UNIVERSITI PUTRA MALAYSIA

DESCRIPTIVE STUDY ON THE ORIGIN AND EVOLUTION OF BETA- DEFENSINS COPY NUMBER VARIABLE GENE USING MULTIGENERATIONAL FAMILY PEDIGREES

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By

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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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Chairman : Suhaili binti Abu Bakar @ Jamaludin, PhD
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Beta-defensins (*DEFB*) consists of *DEFB4, DEFB103, DEFB104, DEFB105, DEFB106, DEFB107, DEFB108, DEFB109, DEFB114, DEFB118, DEFB123*, *DEFB126* and *SPAG11* that are clustering on chromosome 8p23.1. *DEFB* mainly involves as the first line of defence against microorganism, especially in the mucosal and epithelial tissues. *DEFB* is one of the copy number variable gene (CNVs) and has been in close association with disease susceptibility especially in higher copy number. *DEFB* commonly varies between two and seven copies per diploid. Hence, it has been reported that an individual with a higher copy number (more than four) is more susceptible towards psoriasis but protective against HIV disease. Moreover, as *DEFB* is varying in copy number, for an individual with three *DEFB* diploid copies, the haploid copies contributed from each parent is ambiguous whether in a combination of 0+3 or 1+2. Thus, family pedigree was used in this study to follow the *DEFB* segregation in haploid copies from parents to children. The family pedigree is a diagram that listing the members and ancestral relationship in a family and commonly used in the study of human hereditary. Furthermore, pedigree does assist in the determination of the best treatment and clinical decision of an individual as a specific genetic makeup can be determined for each individual as consideration for the personalize medicine. Therefore, in this study, *DEFB* copy number both in diploid and haploid copies were measured and haplotype transmission from parents to children were followed for all families. Hence, eight (8) multigenerational families consist of four (4) Malay and four (4) Chinese families with a total of 51 individuals were selected to achieve the objectives of this study. The diploid copy numbers of *DEFB* was quantified using two Paralogue Ratio Tests (PRTs). Additionally, three multiallelic markers; two microsatellites, EPEV1 and EPEV3, and one indel (rs5889219) were used to validate the diploid copy number and to follow the segregation of haploid copies. This present study found *DEFB* copy number varies between three to six per diploid with five copies as the most common. The copy number is not widely varying due to close-related relationship within the family pedigrees. Segregation analysis from multiallelic markers has been successfully inferred

the haploid copies by following the segregation of the alleles throughout the generation except for three (3) families and found *DEFB* varies between one to four copies per haploid with two and three as the most common. Alleles size range from 171 bp to 189 bp and 135 bp to 145 bp have been noted in EPEV1 and EPEV3 respectively. Meanwhile, allele 185 bp for EPEV1 and 143 bp for EPEV3 continuously appeared in each generation. From the alleles collected, haplotype for each individual was successfully determined by following the inheritance pattern from parents to children in five (5) families. The recombinant event was discovered in one of the pedigrees where the children inherited the recombinant haplotype from the father, but it did not change the *DEFB* copy number of the affected children. In conclusion, the origin of the *DEFB* was successfully determined by following the haplotype transmission from the parents to the children. Moreover, *DEFB* copy number was successfully quantified in both diploid and haploid copies.

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KAJIAN DESKRIPTIF TENTANG ASAL USUL DAN EVOLUSI NOMBOR SALINAN VARIASI GEN BETA-DEFENSIN MENGGUNAKAN SUSUR GALUR KETURUNAN PELBAGAI GENERASI

