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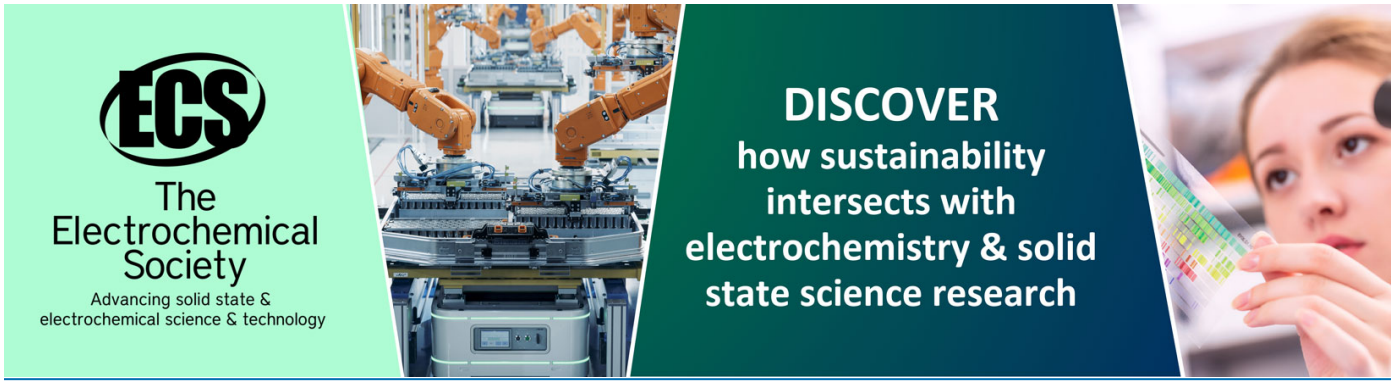

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Genotyping SNP rs7903146 *TCF7L2* gene for detection T2DM in Indonesian melayu ethnic

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Abstract. The prevalence of T2DM is quite high in Indonesian Melayu ethnic. T2DM is a metabolic disorder that is difficult to cure but can be inhibited by its expression. T2DM expression inhibited by knowing its genetic markers. The strong genetic marker for T2DM and widely studied in other countries is SNP rs7903146 TCF7L2 gene. The aim of the research was to identify SNP rs7903146 in TCF7L2 gene as a genetic marker of T2DM in Indonesian Melayu ethnic. Identification of SNP rs7903146 TCF7L2 uses direct DNA sequencing methods and then bioinformatics analysis. SNP rs7903146 in TCF7L2 gene can be a genetic marker of T2DM in Indonesian Melayu ethnic.

1. Introduction

Transcription Factor 7 like 2 (*TCF7L2*) is a strongly related gene with type 2 diabetes mellitus (T2DM) on chromosome 10q. The SNP variant rs7903146 *TCF7L2* is very widely reported as a genetic marker of T2DM. The right genetic markers will be able to provide an indication of go up susceptibility of individual to suffer T2DM. Susceptibility to T2DM increased by the presence of T alleles at rs7903146 [1-3]. Studies of SNP rs7903146 *TCF7L2* in Minang ethnic in West Sumatera [4, 5] and Batak ethnic in North Sumatera [4] have been carry out, therefore the researchers wanted to continue this study on Melayu ethnic in Riau with prevalence of T2DM above the national prevalence rate [6].

Polymorphism can be a change in *TCF7L2* nucleic acids, such as the insertion or removal of nucleotides from a polynucleotide chain. A nucleotide changes resulting in changes in the coding of amino acids. A change of one nucleotide can caused premature generation (truncated polypeptides) because of the stop codon. Removal of several nucleotides, resulting in the removal of one or more amino acids encode by nucleotides. The insertion of one or several nucleotides can cause disruption in coding the gene sequence; duplication of all or parts of genes; transportation of all or parts of gene or rearrangement of all or parts of a gene. More than one of these changes may be in one gene. Such sequence changes can cause differences in polypeptide encoded by *TCF7L2* [3].

