



## ASSOCIATION OF TCF7L2 GENE POLYMORPHISM WITH NON OBESE TYPE 2 DIABETES MELLITUS IN JAVANESE POPULATION

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### ARTICLE INFO

#### Article history

Submission 2023-05-23  
Revision 2023-07-12  
Accepted 2023-10-30

#### Keywords:

2 Type Diabetes  
Polymorphism  
TCF7L2 gene

### ABSTRACT

Type 2 diabetes is characterized by insulin deficiency due to inadequate insulin secretion or decreased tissue response to insulin at one or more points. Three important factors that influence the pathogenesis of type 2 DM are individual factors or ethnic genetics, factors of pancreatic  $\beta$  cell damage and insulin resistance factors. The TCF7L2 gene is a gene that codes for TCF7L2 protein, a group of proteins that act as transcription factors in the Wnt signal pathway. TCF7L2 gene polymorphism occurs when cytosine is replaced with thymine in the intron 3 position in wild-type due to alternative splicing. The purpose of the study was to determine the frequency of TCF7L2 gene polymorphism in Javanese type 2 non-obese DM patients in Semarang. Detection of TCF7L2 gene polymorphism was carried out on 49 people with type 2 non-obese DM patients and 38 people not with type 2 DM using the PCR-RFLP method. The restriction enzyme used is RsaI. The allele frequency of TCF7L2 gene polymorphism was analyzed by Chi-square and analysis of sample characteristics was carried out with Odds Ratio. The results showed that there were significant differences in the allele frequency of TCF7L2 gene polymorphism and in the characteristics of age, KGD and systole between case subjects and control subjects. From this study it was concluded that TCF7L2 gene polymorphism is associated with the type 2 DM through interactions between genetic factors (T allele) and life style.

## INTRODUCTION

Diabetes Mellitus (DM) is a non-communicable disease characterized by chronic hyperglycemia which is caused by an impaired insulin secretion (International Diabetes

Federation, 2017). One type of DM is type 2 DM, this disease is characterized by insulin work deficiency due to inadequate insulin secretion or decreased tissue response to insulin at one point or more (International Diabetes Federation, 2017).

Type 2 Diabetes Mellitus (T2DM), one of the most common metabolic disorders, is caused by a combination of two primary factors: defective insulin secretion by pancreatic  $\beta$ -cells and the inability of insulin-sensitive tissues to respond appropriately to insulin (Garcia *et al.*, 2020) Previously, health experts thought only obese people are at risk for type 2 diabetes yet a recent study denied it. According to the researcher from the University of Florida, thin people are also at risk of developing type 2 diabetes if they have sedentary lifestyles or sitting all day long (Mainous, 2016).

In 2017, the average prevalence of DM in Tawa Tengah was 19.22%, with the number of DM cases in Semarang City as many as 117037 (Dinas Kesehatan Provinsi Jawa Tengah, 2017). TCF7L2 is the most potent locus for type 2 diabetes (T2D) risk and the first locus to have been robustly reported by genomic linkage studies. TCF7L2 is a transcription factor that forms a basic part of the Wnt signaling pathway (Bosque-Plata *et al.*, 2021). The TCF7L2 protein is a key transcriptional effector of the Wnt/ $\beta$ -catenin signaling pathway, which is an important developmental pathway that negatively regulates adipogenesis (Chen, 2018). TCF7L2 gene polymorphism occurs when cytosine is replaced with thymine in the intron 3 position in wild-type due to alternative splicing. Wnt is a stimulus which consists of  $\beta$ -catenin component and part of TCF (TCF7, LEF-1, TCF7L1 dan TCF7L2) group. Wnt signal functions for regulating proglucagon gene expression, Insulin Receptor Substrate-1 (IRS-1) gene expression and secretion of Glucagon Like Peptide-1 (GLP-1) (Liu *et al.*, 2022). Glucagon-likepeptide-1 (GLP-1) is a peptide of 30amino acids, which is formed from proglucagon,the precursor of the well-known pancreatic hormone, glucagon (Holst, 2022).

Polymorphism is a variance of DNA sequence that can cause change of protein function in the body. Polymorphism can be caused by environment exposure and mutagen. Polymorphism impact causes susceptible of a population against a disease. The polymorphism that occurs will continue to be lowered so that the frequency of polymorphisms in each ethnicity varies. Many controversies about TCF7L2 polymorphism in the case of type 2 DM, but Li *et al.* (2021) speculate that the TCF7L2 can promote GLP-1 expression and sensitize pancreatic  $\beta$ -cells to blood glucose, but

