

Polymorphism of Transcription Factor 7-Like 2 Gene and HOMA- β Level of Individuals With and Without Type 2 Diabetes Mellitus Family History

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Abstract

Family history has considered as a risk factor of type 2 diabetes. Transcription factor-7 like 2 (TCF7L2) has role to regulates insulin secretion and blood glucose homeostasis. The aim of current study was to determine the rs7903146 polymorphism of *TCF7L2* gene and *homeostatic model assessment- β* (HOMA- β) level on individual with and without type 2 Diabetes Mellitus (DM) family history. This work is a case-control study. Thirty six subjects with type 2 DM family history and 36 subjects without type 2 DM family history were recruited. HOMA- β measure to analyze the insulin secretion. Polymorphisms of *TCF7L2* gene was analyzed by using PCR-RFLP method. Statistical analysis was performed by using T-test, Mann-Whitney and Chi-square with significance level 0.05. The frequency of the T allele of the cases were 4.2% and the controls were 2.8% ($p=0.500$). The odd ratio was 0.649 (CI;95%:0.106-4.055). The HOMA- β levels of the cases were significant low (132.56 ± 62.48) compared with the controls (266.09 ± 1.68) with $p=0.000$. The subjects with type 2 DM family history have a similar frequency of having T alleles and CT/TT genotypes. The subjects with type 2 DM family history has significantly lower HOMA- β levels than subject without DM family history.

Keyword: Type 2 DM family history, *TCF7L2* gene, rs7903146 polymorphism, HOMA- β

Introduction

Diabetes mellitus (DM) is a multi-factorial disease which has a complex interaction between genetic and environment factors. Genetic factor is an important role to determine the incidence of familial diabetes (Bener *et al.*, 2013). Erasmus *et al.* (2001) has reported that the incidence of type 2 diabetic was contributed by genetic role and family aggregation in some population.

Family history is considered as a risk factor in type 2 DM (Bener *et al.*, 2013).

Harrison *et al.* (2003) has shown that diagnosis of DM will be increase 2-4 fold if one or both parents was suffering from diabetes mellitus.

The Transcription Factor 7-like 2 (TCF7L2) is a gene which contributed in the developing of type 2 DM. The *TCF7L2* gene has played a role to encoding the transcription factor which involved in Wntless-type mouse mammary tumor virus (MMTV) integration site family member (Wnt) of signal pathway (Lyssenko *et al.*, 2007). The Wnt signal has an important action to regulate several genes through *TCF7L2* activation, such as the expression of pro-glucagon gene which encoding the insulin-tropic hormone, glucagon-like peptide-1 (GLP-1). The *TCF7L2* activates pro-glucagon

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gene expression lead to GLP-1 secretion (Tong *et al.*, 2009). The glucagon-like peptide-1 has played a role in blood glucose homeostasis and elevates insulin secretion, so this could be explained that *TCF7L2* has an indirect role as a regulation factor of insulin secretion and blood glucose homeostasis (Tong *et al.*, 2009; Yu *et al.*, 2009).

The rs7903146 (IVS3C/T) polymorphism contributed 10-25% in all of diabetes cases (Xavier *et al.*, 2009). The rs7903146 polymorphism of *TCF7L2* gene has been related with type 2 diabetes which lead to impaired insulin secretion and elevated hepatic glucose production (Wegner *et al.*, 2008).

A number of studies have demonstrated that individuals with type 2 DM family history has 2-fold risk to suffering from type 2 DM than individuals without type 2 DM family history (Wicaksono, 2011). The genetic polymorphism of individuals with type 2 DM family history is interested to be investigated in Indonesian population. The aim of this study was to investigate the rs7903146 polymorphism of *TCF7L2* gene among individuals with and without type 2 DM family history in Indonesian population.

Materials and Methods

This study is a case-control study. The subject was divided into case and control groups. The case group consist of patient with type 2 DM family history was recruited from outpatients clinic of Dr. Sardjito Hospital Yogyakarta. The control group is subjects without type 2 DM family history which was recruited from community in Yogyakarta. This study has been approved by Medical and Health Research Ethics Committee, Faculty of Medicine, UGM.

Inclusion criteria of this study were healthy subjects, male or female at age 19-39 years old, BMI ≤ 24 kg/m² and signing the informed consent form.

