

**OBESITY STUDY IN MALAYSIAN MALAYS WITH FOCUS ON
CANDIDATE GENES AND BIOMARKERS**

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Dedication

*I would like to dedicate my thesis to
my parents, Mr. Apalasaamy & Mrs. Subbamaah,*

And

To Lord Shiva & Rudra

Abstract

Obesity is in rising prevalence globally and it has been associated with high mortality rate and other comorbidities. Obesity is a highly heritable disorder but genes responsible for hereditary variations remain to be largely elusive. Recent findings have shown that single nucleotide polymorphisms (SNPs) in the following genes predispose to the increased risk of obesity and obesity-related traits: leptin (LEP), melanocortin-4 receptor (MC4R), β_2 -adrenoceptor (ADRB2), insulin-induced gene 2 (INSIG2), syndecan 3 (SDC3), fat mass and obesity associated (FTO), resistin (RETN) and adiponectin (ADIPOQ). Biomarkers such as adiponectin, leptin and resistin have been shown to be tightly linked to obesity related metabolic pathways. This study was aimed to investigate association between obesity and obesity-related parameters such as Body Mass Index (BMI), body weight, height, waist circumference, hip circumference, waist hip ratio, blood pressure, cholesterol and lipid parameters with SNPs and biomarker levels. A total of 672 Malaysian Malay subjects were studied. Genotyping was carried out using two methods, namely, Real-Time PCR Taqman[®] SNP genotyping assays and also Sequenom MassARRAY. Data were analyzed using SPSS 16.0 statistical software and Haploview version 4.2. After adjustment with age, gender, related biomarker levels and Bonferroni correction, the present study exhibited significant associations between FTO rs17817288 with LDL-Cholesterol; MC4R rs571312 with logBMI and systolic blood pressure; MC4R rs2229616 SNP with total cholesterol; ADRB2 rs1042714 SNP with diastolic blood pressure, RETN rs3219175 and rs34861192 SNPs with weight and log-resistin levels. Strong linkage disequilibrium (LD) pattern was observed in resistin, FTO, ADRB2 and LEP gene. There is low LD in ADIPOQ and MC4R gene regions in this population. The leptin haplotype designated as GCCGGAA in this study, was associated with obesity in

Malaysian Malays. This study suggests that the variants of the MC4R, ADRB2, LEP, RETN and FTO genes may have important roles for the development of obesity-related metabolic traits in the Malaysian Malay population. Levels of adiponectin and leptin in plasma were linked to obesity and metabolic abnormalities in Malaysian Malays but resistin appeared have less effect on obesity and metabolic abnormalities.

Abstrak

Obesiti kini adalah pada kadar yang semakin meningkat di seluruh dunia dan ianya dikaitkan dengan kadar kematian yang tinggi dan juga ko-morbiditi yang lain. Obesiti adalah satu gangguan kesihatan yang nilai pewarisannya amat tinggi tetapi faktor-faktor genetik utama yang benar-benar terlibat adalah sukar untuk dipastikan. Penemuan terkini telah menunjukkan bahawa polimorfisma- polimorfisma nukleotida tunggal (SNPs) pada gen-gen berikut mempengaruhi obesiti dan ciri-ciri yang berkaitan dengan obesiti: leptin (LEP), reseptor melanocortin-4 (MC4R), β_2 -adrenoseptor (ADRB2), perangsang insulin 2 (INSIG2), syndecan 3 (SDC3), gen kaitan jisim lemak dan obesiti (FTO), resistin (RETN) dan adiponectin (ADIPOQ). Biomarker seperti adiponectin, leptin dan resistin telah dikait rapat dengan laluan metabolik obesiti yang berkaitan. Tujuan kajian ini adalah untuk menyiasat perkaitan antara obesiti dan parameter-parameter yang berkaitan dengan obesiti seperti Index Jisim Badan, berat badan, ketinggian, lilitan pinggang, lilitan pinggul, nisbah pinggang pinggul, tekanan darah, kolesterol dan parameter lipid dengan SNP dengan aras biomarker. Sebanyak 672 subjek Melayu Malaysia telah dikaji. Ciri genotaip (“genotyping”) telah dijalankan dengan menggunakan dua cara iaitu Real-Time PCR Taqman[®] ujian SNP dan juga Sequenom MassARRAY. Data dianalisiskan dengan mengguna SPSS 16.0 statistik perisian dan version Haploview 4.2. Selepas pelarasan dengan umur, jantina, aras biomarker dan pembetulan Bonferroni, kajian ini menunjukkan bahawa terdapat signifikans antara FTO rs17817288 dengan LDL-Kolesterol; MC4R rs571312 dengan logBMI dan dengan tekanan darah sistolik; MC4R rs2229616 dengan jumlah kolesterol; ADRB2 rs1042714 dengan tekanan darah sistolik; SNP-SNP RETN rs3219715 dan rs34861192 dengan berat badan dan tahap log-resistin. “Linkage disequilibrium” (LD) yang nyata diperhatikan bagi gen-gen resistin, FTO, ADRB2 dan