TCF7L2 rs7903146 causes a decrease in protein levels. These underlying mechanisms are consistent with the function and pathological changes in the islets of T/T allele homozygotes. The presence of the risk allele (T) TCF7L2 causes the Wnt signal to be unable to regulate proglucagon gene expression, so the proglucagon gene cannot encode the GLP-1 protein. It results in a decrease in ATP concentration in pancreatic  $\beta$  cells, so that the  $K^+$  canal opens and depolarizes the pancreatic  $\beta$  cell membrane, and closes the  $Ca^{2+}$  canal. The low concentration of  $Ca^{2+}$  in pancreatic  $\beta$  cells results in insulin-filled granules ready to be released or Readily Releasable Granules (RRG) unable to fuse with the plasma membrane. As a consequent, insulin is not secreted outside pancreatic  $\beta$  cells. Decreasing insulin secretion causes an increase in hepatic sugar production and a decrease in glucose use by tissues, resulting in chronic hyperglycemia. As  $\beta$ -cells are attacked, the body fails to produce insulin, resulting in insulin deficiency, chronic hyperglycemia, and long-term complications (Khin *et al.*, 2023). Numerous studies worldwide have evaluated the link between TCF7L2 gene and a high risk of T2DM (Elhourch *et al.*, 2021). In Indonesia, there is no data on the genotypic distribution of type 2 non-obese Javanese DM patients in Semarang based on TCF7L2 gene markers. TCF7L2 gene polymorphism can be used as a marker for early detection of non-obese type 2 DM disease, thus minimizing further risk of the disease. The purpose of this study was to determine the frequency of TCF7L2 gene polymorphism in non-obese Javanese patients of type 2 DM in Semarang City.

## **MATERIALS AND METHODS**

The research was conducted at the Laboratory of Molecular Biology, Biology Department, FMIPA, Universitas Negeri Semarang, Semarang and the Laboratory of Biochemistry, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Jogjakarta in January-October 2018.

The equipment in this study were Sterile Sput, Vacutainer tube containing K3EDTA, Ice-box, Ice gel/ice cool, tourniquet, digital scales, weigh paper, autoclave, erlenmeyer 250 ml, measuring cup, microtube, micropipette, tip, centrifuge, incubator, Thermocycler, spindown, microwave, -20oC deep freezer, horizontal electrophoresis and doc gel.

The materials in the study were absolute ethanol, alcohol swab, DNA isolation kit [GENEJET Genomic Purification Kit]: Lysis Solution, Proteinase K, Wash Buffer I,

Wash Buffer II, 96-100% Ethanol, Elution Buffer, 70% alcohol, tissue, blood sample, forward & reverse primer, PCR Mix and dH<sub>2</sub>O free nuclease (Thermoscientific), RsaI [Biolab] enzyme, NE Buffer, ddH<sub>2</sub>O free nuclease, 3% Agarose Gel, 1X TBE buffer, fluorosave, 50 bp DNA ladder, parafilm paper and loadingdye.

The population in this study were non-obese type 2 DM patients, while the sample in this study was 49 patients non-obese type 2 DM and 49 not patients of non-obese type 2 DM who met the criteria. The determination of sample size in this study was based on inclusion and exclusion criteria. The determination of Puskesmas (health center) and clinics were based on the number of patients and the schedule of Prolanis, so that Puskesmas Pegandan, Pandanaran, Ngaliyan, Gunungpati, Unnes Polyclinic, and Nayaka Husada clinics are selected that meet the inclusion criteria: type 2 DM patients, non-obese, not hypertension, 30-65 years Javanese ethnic. Exclusion criteria is criteria from the research subject which should not be exist and if subject has exclusion criteria therefore subject must exclude from the research. Exclusion criteria in this research are: patients with type 2 DM who are not willing to be the subject of research and absent at the time of data collection.

### ***Blood Sampling***

Before this research is conducted beforehand, the proposal is sent to the Research Ethics Commission, Research and Development Unit of Semarang State University (UNNES) to obtain information on ethical appropriateness. Then asked for permission to conduct research to KESBANGLITMAS, Head of Semarang City Health Office and Head of Puskesmas to be used as a research place. The study is conducted after obtaining permission for Research and Ethical Clearance from the Biomedical Research Ethics Commission on Humans at the State University of Semarang and after Type 2 DM patients were asked for approval and willingness to take part in research and provide informed consent. In each patient who will be included in this study are explained the background and purpose of this study :

- a. Given the freedom to choose whether to take part or not in this research.
- b. Patients who are willing to take part in this study are asked to fill out informed consent.
- c. Prioritized of service by always heeding applicable ethics

Every patient who entered the health center was seen in his medical record, then patients who met the inclusion criteria were collected until 49 samples. Respondents as a case and control group after signing informed consent were taken blood by officers of the CITO Laboratory (laboratories that collaborated in the Prolanis program at Pegandan, Pandanaran, Ngaliyan, Gunungpati, Puskesmas, Unnes Polyclinic, and Nayaka Husada Semarang City). Inclusion criteria from the characteristics of the samples taken: type 2 DM patients, ages 30-65 years, BMI  $\leq 25$  kg/m<sup>2</sup>, fasting blood sugar levels  $\leq 126$  mg/dL, no hypertension ( $\leq 140$  mmHg), and ethnic Javanese. 3 ml of blood was taken from each respondent by the aseptic method using a sterile syringe, put in Vacutainer tube containing K3EDTA and absolute ethanol. Blood samples were stored in a freezer at -20°C until DNA isolation is carried out.