Frame shift mutation can cause changes in amino acids, which result in premature generation (truncated polypeptides) due to the stop codon. *TCF7L2* nucleic acid polymorphisms associated with disease or conditions of susceptibility to a disease can be synonymous with changes in one or more nucleotides (i.e. a change that does not result in changes in polypeptides code by *TCF7L2* nucleic

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acids). Such as splicing polymorphism can change the site, affect the stability or transportation of mRNA, or influence transcription or gene translation [3]

Individual susceptibility detection methods for T2DM can be done by identifying the presence or absence of alleles in a marker associated with *TCF7L2* in individuals. A good marker or haplotype give an indication of the increasing susceptibility of individuals to T2DM. Relative risk at 1.2-1.4 can be a mark of increased susceptibility of T2DM. For SNP rs7903146 the presence of T alleles is an marker of increased susceptibility to T2DM

Genetic markers also used as an indication of decreased T2DM susceptibility. The decrease in susceptibility in one embodiment characterized by a relative risk of less than 0.8 or 0.7 markers selected from group rs7903146 and where the presence of C allele at rs7903146 is an indication of a decrease in susceptibility to T2DM [1, 3]

Based on background, formulation of research problems can be state as follows: Is there a relationship SNP rs7903146 *TCF7L2* with T2DM in Indonesian Melayu ethnic. The goal of the study was to identify the relationship of SNP rs7903146 *TCF7L2* with T2DM in Indonesian Melayu ethnic.

2. Method

Type of this study was a case control study, by analyzing SNP rs7903146 *TCF7L2* gene as a genetic marker of type 2 diabetes mellitus (T2DM) (66 people) then controlled by healthy subject without T2DM (66 people) in the Indonesian Melayu ethnic. Blood sampling by consecutive sampling with details of 66 people with T2DM and 66 people in control were not T2DM in Indonesian Melayu ethnic. Criteria for T2DM samples with fasting blood glucose levels (FBG) after fasting for at least 8 hours is 126 mg / dL, 2-hour postprandial glucose (2HPP) ≥ 200 mg / dL. Control criteria with FBG <100 mg / dL and 2HPP <140 mg / dL. FBG and 2HPP measured enzymatically by measuring digital spectroscopy. The study was conducted in the Biomedical Laboratory of the postgraduate program at Faculty of medicine, Andalas University and the Biotechnology laboratory of FMIPA Padang State University.

The initial phase of the study was to ask approval of research ethics committees *(ethical clearance)*, and then collected the sample of 66 patient with T2DM and 66 people without T2DM. The prospective respondent explained the research objectives and procedures and asked to be willing to volunteer the research by signing the *Informed Consent*.

Anthropometry measurement was done to each sample. Measurements of body weight were carried out using a calibrated weight scale. Measurements done twice and the mean values are determined. The position of sample when weighed was stands upright with a straight view and in a calm state. In addition to weight, height was also measured. Body weight (kg) compared to height in squared (m^2) is called the body mass index.

Venous blood of each sample were taken twice, 5 mL before meals and 2.5 ml 2 hours after meal. Before taking the blood, the skin at the site of blood collection was disinfected using alcohol swaps. The first 2.5 mL venous blood was used to determine FBG meanwhile the second one that take 2 hours after meal was used to identify 2HPP blood glucose. Enzymatic standard method was used to determine the level of blood glucose. The remaining 2.5mL of blood was used to test the genetic variant polymorphism of the SNP rs7903146 *TCF7L2* in the Biomedical laboratory of the Medical Faculty of Andalas University. The sequencing was conducted at the Macrogen laboratory in Seoul, Korea.

DNA isolation and Electrophoresis was done for each sample. Pure Link Genomic DNA Kits from Invitrogen USA was used to extract the DNA. The success and the effectiveness of genomic DNA isolation were controlled using electrophoresis techniques. Electrophoresis on 1.5% agarose gel with a voltage of 100 volts for 30 minutes.