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Beta-defensins (*DEFB*) terdiri daripada *DEFB4, DEFB103, DEFB104, DEFB105, DEFB106, DEFB107, DEFB108, DEFB109, DEFB114, DEFB118, DEFB123, DEFB126* dan *SPAG11* yang berkumpul di kromosom 8p23.1. Kebiasaannya, *DEFB* terlibat sebagai barisan pertahanan yang pertama dalam melawan mikroorganisma, terutamanya di tisu mukosal dan epitelium. *DEFB* adalah satu daripada gen pelbagai bilangan salinan (CNV) dan kerap dikaitkan dengan beberapa jenis penyakit terutamanya dibilangan salinan yang tinggi. *DEFB* biasanya berbeza antara dua hingga tujuh salinan bagi satu genom diploid. Telah dilaporkan bahawa bilangan salinan tinggi (lebih dari empat salinan), seorang individu akan mudah terdedah dengan psoriasis tetapi lebih terlindung dari jangkitan HIV. Disebabkan *DEFB* adalah gen pelbagai bilangan salinan, bagi individu yang mempunyai tiga salinan diploid *DEFB*, salinan haploid yang disumbangkan oleh setiap ibu bapa adalah samar-samar sama ada dalam hasil gabungan 0+3 atau 1+2. Oleh itu, salasilah keluarga digunakan dalam kajian ini untuk mengikuti pengasingan *DEFB* dalam salinan haploid. Salasilah keluarga merupakan gambar rajah yang menyenaraikan hubungan ahli keluarga dan juga susur galur keturunan dalam sesebuah keluarga dan kebiasaannya digunakan dalam kajian mengenai keturunan manusia. Selain itu, salasilah keluarga juga dapat membantu dalam menentukan rawatan serta keputusan klinikal bagi seorang individu. Hal ini berikutan setiap individu mempunyai genetik yang spesifik yang membolehkan perubatan yang khusus direka untuk mereka. Oleh yang demikian, *DEFB* bilangan salinan bagi diploid dan haploid ditentukan, dan penurunan haplotip dari ibu bapa kepada anak-anak diikuti bagi semua keluarga. Dengan itu, keluarga berbilang generasi yang terdiri daripada empat (4) keluarga Melayu dan empat (4) keluarga Cina dengan sejumlah 51 individu dipilih untuk mencapai objektif kajian ini. Nombor salinan diploid *DEFB* ditentukan dengan menggunakan dua penanda ujian nisbah paralog (PRT). Tiga penanda multialelik turut digunakan; dua mikrosatelit, EPEV1 dan EPEV3, dan satu indel (rs5889219) untuk mengesahkan nombor salinan diploid dan juga untuk mengikuti pengasingan *DEFB*

dalam salinan haploid. Kajian ini mendapati nombor salinan *DEFB* bervariasi antara tiga hingga enam setiap diploid dengan lima salinan yang paling kerap. Nombor salinan didapati tidak terlalu bervariasi berikutan hubungan rapat dalam salasilah keluarga. Analisis pengasingan daripada penanda multialelik telah berjaya menyimpulkan salinan haploid dengan mengikuti pemisahan alel sepanjang generasi kecuali untuk tiga (3) keluarga. Oleh itu, pemerhatian telah menunjukkan *DEFB* bervariasi antara satu hingga empat salinan setiap haploid dengan dua dan tiga salinan merupakan yang paling kerap. Julat saiz alel dari 171 bp ke 189 bp untuk EPEV1, dan 135 bp ke 145 bp untuk EPEV3 telah dicatatkan. Sementara itu, alel 185 bp untuk EPEV1 dan 143 bp untuk EPEV3 terus muncul dalam setiap generasi. Daripada alel yang diikuti, haplotip untuk setiap individu dapat ditentukan dengan mengikuti susur galur daripada ibu bapa kepada anak-anak untuk lima (5) keluarga. Rekombinasi ditemui disalah satu keluarga dimana anak-anak telah mewarisi rekombinasi haplotip daripada ayah, namun ia tidak mengubah nombor salinan *DEFB* anak-anak yang terlibat. Konklusi, asal usul nombor salinan *DEFB* dapat dijejaki dengan mengikuti pengagihan haplotip dari ibu bapa kepada anak-anak. Selain itu, nombor salinan *DEFB* dapat ditentukan bagi diploid dan haploid.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
3’	Three prime
5’	Five prime
A	Adenine
ACD	Avellino Corneal Dystrophy
array-CGH	Array-based Comparative Genomic Hybridisation
ADHD	Attention-Deficit/Hyperactivity Disorder
AMP	Antimicrobial Peptides
AMY	Amylase gene
ARNSHL	Autosomal Recessive Non-Syndromic Hearing Loss
ASD	Autism Spectrum Disorder
bp	Base Pair
C	Cytosine
CCL3L1	Chemokine Ligand-3-like 1
CE	Capillary Electrophoresis
CEPH	Centre de’Etude du Polymorphisme Human
CN	Copy Number
CNVs	Copy Number Variations
CNVR	Copy Number Variation Region
DEFA	Alpha-defensin
DEFB	Beta-defensin
DEFT	Theta-defensin