### ***DNA Isolation***

The DNA extraction method is the GeneJET Genomic DNA Purification Kit. A spoonful of spatula blood sample was crushed until smooth using a mortar, then added 400  $\mu$ L Lysis Solution and refined again. Then the mixture was transferred to a 1.5 mL microtube and added with 20  $\mu$ L Proteinase K Solution. Then the solution is homogenized using vortex. The mixture was incubated at 56°C for 10 minutes and every 5 minutes the mixture was shaken to form a number 8. Precipitation of DNA was carried out by adding 200  $\mu$ L of Ethanol 96% to the sample and being vortexed. Then the suspension was transferred into the column and collection tube, the suspension was centrifuged at a speed of 6000 x g for 1 minute. The liquid in the collection tube is removed and the collection tube is reinstalled. 500  $\mu$ L Wash Buffer I was added to the suspension, then centrifuged at 8000 x g for 1 minute. The liquid in the collection tube is removed and installed again. After that, 500  $\mu$ L of Wash buffer II was added to the suspension and centrifuged at a speed of 12000 x g for 3 minutes. The suspension was re-centrifuged at a speed of 12000 x g for 1 minute. Then the collection tube is removed and the column is moved to a 1.5 mL microtube. The column suspension was added to 100  $\mu$ L Elution Buffer and incubated at room temperature for 2 minutes. The suspension was centrifuged at 8000 x g for 1 minute. Then the suspension was centrifuged again at a speed of 8000 x g for 1 minute. The column was removed and the DNA is stored on a 1.5 mL microtube which has been labeled at -20°C.

### ***Amplification TCF7L2 gene***

Amplification of the TCF7L2 gene was carried out with the composition of 7.5  $\mu$ L PCR master mix kits, 3.5  $\mu$ L ddH<sub>2</sub>O, Forward and Primer Reserve primers of 0.5  $\mu$ L and 3  $\mu$ L template DNA respectively. Sequence for TCF7L2, Forward gene amplification: 5'-GAG AGC TAA GTA CTT AGG TA-3' and Reverse: 5'-CTG ACA TTG ACT AAG TTA CTT GC-3'. Amplification of the TCF7L2 gene was carried out using a Thermocycler PCR machine [Biosystems<sup>TM</sup> SimpliAmp TMA24811].

The amplification process of TCF7L2 gene on the thermocycle PCR machine (Eppendorf) are; first step, Hotstart with a temperature of 95°C for 10 minutes, second step denaturation at 95°C for 1 minute, annealing (attaching primer) at 58°C for 1 minute, the next step is extension at 72°C for 1 minute, the final step is the final extension at 72°C for 7 minutes. Gene TCF7L2 Amplification with specific primer will result DNA fragment size 113 bp.

### ***Restriction Fragment Length Polymorphism (RFLP)***

Detection of polymorphism was done using the PCR-RFLP method. The amplified product was cut using the RsaI restriction enzyme (Biolabs Inc, New England). 3  $\mu$ L PCR products were taken using a micropipette and inserted into a 0.2 mL microtube, then added 0.5  $\mu$ L RsaI enzyme, 1  $\mu$ L NE Buffer and 5.5  $\mu$ L ddH<sub>2</sub>O free nuclease. The mixture was incubated for 1 hour at 37°C.

### ***Electrophoresis***

The results of PCR-RFLP were electrophoresed using 3% agarose gel to determine whether DNA fragments were amplified and cut according to the target. Agarose gel electrophoresis performed at voltages of 100 volts for 30 minutes. The electrophoresis results were checked using UV illuminator [Alpha Innotech] then.

### ***Data Analysis***

Molecular data analysis of TCF7L2 gene polymorphism in Javanese ethnic type 2 non-obese DM patients included qualitative and quantitative analysis. Qualitatively by looking at the DNA band of PCR-RFLP results from TCF7L2 gene in gel electrophoresis. While quantitatively is seeing whether there is a difference in frequency of TCF7L2 gene polymorphism in type 2 non-obese DM patients with controls using Chi-Square test and

analysis of the relationship between sample characteristics of type 2 DM patients non-obese with Chi-Square test and Odds Ratio quantitatively begins by calculating allele frequency and frequency of genotype.