Design of primer for SNP rs7903146 *TCF7L2* gene identification, was done by a primary design software. Forward and reverse primers were used to identify the SNP. Each primer ware tested with Genious and Primer Blast software from NCBI to identify the suitability of the primer. The mix

composition of PCR reaction include DNA template, $MgCl_2$, buffers and primer determine the success of the PCR reaction.

The isolated DNA were amplified using the Mix PCR PureTaq Ready-To-Go PCR Beads (RTG) from GE Healthcare. The basic of PCR is to multiply a certain nucleotide sequence exponentially in vitro. The primary will multiply a certain site of nucleotide by thousands or even millions of copies about 10^6-10^7 times, and then electrophoresed was done to make sure that the DNA have been amplified.

The research data in the form of SNP rs7903146 *TCF7L2* polymorphism was obtained using sequencing method. Analysis of sequencing and bio informatics results from *TCF7L2* gene variants in the Melayu ethnic group was carried out qualitatively. The qualitative phase of analysis included: analysis of kontig to determine the good or bad quality of sequences of DNA bases resulting from sequencing. Kontig analysis was done using Genious software. Blast analysis was used to determine the validity of the results of sequencing of the research object species. Blast analysis was done using the Blast program at NCBI. Alignment was used to determine single nucleotide polymorphism between DNA base sequences as sequenced with SNP rs7903146 *TCF7L2*. Alignment was done using software.

Chi square test was used to determine the relationship between SNP rs7903146 *TCF7L2* with type-2 diabetes mellitus in Indonesian Melayus. Independent variables of the test was the existence of the polymorphism rs7903146 *TCF7L2*, while the dependent variable is the incidence of T2DM patient and non-diabetic patients.

Ethical clearance

The present study Obtained ethical clearance from the Medicinal faculty, Universitas Andalas with register number: 136 / KEP / FK / 2018

3. Result and Discussion

Research of polymorphism of Transcription Factor 7 like 2 (*TCF7L2*) gene to patients with type 2 diabetes mellitus (T2DM) in Indonesian Melayu ethnic has done. The total sample of the research is 132 sample, which consisted of 66 cases (T2DM sufferers) and 66 controls.

The success and effectiveness of DNA isolation results are controlled using electrophoresis techniques. Electrophoresis was run on an agarose gel 1.5% with a voltage of 100 volts for 30 minutes. The results of DNA extraction and electrophoresis for seven samples as seen as Figure 1.

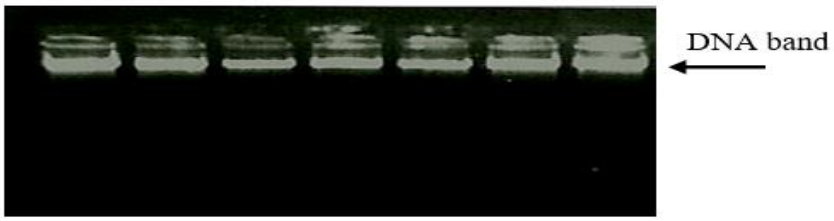


Figure 1. Electrophoregram of genomic DNA of the research subject.

As seen at figure 1, the success of DNA extraction is show by the arrows. The results of DNA isolation stored in a freezer with a temperature of -20ºC and used as template SNP testing sequencing rs7903146 *TCF7L2* gene.

One of the most important things in a PCR reaction is the construction or selection of the right DNA primer. The primer is responsible for recognize and mark the DNA segment of the template. A software was used to confirm the specificity of the primary construction. The analysis was done to prevent the possibility of miss-priming primary with other site of the *TCF7L2* gene other than the site that want to be amplified.

The forward and reverse primer amplified DNA fragments containing the SNP sequence rs7903146 *TCF7L2* with size ± 630bp. The constructed primer tested in in silico and invitro. Testing of in silico primer using Genious and BLAST primer from NCBI, Figure 2.