DM	Diabetes Mellitus
DNA	DeoxyriboNucleic Acid
dNTPs	DeoxyriboNucleotide Triphosphates
DZ	Dizygotic Twins
EDTA	EthyleneDiamine Tetra-Acetic acid
G	Guanine
CWAS	Genome-wide Association Study
HIV	Human Immunodeficiency Virus
HL	Hearing Loss
HRC	Human Random Control
Indel	Insertions and deletions
MAPH	Multiplex Amplifiable Probe Hybridisation
MLPA	Multiplex Probe Ligation Assay
ml	Millilitre
mM	Mili-Molar
MS	Multiple Sclerosis
MZ	Monozygotic Twins
ng/µl	Nanogram per microlite
PCR	Polymerase Chain Reaction
PRT	Paralogue Ratio Test
PPRT	Pyrosequencing Paralogue Ratio Test
RA	Rheumatoid arthritis
REDVR	Restriction Enzyme Digest Variant Ratio
rpm	Rotation per minute

RTU	Ready To Use
SLE	Systemic Lupus Erythematous
SNPs	Single Nucleotide Polymorphism
SSLP	Simple Sequence Length Polymorphism
SSR	Simple Sequence Repeats
STRs	Short Tandem Repeats
T	Thymine
Taq	Thermos aquatics
TBE	Tris/Borate/EDTA
UV	Ultra Violet

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Mendel's law of segregation stated that two alleles of one gene will segregate independently into a gamete of an individual. In other word, an individual will receive one allele from each parent to give a total of two alleles for a gene. Each allele will carry a different copy number that later on will combine to give a diploid copy number that is different between parents and children, and even among siblings because of normal segregation of homologous chromosome for every individual (Wain *et al*., 2009).

Family pedigree by definition is a genetic representation of a family tree by showing a genetic relationship between family members in a diagram form. The pedigree can be helpful in tracking the inheritance pattern of a trait or disease from one generation to the following generation. In term of copy number, the pedigree can be helpful in a deduction of copy number genotype for each individual (Hollox, 2008) and to determine the inheritance pattern of a gene.

Beta-defensins (*DEFB*) consist of multiple genes including *DEFB4, DEFB103, DEFB104, DEFB105, DEFB106, DEFB107, DEFB108, DEFB109* and *SPAG11* that clustering on chromosome 8p23.1 (Wain *et al*., 2014; Bentley *et al*., 2010; Hollox *et al*., 2008; Premratanachai *et al*., 2004; Taudien *et al*., 2004;). *DEFB* act as a first line of defence in innate immune system against a microorganism specifically at epithelial tissues such as lung, kidney, pancreas, trachea and mammary gland (Bensch *et al*, 1995; Zhao *et al*., 1996; Klotman & Chang, 2006; Ferris *et al*., 2013). *DEFB4* also act as a chemokine for cells of the adaptive immune response (Biragyn *et al*., 2002; Yang *et al*., 2002; Yang *et al*., 1999). *DEFB* span at least 260 kb in size when measured by pulsed-field gel analysis (Hollox *et al*., 2003). *DEFB* copy number varies between two to seven copies per diploid genome (Wain *et al*., 2009; Linzmeier & Ganz, 2005; Hollox *et al*., 2003). Based on seven populations include Yorubans, German, Dutch, British, Utah, Japanese and Chinese, *DEFB* showed modal diplotype of four copy number (Hollox, 2008; Hollox *et al*., 2008).

DEFB copy number can be quantified using a triplex PCR system involves two paralogue ratio tests (PRTs) and one multiallelic indel marker. PRT has an ability to amplified two chromosomes simultaneously; one from the test chromosome and one from reference chromosome by using a single primer (Armour *et al*., 2007). By taking the ratio of test to reference peak height or area, *DEFB* copy number per diploid can be estimated. Meanwhile, indel (rs5889219) a 2 bp/5 bp deletion at chromosome 8 was selected to validate the diploid *DEFB* copy number within the pedigrees (Abu Bakar *et al*., 2009). Microsatellite analysis is used to collect the *DEFB* alleles and act as a good

supporter to support the diploid copy number measured by determine the allele ratio of an individual (Abu Bakar *et al.,* 2009; Hollox *et al*., 2005). Microsatellite is a short DNA sequence from 1 to 5 nucleotides long that are tandemly repetitive (Powell *et al*., 1996) and commonly known as short tandem repeats (STRs).