### RESULTS AND DISCUSSION

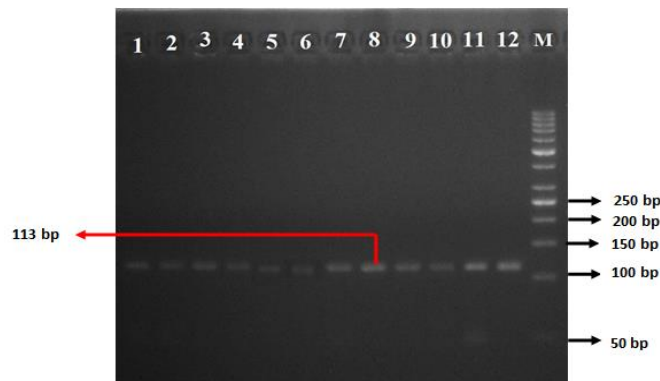
This study involved 87 subjects, consisting of 49 people with type 2 diabetes mellitus non obese as the case subject and 38 non-obese people who did not suffer from type 2 diabetes as control subjects. The characteristics of the research subjects can be seen in **Table 1**.

**Table 1.** Characteristics of subjects with type 2 DM and non-type 2 DM

Variable	Type 2 DM N= 49	Non Type 2 DM N= 38	p value	OR
Gender (L/P)	17/32	12/26	-	0,869
Age (years)	54,87±1,10	45,31±1,71	-	4,149
IMT (kg/m <sup>2</sup> )	22,64±0,27	21,54±0,43	0,192	-
Blood sugar (mg/dL)	154,18±10,65	94,26±2,06	-	-
Systole (mmHg)	127,00±2,06	113,26±2,55	0,002	-
Diastole (mmHg)	82,33±1,13	83,58±1,92	0,078	-

Statistically, different factors between case and control subjects were age and blood sugar levels. While systolic blood pressure was considered different due to DM differences. High blood sugar levels cause thickening of blood vessels which causes narrowing of blood vessel diameter due to increased blood pressure and hypertension.

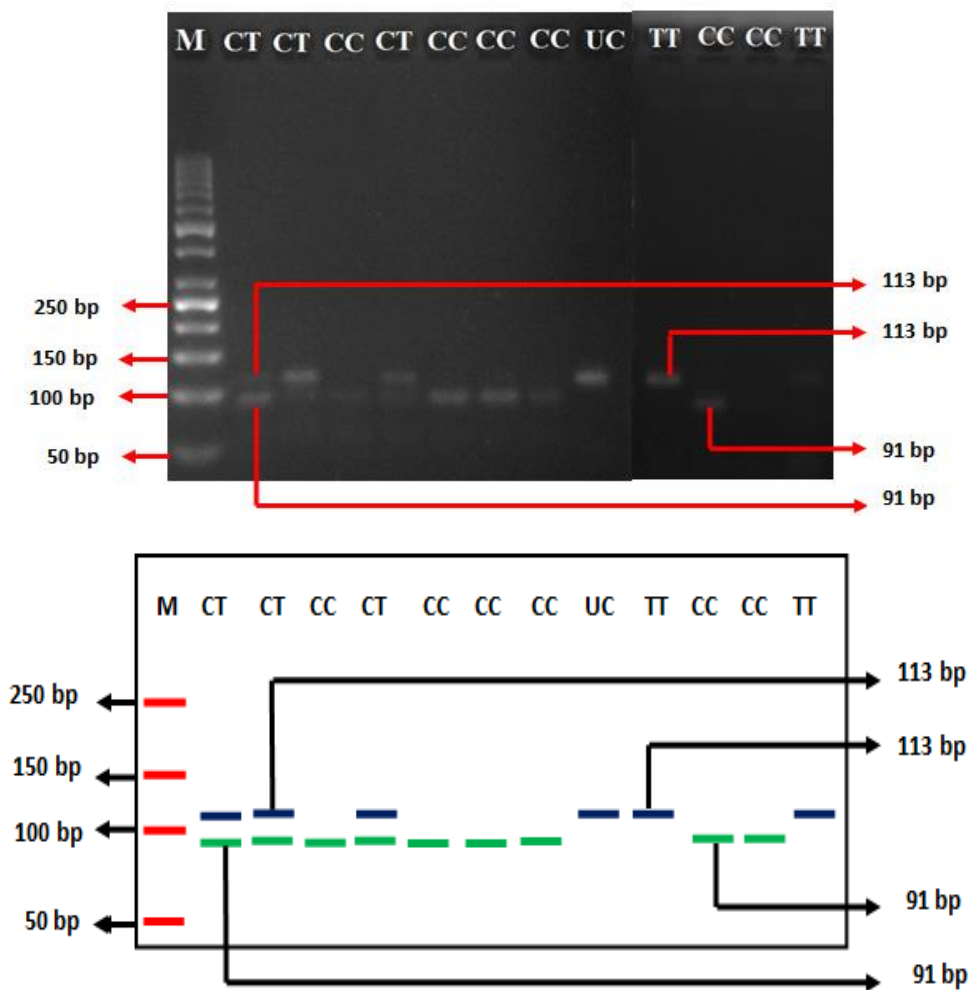
Amplification of the TCF7L2 gene using specific primers F: 5'-GAG AGC TAA GTA CTT AGG TA-3 'and R: 5'-CTG ACA TTG ACT AAG TTA CTT GC-3' was successfully carried out using DNA fragments in **Figure 1**.