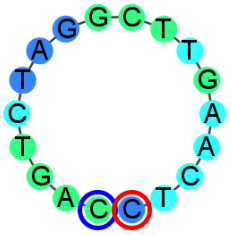
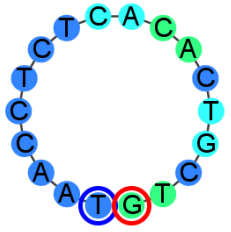
No.	Primary character	DNA Fold
1	Forward Primary rs7903146 Sequence (5'to3 '): CAGTCTAGGCTTGAACTC Type: Primary Lenght: 18 % GC: 50.0 TM: 51.7 Hairpin TM: None Self Dimer TM: 05	
2	Reverse Primer rs7903146 Sequence (5 'to3'): TAACCTCTCCACACTGCT Type: primary Length: 18%GC: 50.0 TM: 53.5 Hairpin TM: None Self dimer TM: None	

Figure 2. Sequence primary SNP rs7903146 *TCF7L2* and test results in silico

Visualization of rs79F primary partner testing and rs79R proves that primers that have been designed can function well in PCR reactions, so that primer that attach another site of *TCF7L2* gene other than the desired site can be avoided. Primary alignment shows that the primary attachment position is sequence 103894 from DNA *TCF7L2*. The primer attachment at this position is in accordance with what was previously predicted that the rs79C internal primer will recognize the region experiencing SNP. Primer rs79C would attach at position 103894 without mispriming.

Concentration or amount of each material in the PCR mix is an important thing to reach the optimum result of PCR reaction. Each component has a different and important role. The success of the reaction depends on the amounts of enzymes, templates, dNTP, $MgCl_2$, buffers and primers. SNP mix rs7903146 *TCF7L2* composition, Table 1.

Table 1. Mix PCR reactions for SNP rs7903146 *TCF7L2*

Reagents	Stock (µM)	Volume (µM)	rs7903146 (µM)
Go Green MM Tag	02	1.0	12.5
Primer F	10	0.1	0.25
Primer R	10	0.4	01.0
$MgCl_2$	50	3.0	01.5
DDH_2O			7.25
DNA template			01.0

Invitro test with PCR reaction also done to test the success of primary construction to detect SNP rs7903146 in *TCF7L2* gene. The basic of PCR is to multiply a certain nucleotide sequence exponentially in vitro. The primary will multiply a certain site of nucleotide by thousands or even millions of copies about 10^6-10^7 times and visualized by electrophoresis as seen as Figure 3.

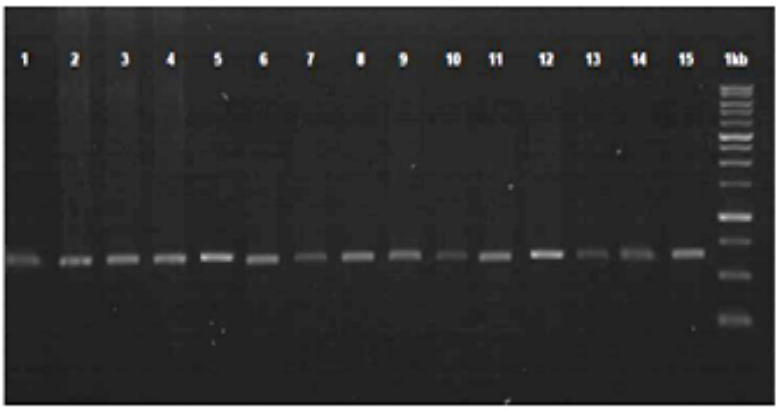


Figure 3. Visualization electropherogram PCR products use primary pairs rs79F and rs79R

Visualization of electropherogram PCR products using primary pairs rs79F and rs79R proves that primers that have been designed can function well in PCR reactions where the formation of a single band on electropherogram visualization.