Thus, this study firstly aimed to quantify *DEFB* diploid copy number by PRT assay. Then information from microsatellites and indel assay are used to collect all *DEFB* alleles and to follow the segregation of the haplotype for each individual which indirectly helps in the determination of haploid copy of *DEFB*. By following the haplotype transmission from parents to children, the inheritance pattern of *DEFB* for each pedigree participate can be observed.

1.2 Problem Statements

Many previous studies on copy number variation only reported the diploid copy of the gene, and less number of studies are carried out to determine the haploid copy number. Theoretically, each gene exists as two copies, where an individual inherits one copy from the mother and one copy from the father that gives a diploid copy of two. However, there are few genes that have been discovered to show diploid copy more than two. They are known as copy number variations (CNVs) gene such as *DEFB*, *CCL3L1,* and *AMY1* (Aldhous *et al*., 2010; Walker *et al*., 2009; Perry *et al*., 2007). For these genes, the theory of each parent contributes only one copy of allele for their child is not applicable. If their child has five copy number of *DEFB*, there are three possible haplotype combination from their parents to give the total copy number of five; either 2+3 or 1+4 or 0+5. This event could only be solved by segregation analysis of copy number alleles in a family pedigree. Based on the law of segregation, every child will inherit their genetic traits from both parents. Thus, contribution copy numbers from each parent could also be identified through haplotype segregation to give a total of diploid copy for the children. Furthermore, by using pedigree analysis, the origin of copy number haplotype that is passing down through generations can be identified.

Therefore, this study was designed to further investigate the haploid copy of *DEFB* as in Malaysia it had been found that *DEFB* can vary between two and eight copies per diploid genome. However, there is a lack of information on haploid copies that have been reported.

1.3 Significance of the Study

Significance of this study could be viewed from multiple prospective. The first prospective is on the inheritance pattern of *DEFB* copy number. Within a pedigree that both parents showed higher copy number, their children have higher tendency to inherit the high copy number too. For example, the father has five copies of *DEFB,* and the mother also has five copies. Both give a possible haplotype combination either 0+5 or 1+4 or 2+3. When the father has a combination of 1+4 and the mother has a combination

of 2+3, the children will inherit a copy number range between three and seven which commonly considered as high copy number. Similar goes to the parents with low copy number, probability of their children to inherit low copy number is increasing Meanwhile, if one parent has a high copy number and another parent has a low copy number, the pattern of the children copy number can vary between low to high copy number. Hence, by knowing the *DEFB* copy number of the parents, we can actually predict the copy number's range of the children directly, and indirectly the future copy number that would possibly generated in Malaysian.

The second prospective was on the association of copy number with disease susceptibility. To date, it has been found that *DEFB* copy number variation plays a major role in diseases susceptibility especially at high copy number. The high *DEFB* copy number has been associated with susceptibility of autoimmune disease such as psoriasis (Hollox *et al*., 2008), but protective against infectious disease such as human immunodeficiency virus (HIV) (Sun *et al*., 2005). Hence, by knowing the copy number, a precaution step can be made for the individual involved. For example, if an individual has *DEFB* copy number of six, he or she may have a higher risk of getting psoriasis compared to individual who has a copy number of two. Thus, that individual can be more aware of the symptoms and causes of the disease, and therefore would be more cautious with the surrounding.

1.4 Research Objective

1.4.1 General Objective

To investigate the origin and evolution of *DEFB* copy number variable genes using family pedigree among normal subjects in Malaysia.

1.4.2 Specific Objective

1. To quantify *DEFB* diploid copy number in family members of multigenerational pedigrees by using Paralogue Ratio Test (PRT)
2. To determine the haploid copies of *DEFB* by following the transmission of microsatellites alleles collected within the region among the family's members.
3. To construct the haplotype of *DEFB* for the whole family pedigrees.

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