**Figure 1.** The Visualization Result of PCR Gene TCF7L2 Product; DNA Ladder Marker 50 bp (M), DNA fragment size 113 bp (1-12).

The length of the fragment can be seen by comparing the length of the DNA fragment in the 50 bp ladder marker with the results of electrophoresis visualization. The results of PCR product electrophoresis visualization in Figure 1 show amplified DNA by producing a DNA fragment measuring 113 bp.

Detection of TCF7L2 gene polymorphism was carried out using the RsaI restriction enzyme. The RFLP results were then separated by electrophoresis using a 3% agarose gel at 100 Volt for 30 minutes. The cutting results of the TCF7L2 gene using the RsaI enzyme as shown in **Figure 2**.



**Figure 2.** Visualization and illustration of polymorphism gene TCF7L2 after RsaI enzyme restriction. DNA Ladder Marker 50 bp (M). UC= uncut (PCR product before restriction with enzyme). Red ribbon: DNA marker, green ribbon: 91 bp DNA fragment and blue ribbon: 113 bp DNA fragments

The results of visualization of restriction products in Figure 2 showed that the Wild type (CC) had two DNA fragments measuring 91 bp and 22 bp (but 22 bp is not visible),



homozygous (TT) had one DNA fragment measuring 113 bp, and heterozygous (CT) had three fragments DNA 113 bp, 91 bp, and 22 bp (but 22 bp is not visible).

The results of the calculation of the frequency of TCF7L2 gene polymorphism in patients with type 2 DM and controls performed on all samples (49 sufferers and 38 controls) are seen in **Table 2**.

**Table 2.** Frequency of Genotype and Allele Gene TCF7L2

Variable		Type 2DM N=49	Non Type 2 DM N=38	<i>p value</i>
Genotype	CC (%)	30 (61.2%)	28 (73.7%)	0,761
	CT (%)	13 (26.5%)	10 (26.3%)	
	TT (%)	6 (12.24%)	0 (0%)	
Allele	C (%)	73 (74.4%)	66 (86.8%)	0,044*
	T (%)	25 (25.6%)	10 (13.1%)	

(\*) = significant difference ( $p < 0.05$ )

Genotypes found in type 2 DM subjects were CC, CT and TT genotypes. While in non-type 2 DM subjects (controls) only found two genotypes, CC and CT genotypes, while TT genotypes were not found. Based on the results of the frequency analysis of genotypes and alleles in Table 3, the frequencies of C and T alleles from both samples showed significant differences. Thus, the T and C alleles play a role in the appearance of type 2 DM on Javanese ethnic, but it is likely not a major risk factor for Javanese type 2 DM.

Based on the results of allele frequency analysis in all samples showed that the frequencies of T and C alleles were significantly different with  $p = 0.044$ . This showed that the T and C alleles play a role in the appearance of type 2 DM in Javanese ethnic groups. Based on this study, the possibility of TCF7L2 gene polymorphism is a risk factor for the occurrence of non-obese type 2 DM in ethnic Javanese. The Asian population has shown positive results regarding the relationship of TCF7L2 gene polymorphism with DM type 2. Other studies with similar results where TCF7L2 gene polymorphisms are positively associated with the occurrence of type 2 DM in the French population (Cauchi *et al.*, 2017), African-Americans (Sale *et al.*, 2017), Finland (Scott *et al.*, 2016), UK (Groves *et al.*, 2016), Japan (Miyake *et al.*, 2018), and China (Chang *et al.*, 2017). Each population has a different frequency of polymorphism.

T allele in the TCF7L2 gene is referred to as risk alleles and is associated with impaired insulin secretion (Alibegovic *et al.*, 2016). T alleles in the TCF7L2 gene are often associated with pancreatic  $\beta$  cell dysfunction and type 2 DM (Grants *et al.*, 2016).

The presence of the risk allele (T) TCF7L2 causes the Wnt signal to be unable to regulate proglucagon gene expression. As a result, the glucagon gene cannot code GLP-1 protein and results in decreased cAMP levels in pancreatic  $\beta$  cells. The reduced cAMP concentration in pancreatic  $\beta$  cells cannot activate Protein Kinase A (PKA) and The cAMP regulated guanine nucleotide exchange factor (cAMP-GEF) (Epac) which causes the  $K^+$  canal to remain open and the  $Ca^{2+}$  canal remains closed. This results in high concentrations of  $K^+$  in pancreatic  $\beta$  cells and vice versa the concentration of  $Ca^{2+}$  in pancreatic  $\beta$  cells is low (Meloni *et al.*, 2016).