SNP polymorphism was Rs7903146 *TCF7L2* tested by method *direct DNA sequencing.* The results of sequencing of SNP samples rs7903146 *TCF7L2* were analyzed bio informatics. To tidy up the results of sequencing data, the contig uses the program *Genious.* The results of the contig on the BLAST (*Basic Local Alignment Search Tool*) use the program at NCBI.

BLAST results rs7903146 *TCF7L2* shows that the sequencing sequence has a query value of 200. This result shows that the PCR results with the construction primers are true fragments of the *TCF7L2* gene. Sequencing then be alignment with nucleotides profile rs7903146. *TCF7L2* alignment prove that PCR products with primary forward rs79 and reverse primer rs79 have true fragments of rs7903146 *TCF7L2* so that it can be used to detect the SNP Figure 4.

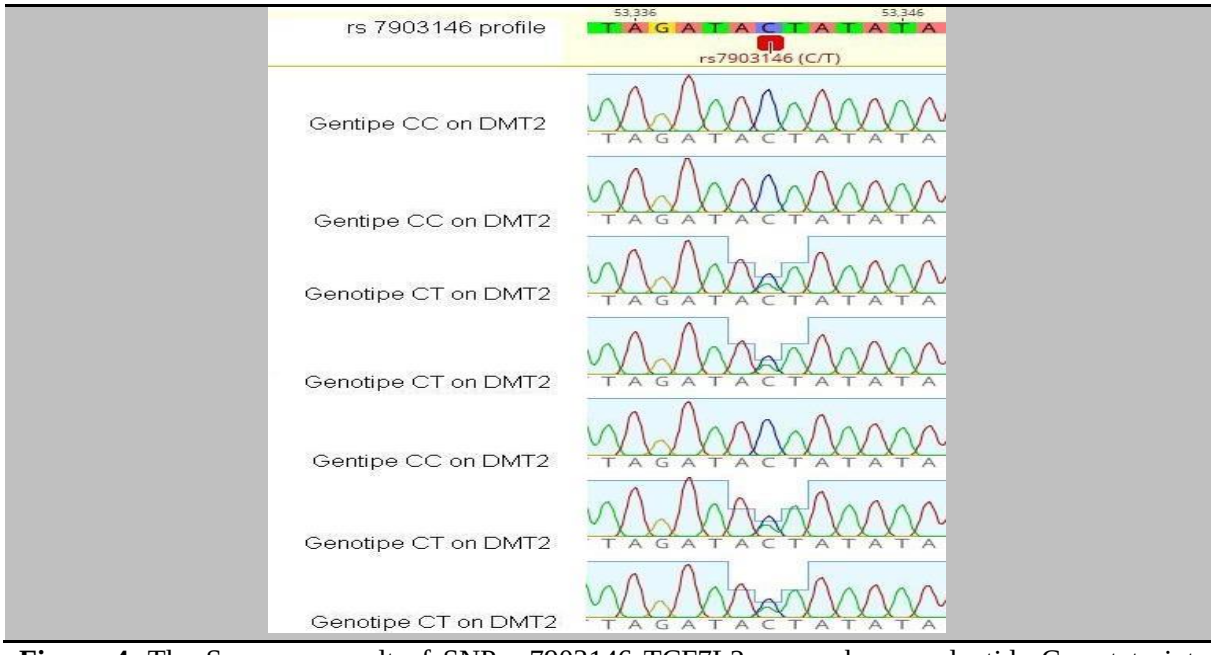


Figure 4. The Sequence result of SNP rs7903146 TCF7L2 gene where nucleotide C mutate into nucleotide T

The results of statistically analysis of *TCF7L2* variant SNP rs7903146 *TCF7L2* in 66 subjects with T2DM and 66 non-T2DM subjects of Indonesian Melayu ethnic (Table 2).

