ASSOCIATION OF *TCF7L2* VARIANTS WITH TYPE 2 DIABETES MELLITUS SUSCEPTIBILITY AND TREATMENT OUTCOME IN A MULTI-ETHNIC MALAYSIAN POPULATION

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a multifactorial disease, where a combination of risk factors interacts and plays different roles, culminating in the disease proper. One such risk factor is genetics. Single nucleotide polymorphisms (SNPs) in several genes have been implicated in rising T2DM risk; one such gene is the transcription factor 7-like-2 (*TCF7L2*), which has been showed to blunt incretin action (specifically glucagon-like peptide-1, GLP-1), leading to impaired glucose-induced insulin secretion. Furthermore, these SNPs were also associated with an impaired therapeutic response to the sulfonylurea group of oral antidiabetic agents. To date, there is only one small scale study that looked at the effect of SNPs in *TCF7L2* on T2DM in a Malaysian population, and no studies looking at the effect of these SNPs on therapeutic response to any antidiabetic agent in T2DM subjects. Our study attempted to address these deficiencies.

A total of 1008 subjects comprising of 642 T2DM and 366 non-T2DM subjects, who fulfilled the inclusion and exclusion criteria, gave written informed consent to be included in this cross-sectional case-control study. Five SNPs in TCF7L2 (rs7903146, rs12255372, rs11196205, rs7901605 and rs4506565) were genotyped from peripheral leukocytes of the subjects using TaqMan SNP genotyping assays on a real-time PCR platform. Anthropometric and treatment data were obtained from interviews and medical records. Glycated haemoglobin levels were measured in the T2DM subjects. All data were analysed using IBM SPSS Statistics (version 19) and Haploview (version 4.2) softwares.

This study showed that in a Malaysian population, the variant alleles of the SNPs examined were significantly more common in T2DM compared to non-T2DM subjects. The strongest association was observed in rs4506565 (p=5e-4; OR 1.97, CI 1.35 – 2.88). The haplotype TT containing both variant alleles of rs4506565 and rs7903146 were significantly more common in the T2DM subjects (p=9.97e-5; OR 1.98, CI 1.41 – 2.84). A significantly higher mean HbA1c was observed in patients on insulin therapies (alone or with oral medications) carrying the TT genotype of rs4506565. In a subset of subjects on a combination of metformin and a sulfonylurea drug, there were significantly higher mean HbA1c values in heterozygous genotype carriers of rs7903146, rs11196205, rs7901695 and rs4506565. In the same subset, heterozygous genotype carriers of rs11196205, rs7901695 and rs4506565 were associated independently with failure to achieve HbA1c target of 6.5%.

In conclusion, TCF7L2 gene polymorphism is associated with T2DM in a Malaysian population. Furthermore, carriers of the variant alleles are associated with higher HbA1c levels, which could indicate more severe disease.

ABSTRAK

Penyakit diabetes jenis 2 (T2DM) adalah penyakit yang mempunyai faktor risiko yang pelbagai, di mana kombinasi faktor-faktor ini berinteraksi dan memainkan peranan berbeza, yang akhirnya menghasilkan penyakit tersebut. Salah satu faktor risiko itu ialah genetik. Polimorfisme tunggal nukleotida (PTN) dalam beberapa gen telah dikaitkan dengan peningkatan risiko T2DM; salah satu gen tersebut adalah *transcription factor 7-like-2* (*TCF7L2*) yang menyebabkan pengurangan tindakan hormon-hormon *incretin* (seperti *glucagon like peptide-1*, GLP-1) lantas menjejaskan rembesan insulin yang didorongi glukosa. Selain itu, PTN ini juga dikaitkan dengan pengurangan kesan terapi dengan ubat antidiabetik daripada kumpulan *sulfonylurea*. Pada masa ini, hanya terdapat satu kajian kecil yang pernah dijalankan untuk melihat kesan PTN dalam gen *TCF7L2* ke atas T2DM di kalangan penduduk Malaysia. Tambahan pula, belum lagi terdapat kajian yang melihat kesan PTN ini terhadap kesan terapi dengan menggunakan mana-mana ubat antidiabetik ataupun insulin dalam pesakit T2DM.

Sejumlah 1008 subjek yang terdiri daripada 642 pesakit T2DM dan 366 subjek tanpa T2DM yang memenuhi kriteria kemasukan dan pengecualian, telah memberi kebenaran secara bertulis untuk dimasukkan ke dalam kajian ini. Lima PTN dalam gen *TCF7L2* (rs7903146, rs12255372, rs11196205, rs7901605 dan rs4506565) dianalisa daripada DNA sel darah putih subjek-subjek tersebut dengan menggunakan kaedah *TaqMan* pada platform *real-time PCR*. Maklumat antropometri dan rawatan diperolehi melalui temuduga subjek dan rekod perubatan mereka. Tahap *glycated haemoglobin* juga diukur pada subjek T2DM. Semua data dianalisis dengan menggunakan perisian *IBM SPSS Statistics* (versi 19) dan *Haploview* (versi 4.2).