The low concentration of  $Ca^{2+}$  in pancreatic  $\beta$  cells results in insulin-filled granules ready to be released or Readily Releasable Granules (RRG) unable to fuse with the plasma membrane, consequently, insulin is not secreted outside pancreatic  $\beta$  cells. Decreasing insulin secretion causes an increase in hepatic sugar production and a decrease in glucose use by tissues, resulting in chronic hyperglycemia. Chronic hyperglycemia will lead to hyperstimulated metabolism, which will be constitutively coupled to hyperexcitability and chronically elevated  $Ca^{2+}$ , factors that have all been suggested to play a role in diabetic loss of  $\beta$ -cell function (Shyr *et al.*, 2019).

Based on the results of the study, the gender average of women was greater than men, both in the case subjects and in the control subjects. Based on the analysis of the odds ratio, the possibility of women having a risk of 0.869 times for the occurrence of type 2 DM compared to men. Type 2 diabetes mellitus, it is highlighted that it is more frequently diagnosed in men who have a lower age and body mass index than in women (Ciarambino *et al.*, 2022), this occurs because men are influenced by body fat distribution, fat accumulation is concentrated around the abdomen so that it triggers central obesity which is more at risk of triggering metabolic disorders and the emergence of type 2 DM. The incidence of type 2 DM varies between the sexes in one population with another population. This difference is not significant unless it is affected by lack of physical activity and central obesity. Changes in lifestyle are associated with an increase in the frequency of type 2 DM.

The appearance of type 2 DM is often associated with age factors. The results of the analysis showed that the average Javanese type 2 DM subjects aged  $> 45$ , while the average control subject was  $<45$  years old. Based on the statistical analysis in this study, people aged  $> 45$  years had a risk of 4,149 times for the occurrence of type 2 diabetes

compared to those aged <45 years. Research conducted by Magliano *et al.*, (2020) showed an accumulating data suggest that type 2 diabetes mellitus (T2DM) in younger people (aged <40 years), referred to as young-onset T2DM, has a more rapid deterioration of  $\beta$ -cell function than is seen in later-onset T2DM.

Research conducted by Kurniawaty & Bella (2016) has a significant relationship between age and incidence of type 2 DM, where people > 45 years have 9 times the risk of the occurrence of type 2 DM compared to those aged < 45 years. The existence of the aging process causes a reduction in the ability of pancreatic  $\beta$  cells to produce insulin. In addition, there is a decrease in mitochondrial activity in muscle cells by 35% in older individuals. This is associated with an increase in muscle fat levels by 30% and triggers insulin resistance (Pardede *et al.*, 2017).

Based on the results of the characteristic analysis between type 2 non-obese DM subjects and control subjects showed that KGD (blood sugar levels) was associated with the emergence of type 2 DM in Javanese ethnic. Various factors, including genetics, weight, age, sex, alcohol intake, coffee and caffeine intake, and smoking habits can affect KGD which triggers the occurrence of type 2 diabetes. An imbalance between high-energy food intake and energy expenditure for long-term activities allows the occurrence of insulin resistance and type 2 diabetes. Intake of high energy food can stimulate insulin resistance through increased blood sugar levels.

Some of the evidence underpinning these concerns is derived from cross-sectional surveys or ecological observations, but there are also prospective cohort studies and controlled intervention trials which have addressed this issue. This brief narrative review arises from a Symposium on Sugars and Health held at the European Nutrition Conference in October 2015 (Macdonald, 2016). Reducing carbohydrate intake can increase insulin sensitivity in healthy individuals and decrease fasting blood sugar levels in type 2 DM patients (Bolla *et al.*, 2016). Too much reduction in the number of carbohydrates consumed is not allowed for people with type 2 diabetes.

The TCF7L2 gene is not the only gene that can cause DM type 2. Some gene variants that play a role in type 2 DM include the Peroxisome Proliferator-Activate Receptor gene PP (PPARG), Potassium inwardly-rectifying subfamily channel J member 11 (KCNJ11), Calpain 10 (CAPN10), Transcription Factor 7-like 2 (TCF7L2), Fat mass

and obesity-associated (FTO), Cyclin-dependent kinase 5 regulatory subunit-associated 1-like 1 proteins (CDKAL1) and others (Mc Carthly, 2016).

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The efforts in controlling DM can be done in several ways such as education, physical exercise, Medical Nutrition Therapy (TNM), and pharmacological therapy. In DM management, besides doctors and other medical personnel, the role of sufferers/patients and families is very important. The main pillars of DM control include is counselling, food planning, physical training and hypoglycemic drug (Fatimah, 2016).

## **CONCLUSION**

Polymorphism TCF7L2 gene relates to the type 2 DM incident on Javanese ethnic is seen from obtained genotype frequency, that is CC (61.2%), CT (26,5%) and TT (12,2%) genotypes for Type 2 non-obese DM patients. While, CC (73.7%) and CT (26.3%) genotypes were found on the non-DM subjects. Statistically, there is significant difference of polymorphism frequency TCF7L2 gene on Type 2 non-obese DM Javanese ethnic patients.