Blood chemical examination

Whole blood samples which obtained from the subjects after informed consent has been done. Glucose oxidase-p-amino phenazone (GOD-PAP) method was used for measuring blood glucose line. Insulin level was analyzed from blood serum by ELISA method (DRG[®] kit) to obtain the HOMA- β level. The HOMA- β was calculated to determine the function of pancreatic beta cell with the following below (Chen *et al.*, 2012; Oya *et al.*, 2014):

DNA isolation

DNA was isolated using Wizard[®] Genomic DNA Purification Kit (Promega). DNA was isolated from 400 μ L leucocytes sample and was briefly performed by mixed the 900 μ L erythrocyte lyses buffer, 300 μ L nucleid lyses solution, 100 μ L protein precipitation solution, 300 μ L isopropanol, 300 μ L ethanol 70%, 100 μ L DNA rehydration solution and incubate for 12 hour at 4°C.

Polymerase chain reaction (PCR)

Amplification of DNA was performed by mixed the 15 μ L PCR mix, 11 μ L H₂O, 2 μ L DNA in a tube, then spin down for a minute at 3500 rpm. The primers (IDT, Inc) sequence for *TCF7L2* gene segment amplification were 5' - GAG AGC TAA GCA CTT TTT AGG TA - 3' (forward) and 5' - CTG ACA TTG ACT AAG TTA CTT GC - 3' (reverse). PCR amplification conditions were as follows: an initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 30 seconds, final extension at 72°C for 5 minutes and cooling at 4°C. The PCR program was running for 1h 36 minutes.

Restriction fragment length polymorphism (RFLP)

The enzymatic digestion of PCR product was performed by mixed the 0.5 μ L restriction enzyme Rsa1, 4.5 μ L H₂O, 1 μ L tango buffer, 4 μ L DNA in a tube. The reaction mixture was

incubated for 16 hour at 37°C. The C allele was represented by two fragments of 91 and 22 base pairs and T allele was represented by one fragment of 113 base pair.

Electrophoresis

Electrophoresis analysis was performed by using the 3% of agarose with ethidium bromide (EtBr).

Statistical analysis

Statistical analysis was performed by using univariate (mean, SD) and bivariate (independent t-test, Mann-Whitney and Chi-square) analysis.

Results

Subject Characteristic

We enrolled 72 subjects that consisted of 36 individual with type 2 DM family history and 36 individual without type 2 DM family history. The age, body mass index (BMI), blood pressure (systolic blood pressure and diastolic blood pressure), and fasting blood glucose were not statistically difference between subjects with and without type 2 DM family history (Table 1).

The rs7903146 polymorphism of TCF7L2 gene

The result of genotype analysis of rs7903146 polymorphism of TCF7L2 gene in this study was shown on Figure 1.

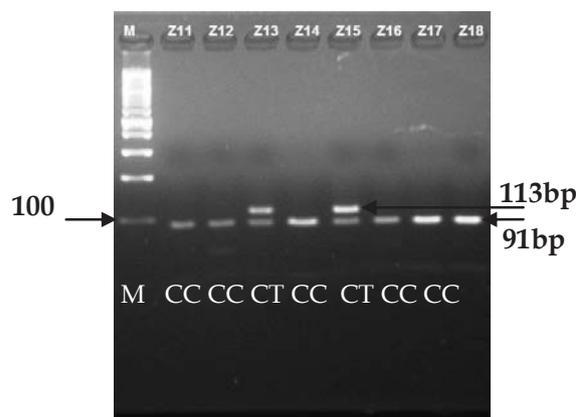


Figure 1. The genotyping result of rs7903146 polymorphism of TCF7L2 gene. M = Marker. CC genotype = wild-type (91 bp and 22 bp), CT genotype = variant (113 bp and 91 bp).

The frequency distribution of genotypes and alleles of rs7903146 polymorphism of TCF7L2 gene

The genotype and allele distribution of rs7903146 polymorphism of TCF7L2 gene is shown in Table 2. The odds ratio (OR) and Hardy-Weinberg is also shown in Table 2.

Data are presented as mean \pm SD. Distribution of data was analyzed by Shapiro-Wilk: $p \geq 0.05$. Independent t-test: $p \leq 0.05$: significantly difference. *Mann-Whitney U test: $p < 0.05$: significantly difference.

