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

S Syamsurizal¹, D Handayani¹, H Kadri² and E Badriyya³*
¹Biology Department, Faculty of Mathematics and Science, Universitas Negeri Padang, Jl. Prof. Dr. Hamka Padang, West Sumatera, Indonesia
²Faculty of Medicine, Universitas Andalas, Limau Manis, Sumatera Barat, Indonesia
³Faculty of Pharmacy, Universitas Andalas, Limau Manis, Sumatera Barat, Indonesia
*badriyyaelsa@gmail.com

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
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 spectrophotometry. 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 is 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 (m²) 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, MgCl₂, 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 Melayu ethnic group. 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.

![Electrophoregram of genomic DNA of the research subject.](image)

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 -20°C 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 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 | ![DNA Fold](image1) |
|     | Sequence (5' to 3'): CAGTCTAGGCTTGAACTC | ![DNA Fold](image2) |
|     | Type: Primary | ![DNA Fold](image3) |
|     | Length: 18 | ![DNA Fold](image4) |
|     | % GC: 50.0 | ![DNA Fold](image5) |
|     | TM: 51.7 | ![DNA Fold](image6) |
|     | Hairpin TM: None | ![DNA Fold](image7) |
|     | Self Dimer TM: 05 | ![DNA Fold](image8) |
| 2   | Reverse Primer rs7903146 | ![DNA Fold](image9) |
|     | Sequence (5' to 3'): TAACCTCTCCACACTGCT | ![DNA Fold](image10) |
|     | Type: primary | ![DNA Fold](image11) |
|     | Length: 18%GC: 50.0 | ![DNA Fold](image12) |
|     | TM: 53.5 | ![DNA Fold](image13) |
|     | Hairpin TM: None | ![DNA Fold](image14) |
|     | Self dimer TM: None | ![DNA Fold](image15) |

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, MgCl2, buffers and primers. SNP mix rs7903146 TCF7L2 composition, Table 1.

| 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         | 0.10           |
| MgCl2             | 50         | 3.0         | 0.15           |
| DDH2O             |            | 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.
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.

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 Region | Genotype / allele | frequency of with type 2 diabetes | P value |
|-------|---------------------|-------------------|-----------------------------------|---------|
| 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 TCF7L2 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 the 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'-CCTTGTGATGTTCTTCTCCTCC-3' ; reverse primer rs79R: 5'-ACATCTTCTCTCCTCCTC-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; MgCl2 (3 µM) 0.15 µL; ddH2O 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 95°C 3 minutes; denaturation 95°C 30 second; annealing 64°C 30 second and elongation 70°C 30 second as much as 5 cycles. Continued denaturation 95°C 30 second; annealing 64-54°C 30 second and elongation of 70°C 30 as much as 10 cycles. Finally, denaturation 95°C 30 second; annealing 54°C 30 second and elongation 70°C 30 resized as many as 20 cycles with the final stage of elongation 70°C 7 minutes and 12°C 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
On this opportunity, we would like to thank the director of the DRPM of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia, as research sponsor contract no. SP DIPA 042.06.1.401516/2018. All the staff at Biomedical Laboratory, Faculty of Medicine, Universitas Andalas and the laboratory of Genetics and Biotechnology, Faculty of Mathematics and Natural Sciences, Padang State University that helped the success of this research.

References
[1] Florez, JC, et al., TCF7L2 Polymorphisms and Progression to Diabetes in the Diabetes Prevention Program. The New England Journal of Medicine, 2006. 355(3): p. 241-250.
[2] Stolerman, ES, et al., TCF7L2 variants are associated with increased proinsulin / insulin ratios but not obesity traits in the Framingham Heart Study. Diabetologia, 2009. 52(1): p. 614-6520.
[3] Grant, SF, et al., Variant of transcription 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nature genetics, 2006. 38(3): p. 320-323.
[4] Syamsurizal and H. Kadri, SNP Genotyping Rs12255372 TCF7L2 Gene Using Three-Primary ARMS-PCR for Detection T2DM in Indonesian Batak Ethnic, in International Conference on Mathematics and Natural Sciences (IConMNS), IWP Astawa, Editor 2018, IOP Conf. Series: Journal of Physics: Conf. Series 1040 (2018) 012003: Bali, Indonesia. p. 1-8.
[5] Syamsurizal, et al., 7-like 2 transcription factor as type-2 diabetes mellitus diagnostic marker in ethnic Minangkabau. Universa Medicina, 2014. 33(3): p. 206-213.
[6] Trihono, Basic Health Research. RISKESDAS 2013, 2013. 1(1): p. 1-305.
[7] Peng, S., et al., TCF7L2 gene polymorphisms and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121 subjects. Mutagenesis, 2012. 28(1): p. 25-37.
[8] Scott, LJ, et al., Association of Transcription Factor 7-Like 2 (TCF7L2) Variants With Type 2 Diabetes in a Finnish Sample. Diabetes, 2006. 55(1).
[9] Haupt, A., et al., Gene Variants of TCF7L2 Influence Weight Loss and Body Composition During Lifestyle Intervention in a Population at Risk for Type 2 Diabetes. DIABETES, 2010. 59(1): p. 747-750.
[10] Sale, MIM, et al., 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, 2007. 56(1): p. 2638-2642.
[11] Guewo-Fokeng, M., et al., Contribution of TCF7L2 rs7903146 (C / T) gene polymorphism to the susceptibility to type 2 diabetes mellitus in Cameroon. J Diabetes Metab Disord, 2015. 14(26).