## **ACKNOWLEDGMENT**

This research project was supported by the Laboratory of Molecular Biology, Biology Department, Faculty of Mathematics and Science Universitas Negeri Semarang and Universitas Gadjah Mada of Medical Sciences. We wish to thank all those who participated in this study.

## **REFERENCES**

Alibegovic AC, Sonne MP, Hojbjerg L, Hansen T, Pedersen O, Hall G. (2016). The T-Allele Of TCF7L2 rs7903146 Associates with a Reduced Compensation of Insulin

Secretion for Insulin Resistance Induced by 9 Days of Bed Rest. *Diabetes*. 59(4): 836-843. DOI: [10.2337/db09-0918](https://doi.org/10.2337/db09-0918)

Bolla AM, Amelia C, Andrea L, Marina S, Lorenzo P. (2019). Low-Carb and Ketogenic Diets in Type 1 and Type 2 Diabetes. *Nutrients*. 11(962): 1-14. doi: [10.3390/nu11050962](https://doi.org/10.3390/nu11050962)

Bosque-Plata LD, Eduardo M, Miguel AE, Claudia G. (2021). The Role of TCF7L2 in Type 2 Diabetes. *Diabetes*. 70: 1220–1228. doi: [10.2337/db20-0573](https://doi.org/10.2337/db20-0573)

Cauchi S, Younes EA, Hélène C, Christian D, Franz K, Raimund W, Chakib N, Wolfgang P, Mohamed CDM, Philippe F. (2017). TCF7L2 is Reproducibly Associated with Type 2 Diabetes in Various Ethnic Groups : A Global Meta-analysis. *Journal of Molecular Medicine*. 85(7):777–782. DOI: [10.1007/s00109-007-0203-4](https://doi.org/10.1007/s00109-007-0203-4)

Chang CY, Chang JT, Jiang DY, Kuo SS, Lee CK, Chiu CK, Chuang LM. (2017). Association Study of the Genetic Polymorphism of the Transcription Factor 7-like 2 (TCF7L2) Gene and Type 2 Diabetes in the Chinese Population. 2017. *Diabetes*. 56(10):2631-2637. DOI: [10.2337/db07-0421](https://doi.org/10.2337/db07-0421)

Chen X, Iriscilla A, Chris S, Marcel F, Nikhil K, Christopher P, Sami H, Luke N. (2018). The Diabetes Gene and Wnt Pathway Effector TCF7L2 Regulates Adipocyte Development and Function. *Diabetes*. 67: 554–568. DOI: [10.2337/db17-0318](https://doi.org/10.2337/db17-0318)

Dinas Kesehatan Provinsi Jawa Tengah. (2017). *Profil Kesehatan Provinsi Jawa Tengah Tahun 2017*. Semarang.

Grant SFA, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. (2016). Variant of Transcription Factor 7-like 2 (TCF7L2) Gene Confers Risk of Type 2 Diabetes. *Nature Genetics*. 38(3):320-323. DOI: [10.1038/ng1732](https://doi.org/10.1038/ng1732)

Groves CJ, Eleftheria Z, Jayne M, Timothy MF, Michael NW, Nigel WR, Graham AH, Mark W, Steven W, Andrew TH, Mark IM. (2016). Association Analysis of 6,736 U.K. Subjects Provides Replication and Confirms TCF7L2 as A Type 2 Diabetes Susceptibility Gene with A Substantial Effect on Individual Risk. *Diabetes*. 55(9):2640–2644. DOI: [10.2337/db06-0355](https://doi.org/10.2337/db06-0355)

Holst J. (2022). Glucagon-like Peptide-1: are Its Roles as Endogenous Hormone and Therapeutic Wizard Congruent. *Journal of Internal Medicine*. 291: 557–573. DOI: [10.1111/joim.13433](https://doi.org/10.1111/joim.13433)

International Diabetes Federation. *IDF Diabetes Atlas 8th ed*. India: Prof. Shaukat Sadikot.

Khin P, Jong H, Hee-Sook J. (2023). Pancreatic Beta-cell Dysfunction in Type 2 Diabetes. *European Journal of Inflammation*. 21: 1–13. <https://doi.org/10.1177/1721727X231154152>