Table 1. Characteristic of subjects with and without type 2 DM family history

Variable	With type 2 DM family history n = 36	Without type 2 DM family history n = 36	p (CI 95%)
Gender (M/F)	9/27	9/27	1,00 (0,34-2,90)
Age (year)	23,64 \pm 3,66	24,19 \pm 3,50	0,37* 0,90
BMI (kg/m ²)	20,45 \pm 2,15	20,51 \pm 1,87	(-1,01-0,89)
Blood pressure (mmHg)			
Sistole	109,50 \pm 9,44	109,33 \pm 8,24	0,93 (-4,00-4,33)
Diastole	72,08 \pm 7,18	73,17 \pm 7,93	0,54 (-4,64-2,47)
Fasting Blood Glucose (mg/dL)	90,64 \pm 12,55	89,61 \pm 9,87	0,70 (-4,28-6,33)

Table 2. The distribution of genotype (TT, CT, and CC) and allele (T and C) of *TCF7L2* gene among subjects with and without type 2 DM family history

Variable		With type 2 DM family history n = 36	Without type 2 DM family history n = 36	OR	p	H-W
Genotype	CC	33 (91.7%)	34(94.4%)	0.647	0.500	1.000
	CT	3 (8.3%)	2(5.6%)			
	TT	0 (0%)	0(0%)			
Allele	C	69(95.8%)	70(97.2%)	0.657	0.500	
	T	3(4.2%)	2(2.8%)			

The Pearson Chi-square-Fishers Exact Test: $p < 0,05$: significantly difference. H-W= Hardy-Weinberg Equilibrium.

Table 3. The difference means of insulin and HOMA- β levels between subject with and without family history of type 2 DM

Variable	With family history DM n = 36	Without family history DM n = 36	p
Insulin (μ IU/mL)	9.77 \pm 6.35	16.18 \pm 3.76	0.00
HOMA- β (%)	132.56 \pm 62.48	266.09 \pm 1.68	0.00

Data are expressed as mean \pm SD. Independent T-test: $p \leq 0,05$. *Mann-Whitney U test: $p \leq 0,05$.

Table 4. The difference means of insulin level on CT and TT genotypes among subject with and without type 2 DM family history

Variable	With type 2 DM family history		P	Without type 2 DM family history		p
	CC (n = 33)	CT (n = 3)		CC (n = 34)	CT (n = 2)	
Insulin level (μ IU/mL)	9.1 \pm 5.3	16.8 \pm 12.9	0.40*	16.2 \pm 3,8	14.6 \pm 0,7	0.54

Data are described as mean \pm SD. Independent T-test: $p \leq 0,05$. *Mann-Whitney U test: $p \leq 0,05$.

Table 5. The difference means of HOMA- β on CC and CT genotypes among subjects with and without type 2 diabetes family history

Variable	With type 2 DM family history		p	Without type 2 DM family history		p
	CC (n = 33)	CT (n = 3)		CC (n = 34)	CT (n = 2)	
HOMA- β (%)	130.5 \pm 59.6	154.3 \pm 1.03	0.53	244.9 \pm 1.12	626.1 \pm 5.51	0.30

Data are presented as mean \pm SD. Mann-Whitney U test: $p \leq 0,05$.

The difference mean of Insulin and HOMA- β levels

The mean of insulin and HOMA- β levels of subjects with type 2 DM family history was significantly lower than subjects without type 2 DM family history ($p=0.00$) (Table 3).

The association of CC and CT genotypes of TCF7L2 gene with insulin and HOMA- β levels

Statistically, the mean of insulin and HOMA- β levels in CC and CT genotypes was not significantly different between two groups (Table 4 and 5).

Discussion

Family history is important in the clinical setting and patient's management, such as modification of environmental factors or lifestyle, increasing diagnosis accuracy, and prevention of expensive medical expenses (Das *et al.*, 2012). A type 2 DM family history may be associated with insulin resistance and dysfunction of pancreatic β -cells (Arslanian *et al.*, 2005). Danadian *et al.* (1999) showed that individuals with type 2 DM family history leads to decrease the insulin sensitivity by 25%.

Rs7903146 polymorphism of *TCF7L2* gene plays a role in the pathogenesis of type 2 DM which affects the regulation of transcription in insulinotropic GLP-1 hormone thereby reducing the secretion of insulin and improve glucose hepatic production. Single nucleotide polymorphism (SNP) rs7903146 in intron 3 is a C allele transformation into T allele. The T allele in *TCF7L2* gene associated with insulin secretion interference and insulin sensitivity enhancement (Alibegovic *et al.*, 2010). Lyssenko *et al.* (2007) observed that the CT/TT genotype was strongly associated with type 2 DM. The T allele is also associated with insulin secretion interference, increasing glucose production. In addition, T allele has a larger role in open chromatin and activity enhancement compared to C allele (Gaulton *et al.*, 2010). The dysfunction of beta cells is caused by protein *TCF7L2* deficiency and post-transcription *TCF7L2* disorders. The basic mechanism that explains the regulation of transcription and translation of *TCF7L2* was still unclear (Shu *et al.*, 2009). The depletion of *TCF7L2* caused a five-fold increase in β -cell apoptosis, a two-fold decline in β -cell proliferation and a two-fold decrease in insulin secretion (Shu *et al.* (2008).