Table 2. Relationship of SNP rs7903146 (C/T) TCF7L2 gene to type-2 diabetes mellitus in Melayu ethnic

SNP	Position Gen (cl)	Gen Region	Genotype / allele	frequency of		*P value*
				Control	with type 2 diabetes	
rs7903146	91.31	Intron 4	TT	0 (0%)	3 (5%)	0.045*
			CT	6 (10%)	16 (26%)	
			CC	56 (90%)	43 (69%)	
			T	6 (7%)	22 (16%)	
			C	118 (93%)	102 (84%)	

Description:
*There is an association between the combination of SNP with the incidence of T2DM

Table 2 shows that SNP rs7903146 TCF7L has an association with T2DM in ethnic Melayu. To support the research, rs7895340 *TCF7L2* gene also found to be associated with the risk of T2DM in scope of Minangkabau ethnic. Our finding has a similar result with other previous studies in some ethnic for example in Asia [7], Finland [8], and Iceland [3].

The polymorphism rs7903146 *TCF7L2* in T2DM patients of the Indonesian ethnic group occurred in 9 samples while in the control there were 6 samples. After the statistical test was obtained p=0.045, thus there was a significant correlation between SNP rs7903146 *TCF7L2* gene with T2DM in Indonesian Melayu ethnic.

RS 7903146 *TCF7L2* has a significant relationship in women with T2DM in the Caucasus nation [9]. There is a significant relationship of rs7903146 *TCF7L2* in weight loss in T2DM populations [9]. Meta analysis of eight research reports and successfully identified the association of rs7903146 *TCF7L2* in increasing the risk of T2DM [7]. A significant association of rs7903146 *TCF7L2* in the population of African American [10]. This phenomenon is also in line with previous research on ethnic Iceland [3], on Finnish [8], and on ethnic Pima Indians [11].

In this study, it was also found OR = 2.51, which means that someone who is not T2DM (normal) when experiencing SNP polymorphism rs7903146 *TCF7L2* has a chance of 2.51 times to suffer from T2DM. Relative risk of 1.2-1.4 show that someone have a bigger probability to suffer T2DM [3].

The success of the discovery of polymorphism SNP rs7903146 *TCF7L2* gene in T2DM patients in Indonesian Melayu ethnic is also supported by the appropriate primary design results. In this study used a pair of primers designed by themselves, namely forward rs79F: 5'-CCTTGTGATGTCTTCTCTCC-3 '; reverse primer rs79R: 5'-ACATCTTCTCTCCTCACTC-3 '. The RS78F primer, the reverse RS78R primer is used to amplify DNA, which covers an area of ± 630bp. In addition to the primer, the mix composition used also determines the success of the study. The mix composition for the ARMS-PCR reaction for SNP rs7903146 *TCF7L2* is as follows: Go Tag Green MM (1 µM) as much as 12.5 µL; Primer rs-79F (0.1 µM) 0.25 µL; Primer rs-79R (0.4 µM) 0.1 µL; $MgCl_2$ (3 µM) 01.5 µL; ddH_2O 7.25 µL and template DNA of 1.0 µL with a total mix of 25 µL.

The PCR program used for SNP rs7903146 *TCF7L2* is touchdown PCR with the following profiles: initial denaturation 95ºC 3 minutes; denaturation 95ºC 30 second; annealing 64ºC 30 second and elongation 70ºC 30 resent as much as 5 cycles. Continued denaturation 95ºC 30 second; annealing 64-54ºC 30 second and elongation of 70ºC 30 as much as 10 cycles. Finally, denaturation 95ºC 30 second; annealing 54ºC 30 second and elongation 70ºC 30 resized as many as 20 cycles with the final stage of elongation 70ºC 7 minutes and 12ºC 10 minutes.

4. Conclusion

SNP rs7903146 *TCF7L2* gene find to be associated with type 2 diabetes mellitus (T2DM) in ethnic Melayu, and can be used as a genetic marker for T2DM.

Acknowledgment

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