- Kurniawaty E, Bella Y. (2016). Faktor-faktor yang Berhubungan dengan Kejadian Diabetes Mellitus Tipe 2. *Majority*. 5(2):27-31.
- Li J, Li Z, Xinping O, Pingping H. (2021). Transcription Factor-7-Like-2 (TCF7L2) in Atherosclerosis: A Potential Biomarker and Therapeutic Target. 8(701279): 1-12. doi: [10.3389/fcvm.2021.701279](https://doi.org/10.3389/fcvm.2021.701279)
- Liu J., Qing X., Jiani X., Chenxi N., Yuanyuan L., Xiaojun Z., Zhengwei Z., Guang S., & Gang Y. (2022). Wnt/ $\beta$ -catenin Signalling: Function, Biological Mechanisms, and Therapeutic Opportunities. *Signal Transduction and Targeted Therapy*. 7(3): 1-23. DOI: [10.1038/s41392-021-00762-6](https://doi.org/10.1038/s41392-021-00762-6)
- Macdonald IA. (2016). A Review of Recent Evidence Relating to Sugars, Insulin Resistance and Diabetes. *Eur J Nutr*. doi: [10.1007/s00394-016-1340-8](https://doi.org/10.1007/s00394-016-1340-8)
- Magliano DJ, Julian W, Jessica L, Edward W, Paul Z, Jonathan E. (2020). Young-onset Type 2 Diabetes Mellitus-implications for Morbidity and Mortality. *Nat Rev Endocrinol*. 16(6): 321-331. DOI: [10.1038/s41574-020-0334-z](https://doi.org/10.1038/s41574-020-0334-z)
- Mainous G, Rebecca J, Ara J, Stephen DA. (2016). Prevalence of Prediabetes and Abdominal Obesity Among Healthy-Weight Adults: 18-Year Trend *Annals Of Family Medicine*.14(4):304-310. DOI: [10.1370/afm.1946](https://doi.org/10.1370/afm.1946)
- McCarthy M. (2016). Genomics, Type 2 Diabetes and Obesity. *New England Journal of Medicine*. 363(24):2339-2350. DOI: [10.1056/NEJMra0906948](https://doi.org/10.1056/NEJMra0906948)
- Meloni AR, Deyoung MB, Lowe C, Parkes DG. (2016). GLP-1 Receptor Activated Insulin Secretion from Pancreatic  $\beta$ -cells: Mechanism and Glucose Dependence. *Diabetic Obes and Metabolism*. 15(3): 15-27. DOI: [10.1111/j.1463-1326.2012.01663.x](https://doi.org/10.1111/j.1463-1326.2012.01663.x)
- Miyake K, Yukio H, Kazuo H, Kazuki Y, Haruhiko O, Hiroto F, Yushi H, Kazuya Y, Yoshinori H, Yoshitomo O, Naoka I, Yasuhiko I, Yuichiro Y, Yutaka S, Hiroshi M, Atsunori K, Ken Y, Katsushi T, Jun T, Hideichi M, Kaishio N, Takashi K. & Masato K. (2018). Association of TCF7L2 Polymorphisms with Susceptibility to Type 2 Diabetes in 4,087 Japanese Subjects. *The Japan Society of Human Genetics and Springer*.53(2):174–180.
- Pardede, Tami E, Dani R, Erwin C. (2017). Gambaran Pengendalian Diabetes Melitus Berdasarkan Parameter Indeks Massa Tubuh dan Tekanan Darah di Poli Rawat Jalan Penyakit Dalam RSUD Arifin Achmad Pekanbaru. *Jurnal Online Mahasiswa Fk*. 4(1):1-14.
- Sale MM, Shelly GS, Josyf CM, Keith LK, Carld DL, Tennille SL, Pamela JH, Donald WB, Stephen SR, Barry IF. (2017). Variants of The Transcription Factor 7-like 2 (TCF7L2) Gene are Associated with Type 2 Diabetes in an African-American Population Enriched for Nephropathy. *Diabetes*.56(10):2638–2642. DOI: [10.1007/s00125-007-0656-6](https://doi.org/10.1007/s00125-007-0656-6)
- Sarah E, Housna A, Nour M, Younes A, Fatima G, Loubna A, Naima E, Lahcen B, Azeddine I, Naoual E, Rachid E. (2021). Significant Association of Polymorphisms

in the TCF7L2 Gene with a Higher Risk of Type 2 Diabetes in a Moroccan Population. *Journal of Personalized Medicine*. 11(461): 1-8. DOI: [10.3390/jpm11060461](https://doi.org/10.3390/jpm11060461)

Scott LJ, Lori LB, Cristen JW, Andrew GS, Anne UJ, Narisu, William JT, Richard NB, Karen LM, Francis SC, Michael B. (2016). Association of Transcription Factor 7-like 2 (TCF7L2) Variants with Type 2 Diabetes in a Finnish Sample. *Diabetes*.55(9):2 z649–2653. DOI: [10.2337/db06-0341](https://doi.org/10.2337/db06-0341)

Shyr1 Z, Zhiyu W, Nathaniel W, Colin G, Maria S. (2019). The Role of Membrane Excitability in Pancreatic  $\beta$ -cell Glucotoxicity. *Nature*. 9(6952): 1-12. doi: [10.1038/s41598-019-43452-8](https://doi.org/10.1038/s41598-019-43452-8)

Unai G, Asier B, Shifa J, Asier L, Haziq S, Kepa B, Helena O, César M. (2020). Pathophysiology of Type 2 Diabetes Mellitus. 21(6275): 1-34. doi: [10.3390/ijms21176275](https://doi.org/10.3390/ijms21176275)