Kajian ini menunjukkan bahawa di kalangan sekumpulan penduduk Malaysia, alel varian daripada PTN yang diperiksa, lebih biasa dijumpai pada subjek T2DM berbanding subjek bukan-T2DM. Perkaitan yang kuat telah diperhatikan bagi rs4506565 ($p = 5e^{-4}$, OR 1.97, CI 1.35 - 2.88). Haplotaip TT, yang mengandungi kedua-dua alel varian rs4506565 dan rs7903146, didapati lebih tinggi frekuensinya dalam subjek T2DM ($p = 9.97e^{-5}$; OR 1.98, CI 1.41 - 2.84). Dikalangan pesakit yang menerima terapi insulin (tunggal atau beserta ubatan oral), purata HbA_{1c} lebih tinggi pada pesakit yang membawa genotip TT rs4506565. Pada subset subjek yang dirawat dengan gabungan *metformin* dan ejen *sulfonylurea*, purata HbA_{1c} adalah lebih tinggi pada pembawa genotip heterozigot rs7903146, rs11196205, rs7901695 dan rs4506565.

Pada subset yang sama, pembawa genotip heterozigot rs11196205, rs7901695 dan rs4506565 juga dikaitkan dengan kegagalan untuk mencapai sasaran HbA_{1c} sebanyak 6.5%.

Kesimpulannya, polimorfisme tunggal nukleotida pada gen *TCF7L2* dikaitkan dengan T2DM di kalangan penduduk Malaysia. Selain itu, pembawa alel varian dikaitkan dengan paras HbA_{1c} yang lebih tinggi, yang mungkin menunjukkan tahap penyakit yang lebih teruk.

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ETHICAL APPROVAL

All participants in this study gave full, written informed consent and were subjected to methods approved by the Medical Ethics Committee of the University of Malaya Medical Centre (UMMC) [Appendix 1].

PUBLICATIONS ARISING FROM THE STUDY

PRESENTATIONS

1. Association of *TCF7L2* Gene Polymorphism with Type 2 Diabetes Mellitus in Malaysia: A Preliminary Report. Oral presentation at the 15th Meeting of Asean Federation of Endocrine Societies (AFES), 28 November – 1 December 2009, Bangkok, Thailand.

2. The Impact of *TCF7L2* Variants on Antidiabetic Treatment Regime Selection: A Malaysian Perspective. Oral presentation for the Young Investigator's Award at WorldPharma 2010, 17 – 23 July 2010, Copenhagen, Denmark [Appendix 2].

3. TCF7L2 polymorphisms: Their Impact on Diabetes and its Treatment. Poster presentation at the 26th Scientific Meeting of Malaysian Society of Pharmacology and Physiology, 18 – 20 May 2012, Penang, Malaysia [Appendix 3].

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ABBREVIATIONS

A	adenosine
ABCC8	ATP-binding cassette, subfamily C, member 8 gene
ANOVA	analysis of variance
ATP	adenosine triphosphate
BD	Becton, Dickinson and company
BF%	body fat percentage
BMI	body mass index
C	cytosine
CDK5	cyclin-dependent kinase 5
CDKAL1	CDK5 regulatory subunit associated protein1-like 1 gene
CDKN2A/B	cyclin-dependent kinase inhibitor 2A/B gene
CI	confidence interval
Cl^-	chloride ion
CYP	cytochrome P450
CYP2C9	CYP family 2, subfamily C, polypeptide 9
DKA	diabetic ketoacidosis
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DPP-IV	dipeptidyl peptidase-IV
FABP	fatty acid binding protein
FTO	fat mass and obesity associated gene
G	guanine
Gab1	GRB2-associated-binding protein 1
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter family
GWAS	genome-wide association study
H^+	hydrogen ion, proton
HbA_{1c}	glycated haemoglobin fraction
HHEX	haematopoietically expressed homeobox gene
HNF4α	hepatocyte nuclear factor 4-alpha gene
IBM	International Business Machines Corporation

IDE	insulin-degrading enzyme gene
IGF2BP2	insulin-like growth factor 2 mRNA binding protein 2 gene
IRS	insulin receptor substrate
K^+	potassium ion
KCNJ11	potassium inwardly-rectifying channel, subfamily J, member 11 gene
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1 gene
LD	linkage disequilibrium
LOD	logarithm (base 10) of odds
Mg^{2+}	magnesium ion
miRNA	micro RNA
MIT	Massachusetts Institute of Technology
MODY	maturity-onset diabetes of the young
mRNA	messenger RNA
MTF	metformin
NHMS III	3rd National Health and Morbidity Survey
OCT	organic cation transport
OR	odds ratio
PCR	polymerase chain reaction
PI3K	phophoinositide 3-kinase
PKC	protein kinase-C
PPARγ	peroxisome proliferator-activated receptor gamma
PTj	Pusat Tanggungjawab
RBC	red blood cell
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rs#	reference SNP identification number
SLC30A8	solute carrier family 30 (zinc transporter), member 8 gene
SNP	single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
Sulf	sulfonylureas
SUR	sulfonylurea receptor
T	thymine
T1DM	type 1 diabetes mellitus

T2DM	type 2 diabetes mellitus
Taq	*Thermus aquaticus*
TCF7L2	transcription factor 7-like-2 gene
UMMC	University of Malaya Medical Centre
USA	United States of America
WHO	World Health Organisation