Genotyping SNP Rs12255372 TCF7L2 Gene Using Three-Primer ARMS-PCR for Detection T2DM in Indonesian Batak Ethnic

Syamsurizal¹ and H Kadri²
¹Universitas Negeri Padang
²Universitas Andalas
E-mail: syam_unp@yahoo.co.id

Abstract. Genetic markers may indicate the increasing of individuals' susceptibility towards type 2 diabetes mellitus. The purpose of this research is to construct a primary SNP rs12255372 variant TGCCCAGGAATAAGGCAAGAATTCC (G/T) ATACCATATTCTGAATTACTCAGGC in the TCF7L2 gene and to determine the primary ability to detect polymorphisms of SNP rs12255372 variant TCF7L2 gene as a genetic marker. A case control study was used as the research method. Samples (62 people) were taken from the people of Batak ethnic with diabetes mellitus type 2 who came to the Metabolic Endocrinology clinic of Dr. M. Djamil Hospital. Controls were taken from healthy people without type 2 diabetes mellitus (62 people). EDTA blood was taken for DNA extraction. Polymorphisms of SNP rs12255372 in the TCF7L2 gene were detected by using three primer ARMS-PCR method and direct DNA sequencing method, and then were Analysed by bioinformatics. Based on the data analysis, it can be concluded that it has successfully constructed three primers ie RS12F forward primer, reverse primer and forward primer RS12R RS12C. All constructed primers are able to recognize SNP rs12255372 in TCF7L2 gene with three primer ARMS-PCR method, however, the SNP rs12255372 is genetic markers of type 2 diabetes mellitus for the Indonesian Batak ethnic.

1. Introduction
Worldwide, diabetes kills more humans than HIV/AIDS. Estimated number of people died of diabetes mellitus in 2000 to reach 6% (3.2 million people). One in ten people in the world died at the age of 35-64 years is a history of diabetes mellitus [1]. Every 10 seconds a person dies from complications of diabetes mellitus and at the same time discovered two new persons with diabetes mellitus [2].

Patients with diabetes mellitus in the world each year has increased, including in Indonesia and West Sumatra. The prevalence of diabetes mellitus in the world in 2000 amounted to 2.8% (171 million) and projected in 2030 by 4.4% (366 million people). Estimated number of people with diabetes mellitus in Indonesia in 2000 amounted to 4.1% (8.4 million Indonesian population of 205 132 000 votes). Projected in 2030 the number of cases of diabetes mellitus in Indonesia will increase by 7.8% (21.3 million out of 273 219 200 people). Estimated number of people with diabetes mellitus in Indonesia ranked fourth after India, China and America [2].

In clinical diabetes mellitus can be divided into four types, types I, II, gestational and other types. Diabetes mellitus type 2 is the most common type found 95% [3]. Diabetes mellitus type 2 occurs because the hormone insulin in the blood does not work effectively, although the amount of insulin
produced by the beta cells of normal pancreatic islets of Langerhans. Glucose entry into the cell so that the cell is reduced so that the shortage of energy resources increased blood glucose [4]. Diabetes mellitus type 2 is influenced by several factors as follows: family history of diabetes, obese, risky lifestyles, lack of rest, and stress [4, 5].

Diabetes mellitus type-2 will appear in a person with a genetic defect after a genetic change in a long time. Acceleration and deceleration process of genetic change is highly dependent on environmental factors that influence it. If the genetic factors do not develop towards deterioration due to environmental factors, then theoretically diabetes mellitus type 2 will not come to the surface. Abnormalities or genetic abnormalities at an early stage without any symptoms that are clinically difficult to recognize [6]. Developing genetic markers toward deterioration but has not led to impaired sugar tolerance (IGT) can be detected through DNA analysis. DNA analysis is required to perform genetic data in the form of genes associated with type-2 diabetes mellitus. Some nations in the world already has a gene bank for diabetes mellitus type 2 such as the Caucasus, Denmark, USA, Britain, France and India [7].

Among the genes associated with type 2 diabetes mellitus are genes' transcription factor 7 like 2 (TCF7L2) "on chromosome 10q. TCF7L2 gene strongly associated with type 2 diabetes mellitus in ethnic Danish, Caucasian, Indian, and ethnicity of nations in Asia [7, 8]. TCF7L2 gene variant may be candidates for genetic markers in Indonesian Batak ethnic people with diabetes mellitus type 2.