LEP. Kajian ini menunjukkan LD di kawasan gen-gen ADIPOQ dan MC4R adalah lemah dalam populasi ini. Haplotaip GCCGGAA bagi gen leptin telah dikaitkan dengan obesiti di populasi Melayu Malaysia. Kajian ini mencadangkan bahawa varian MC4R, ADRB2, LEP, RETN, ADIPOQ dan gen FTO mungkin mempunyai peranan penting untuk pembangunan ciri-ciri yang berkaitan dengan obesiti dan gangguan metabolik dalam populasi Melayu Malaysia. Aras adiponectin dan leptin dalam plasma dikaitkan dengan obesiti dan keabnormalan metabolik tetapi resistin nampaknya mempunyai kesan yang lemah ke atas obesiti dan keabnormalan metabolik dalam populasi Melayu Malaysia.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg-Microgram

µl-Microliter

ADIPOQ-Adiponectin

ADRB2-β₂-adrenoceptor

AGRP-Agouti related peptide

ASW-African ancestry in Southwest USA

BDNF-Brain-derived neurotrophic factor

BMI- Body mass index

BSA-Bovine serum albumin

CADM2-Cell adhesion molecule 2

CETP-Cholesteryl ester transfer protein

CEU-Utah residents with Northern and Western European ancestry

CHB-Han Chinese in Beijing, China

CHD-Chinese in Metropolitan Denver, Colorado

CHD-Coronary heart disease

CHS-Singaporean Chinese

CNVs-Copy Number Variations

CRP- C-reactive protein

CTNBL1-Catenin beta like 1

CVD-Cardiovascular disease

D'-Coefficient of linkage disequilibrium

DBP-Diastolic blood pressure

dbSNP-The single nucleotide polymorphism database

df-Degree of freedom

DNA-Deoxyribonucleic acid

DRD2-Dopamine D2 receptor

EDTA-Ethylenediaminetetraacetic acid

ER-Endoplasmic reticulum

LIST OF ABBREVIATIONS AND SYMBOLS (Cont.).

