seen in T2D. Therefore, this study was carried out with emphasis on beta-defensins copy number variable gene due to limited knowledge in Malaysian context to serve as a reference.

1.3 Significance of study

The findings generated from this study will remarkably contribute particular data and knowledge for beta-defensins gene distribution in Malaysian context for research concerning copy number variations. In addition, this knowledge can be utilised by other researchers to develop personalised medicine for treating T2D or any diseases related to beta-defensins imbalances more effectively.

1.4 Hypothesis

It is hypothesised that there will be a different copy number distribution of beta-defensins between Malaysian and Caucasian control population. Besides that, copy number distribution between major ethnics of Malaysian individuals is speculated to show significant differences. Variation in copy number distribution could modify immune products dosage, whereby indirect attacks on the host cells could be exerted. This mechanism will introduce inflammation in T2D patients.

1.5 Objectives

1.5.1 General objective

To investigate the relationship between copy number variation of beta-defensins with inflammation found in T2D.

1.5.2 Specific objectives

1) To quantify beta-defensins copy number in Malaysian controls and compare it with established copy number distribution in Caucasian individuals as the reference population.
2) To quantify beta-defensins copy number in T2D individuals and compare it with copy number distribution in Malaysian control population.
3) To investigate the risk of developing T2D between major ethnics of Malaysian population with respect to variable copies of beta-defensins gene.
4) To determine the relationship between beta-defensins copy number and WBC count in T2D population.

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