One new surprises found in the Human Genome Project is the single nucleotide polymorphisms (SNPs). SNPs are minor elel the existence of more than 1%. If SNPs occur at the gene coding regions can lead Synonymous (does not cause amino acid changes) or non Synonymous. But in the last few years of research SNPs Synonymous led to the evolution that encourage the occurrence of a disease [9]. SNPs Synonymous can alter the structure, function, expression of the protein. Polymorphisms Synonymous can cause RNA splicing, stability and structure of proteins can be damaged. These changes may cause significant effects on protein function, changes in cellular response. Single nucleotide polymorphisms (SNPs) are DNA sequence variations that may be associated with susceptibility to a disease just as diabetes mellitus type 2. Most SNPs are non-coding region which is the basis of genetic variation in humans and refers to a single base differences between individuals [10].

The marker or haplotype is right will provide an indication of individual susceptibility to type 2 diabetes mellitus. Embodiment increased susceptibility is characterized by a relative risk of at least 1.2-1.4. TCF7L2 gene variant most strongly suspected association with diabetes mellitus type 2 is a variant rs12255372. Sequent rs12255372 is TGCCAGGAATAAGGCAAGAATTCC (G/T) ATACCATATTCTGAAATTACTCAGGC. The presence of the T allele in rs12255372 is indicative of increased susceptibility to diabetes mellitus type 2 [11].

The absence of genetic data on association the rs12255372 variant of the gene TCF7L2 with ethnic Batak people with diabetes mellitus type 2, it becomes its own interest to be investigated. In this research, a method of tracking the SNP rs12255372 with three primer ARMS-PCR method and DNA Direct Sequencing to identify TCF7L2 gene polymorphism in patients with type 2 diabetes mellitus ethnic Batak. The purpose of research is to develop an early warning system with type 2 DM molecular fast, accurate so that it can assist in the prevention or treatment of type 2 DM in ethnic Batak. Specific target research are: primary construct for variants rs12255372. in genes TCF7L2 knowing the primary ability to detect polymorphisms TCF7L2 gene variant rs12255372.

2. Method

This study is a descriptive study, in which researchers describe the results of the primary construction and confirmation of the primary ability to amplify the desired region. The research was conducted in the laboratory of Biomedical Universitas Andalas and the laboratory of Biotechnology Universitas Negeri Padang, West Sumatera, Indonesia.

The tools used in this study is water bath, engine thermo cycler (Polymerase Chain Reaction), micro centrifuge, micropipette, a tube eppendorf, microtube, vortex, chamber, tube rack micro, power
supply, magnetic stirrer, tip, loop, LAFC, PCR tube, camera. Materials used are dNTP's, taq polymerase mL, 10X buffer, MgCl$_2$, H$_2$O, agarose, TAE IX, red gel, aquabidest, tris-base, EDTA, glacial acetic acid, 100bp DNA ladder.

In this study the DNA is derived from human peripheral blood. These isolates are needed to examine whether primary, constructed to work amplifying the DNA fragment of interest. Data were analysed qualitatively, the analysed data is the result of the primary construction and the primary ability to amplify the desired region.

Construction Primer. Primers used to detect SNP rs12255372 on gene TCF7L2 with ARMS-PCR method is constructed using computer software "primary designer". Will produce three results of primary construction RS12F forward primer, reverse primer and forward primer RS12C RS12R. Primary RS12F, RS12R reverse primer used to amplify DNA that includes the region $\pm$ 838 bp (hereinafter referred to as the external primer). Primary RS12C and RS12R used to fragment size and $\pm$ 384 bp, an area that includes the SNP rs12255372 (called internal primer). TCF7L2 gene sequences which will be used for the primary construction was obtained from gene NCBI bank. Possibility misprime then the next primary construction results ready to be synthesis.

Confirmation is done using software to look at the possibility of misprime a primary with other regions in the gene TCF7L2 other than the area to be amplified. If not found zed into the oligonucleotide primer. The primary ability to amplify the desired region. Do the sequence of events as follows: Isolation of DNA using a kit from Invitrogen. Furthermore, the isolated DNA electrophoresis.

Amplification by ARMS-PCR method. DNA obtained from the insulation, then amplified using primers constructed with PCR mix RTG / Go Green Tag. To determine the results of amplification, electrophoresis on a 1.5% agarose gel. The steps performed during the study: 1) Isolation of DNA from the sample. 2) Design of primers for genes TCF7L2 primary use software designer. 3) Optimization of PCR reactions using the primer design results. 4) TCF7L2 gene amplification by PCR. 5) ARMS-PCR and sequencing for analysis of polymorphic sites. 6) bioinformatics analysis

3. Result and Discussion

One of the things that is very important in the PCR reaction is DNA constructs or appropriate primary election. Primary responsibility to recognize and mark the DNA segment template to be amplified. In this study generated three primary pieces that RS12F forward primer, reverse primer and forward primer RS12C RS12R. Primary RS12F, RS12R reverse primer used to amplify DNA that includes the region $\pm$ 838 bp (hereinafter referred to as the external primer). Primary RS12C and RS12R used to fragment size and $\pm$ 384 bp, for more details see Table 1.
fragments formed relatively can be seen in Figure 1.