The subjects enrolled in this study were in normal condition. All subjects blood glucose level was ≤ 126 mg/dL, mean of level of body mass index (BMI) of two groups were 20 kg/m^2 , and subjects blood pressure was $\leq 140/90$ mmHg. This result was associated

with subject characteristic which healthy subject. The PERKENI was reported that diagnose of diabetes was established if fasting blood glucose level ≥ 126 mg/dL (7.0 mmol/L), not obesity ($\leq 25 \text{ kg/m}^2$) and normal blood pressure ($\leq 140/90$ mmHg) (PERKENI, 2011; Annurad *et al.*, 2003).

This study found only two genotypes in each group, there are wild type homozygote genotype (CC) and variant heterozygote genotype (CT). This result was different with previous study that found TT, TC, and CC genotypes at Japanese (Miyake *et al.*, 2007), Indian population (Jyothi *et al.*, 2013), Chinese population (Wang *et al.*, 2013) and Brazilian population (Marquizine *et al.*, 2008).

The frequency distribution of CC genotype was higher than CT genotype. However, it was not statistically different between subjects with and without type 2 DM family history ($p=0.500$). Alsmadi *et al.* (2008) reported that the frequency of genotype and allele of rs7903146 polymorphism at Arab population was not statistically different between case and control subjects ($p=0.573$ and $p=0.675$), respectively. This result was different with previous study at Dutch Breda population. Vliet *et al.* (2007) reported that there was significantly different ($p=0.009$ and $p=0.00004$, respectively). The frequency of genotype and allele of rs7903146 polymorphism in Amish population also reported a similar results ($p=0.008$) by Damcot *et al.* (2006).

The polymorphism is affected by geographical and ethnical differences, so it causes genotype frequency differences. The *TCF7L2* gene genotype frequencies showed variations geographically and ethnically. The population of French, Dutch, Afro-American, and Mexico have a high *TCF7L2* gene rs7903146 genotype frequency (Goodarzi *et al.*, 2007; Bodhini *et al.*, 2007; Moczulski *et al.*, 2007).

In this study, the genotype distribution of the rs7903146 polymorphism of *TCF7L2* gene was not diverge from Hardy-Weinberg equilibrium ($p=1.000$). It can be concluded that genotype and allele frequencies at

this population were distributed normally. Individual with type 2 DM family history have a low frequency of T allele and CT/TT genotypes. The same results were reported at Arabian population (Alsmadi *et al.*, 2008) and Indian Pima population (Guo *et al.*, 2007) that rs7903146 has low risk to type 2 DM (OR=1.04). In another study conducted in France population (Moczulski *et al.*, 2007) and Italian population (Gambino *et al.*, 2010) were reported different result that rs7903146 was a risk factor of type 2 DM.

The mean of insulin levels was lower among subjects with type 2 DM family history than subjects without type 2 DM family history ($p=0.00$). Chen *et al.* (2012) and Arslanian *et al.* (2005) also found that insulin level of subject with type 2 DM family history was lower than subjects without type 2 DM family history. The low insulin level on subjects with type 2 DM family history indicates that there was a decreasing insulin secretion due to abnormality of pancreatic β -cell (Arslanian *et al.*, 2005).

HOMA- β was employed for evaluation of pancreatic β -cell (Chen *et al.*, 2012). HOMA- β values were parallel with the results of insulin levels, which is significantly lower in subjects with a type 2 DM family history than in subjects without a type 2 DM family history. Chen *et al.* (2012) was shown that individuals with type 2 DM family history has impaired function of pancreatic β -cell with low HOMA- β level compared with HOMA- β level of subjects without type 2 DM family history. The decreasing of insulin secretion resulted from impaired function of pancreatic β -cell which caused by several factor, include genetic factors (DeFronzo *et al.*, 2008).

In this study, the number of samples that have been enrolled is much lower than in the other study. Insufficient number of samples caused TT genotype could not be found in this study. Certainly, a limitation of this study.

Conclusion

The polymorphism of *TCF7L2* gene is not risk of individuals with and without type 2 DM family history in Indonesian population. The Insulin secretion on individuals with type 2 DM family history was lower than that of individuals without type 2 DM family history.

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