ETV5-ets variant 5

FANCL-Fanconi anemia, complementation group L

FTO-Fat mass and obesity associated gene

GHRL-Ghrelin

GIANT-Genetic Investigation of Antropometric Traits Consortium

GIH-Gujarati Indians in Houston, Texas

GLM-General linear method

Gln-Glutamine

Glu-Glutamic acid

GNB3G-Protein beta 3 subunit gene

GNPDA2-Glucosamine-6-phosphate deaminase 2

GPRC5B-G protein-coupled receptor, family C, group 5, member B

GWAS-Genome-wide association studies

HapMap-Haplotype Map

HC-Hip Circumference

HDL-C-High density lipoprotein-cholesterol

HMG-CoA-3-hydroxy-3-methylglutaryl-coenzyme A

HRP-Horseradish Peroxidase

HWE-Hardy-Weinberg equilibrium

IDF-International Diabetes Federation

IL-1- Interleukin-1

IL-Interleukin-6

INSIG2-Insulin-induced gene 2

INS-Insulin

INS-Singaporean Indians

JPT-Japanese from Tokyo, Japan

KCTD15-Potassium channel tetramerisation domain containing 15

kg-kilogram

LDL-Low density lipoprotein

LIST OF ABBREVIATIONS AND SYMBOLS (Cont.).

LD-Linkage disequilibrium

LEPR-Leptin receptor

HSL (LIPE)-Hormone-sensitive lipase

LRP1B-Low density lipoprotein receptor-related protein 1B

LWK-Luhya in Webuye, Kenya

m/z-Mass-to-charge ratio

MAF-Minor allele frequency

MALDI-TOF-Matrix-assisted laser desorption ionization –time-of-flight

MAP2K5-Mitogen-activated protein kinase kinase 5

MAS-Singaporean Malays

MC4R- Melanocortin-4 receptor

MCP-1-Monocyte chemotactic protein-1

MEX-Mexican ancestry in Los Angeles, California

ml-milliter

m-meter

mRNA- Messenger ribonucleic acid

MTCH2-Mitochondrial carrier 2

MTMR9-Myotubularin related protein 9

NCR3C1-Glucocorticoid receptor

NEFA-Nonesterified fatty acid

NEGR1-Neuronal growth regulator 1

NHMS- Third National Health and Morbidity Survey

nl-nanoliter

nm-nanometer

NTRK2-Neurotrophic tyrosine kinase, receptor type 2

NUDT3-Nudix (nucleoside diphosphate linked moiety X)-type motif 3

OD-Optical Density

PAI-1-Plasminogen activator inhibitor

PBS-Phosphate buffered saline

LIST OF ABBREVIATIONS AND SYMBOLS (Cont.).

PC1-Proconvertase 1

pg-pigogram

POMC-Pro-opiomelanocortin

PPAR γ -Peroxisome proliferator activated receptor γ

PRKD1-Protein kinase D1

QPCTL-Glutaminyl-peptide cyclotransferase-like

r²-Correlation coefficient

RBP4-Retinol binding protein-4

RETN-Resistin

RPL27A-Ribosomal protein L27a

SBP-Systolic blood pressure

SCAP-SREBP cleavage-activating protein

SDC3-Syndecan 3

sdLDL-Small, dense low-density lipoprotein

SDS-Sequence Detection System

SGVP-Singaporean Genome Variation Project

SH2B1-Adaptor protein 1

SLC39A8-Solute carrier family 39 (zinc transporter), member 8

SNP- Single nucleotide polymorphism

SREBPs-Sterol regulatory element binding proteins

T2DM DM-Type 2 Diabetes mellitus

TC-Total cholesterol

TG-Triglyceride

TMB-Tetramethyl-benzidine

TMEM160-Transmembrane protein 160

TMEM18-Transmembrane protein 18

TNF- α -Tumor necrosis factor-alpha

TNNI3K-TNNI3 interacting kinase

TSI-Toscans in Italy

LIST OF ABBREVIATIONS AND SYMBOLS (Cont.).

UCP1-Uncoupling protein 1

UCP2-Uncoupling protein 2

UCP3-Uncoupling protein 3

UPR-Unfolded protein response

WC-Waist circumference

WHO- World Health Organization

WHR-Waist-Hip-Ratio

YRI-Yoruba in Ibadan, Nigeria

ZNF608-Zinc finger protein 608

α -MSH- α -melanocyte-stimulating hormone

β 2-AR- β 2-adrenergic receptor

β 3-AR- β 3-Adrenergic receptor

χ 2-Chi square