Annealing at that position as predicted earlier that the internal primary rs12C will be amplified subsequently confirmed by the soft ware. This done to avoid the possibility of mis prime a primary with other regions in the gene TCF7L2 other than the region to be amplified [12]. It can be seen that the position of the annealing rs12C are DNA sequences of TCF7L2 103 894. Annaling at that position as predicted earlier that the internal primary rs12C will identify areas that experienced the SNP. Theoretically, annealing primers 12C will start from position 103 894 and there were no possibility mis prime. The position and size of the tape primer / DNA fragments formed relatively can be seen in Figure 1.

| Criteria         | Setting          | Result |
|------------------|------------------|--------|
| % GC             | Min 50, Max 60   | 50 YES |
| Tm C             | Min 55, Max 80   | 70 YES |
| No Hairpins      | Energy cut off 0.0 kcal | - YES |
| No 3 Dimers      | Reject >= 3 matches at the end 3' | 2 YES |
| No Dimers No     | Reject >= 7 homologous base | 3 YES |
| Runs             | Reject >= 3 base runs | 2 No |
| No 3' GC runs    | Reject >= 3 G or C at the end 3' | 1 YES |

Table 1. Results of the primary reconstruction RS12C

| Criteria         | Setting          | Result |
|------------------|------------------|--------|
| % GC             | Min 50, Max 60   | 50 YES |
| Tm C             | Min 55, Max 80   | 70 YES |
| No Hairpins      | Energy cut off 0.0 kcal | - YES |
| No 3 Dimers      | Reject >= 3 matches at the end 3' | 2 YES |
| No Dimers No     | Reject >= 7 homologous base | 4 YES |
| Runs             | Reject >= 3 base runs | 2 YES |
| No 3' GC runs    | Reject >= 3 G or C at the end 3' | 2 YES |

Table 2. The results of the primary construction and RS12RRS12F

| Criteria         | Setting          | Result |
|------------------|------------------|--------|
| % GC             | Min 50, Max 60   | 50 YES |
| Tm C             | Min 55, Max 80   | 70 YES |
| No Hairpins      | Energy cut off 0.0 kcal | - YES |
| No 3 Dimers      | Reject >= 3 matches at the end 3' | 1 YES |
| No Dimers No     | Reject >= 7 homologous base | 2 YES |
| Runs             | Reject >= 3 base runs | 2 YES |
| No 3' GC runs    | Reject >= 3 G or C at the end 3' | 0 YES |

Specificity primary construction made subsequently confirmed by the soft ware. This is done to avoid the possibility of mis prime a primary with other regions in the gene TCF7L2 other than the region to be amplified [12].
Determine the ability of primary constructed in detecting SNPs in the gene TCF7L2 particular SNP rs12255372, then was examined by PCR. The principle of PCR is multiplying exponentially a specific nucleotide sequence in vitro. In order to identify sequences that will be multiplied needed a special and specific primer. The primary area known is what will be multiplied by thousands or even millions of copies, about 10 \(6\) - \(10^7\) times so as to be visible band after electrophoresis of the amplified DNA.

The initial phase of the primary confirmation is carried out separately in accordance with the conditions of each primer pair. Factors that must be considered in obtaining optimum results in the PCR is the amount / concentration mix used. Each of these components has a very important role in a PCR reaction. The composition of the enzyme, template, dNTPs, MgCl\(_2\) buffer and appropriate primers determine the success of a PCR reaction. DNA obtained from the insulation, then electrophoresed on a 1.5% agarose gel to confirm the successful isolation of DNA from the sample. For more details, see Figure 2.

**Figure 1.** The position of primer and the amount of ribbon/DNA fragments formed

DNA obtained from the insulation, then amplified using primers constructed with PCR mix RTG / Go Green Tag. The results of PCR amplification are analyzed using electrophoresis on agarose. Electrophoresis is the movement of charged molecules in an electric field. Speed moving molecules in an electric field depends on the charge, shape and size of the molecule. Agarose and poly acrylamide is a buffer matrix that is widely used for the separation of proteins and nucleic acids. In this experiment, agarose 1.5%. According to Sambrook and Russell, agarose 1.5% is suitable for separating DNA fragment size of 200-300 bases. The location of the DNA contained in the gel can be observed by staining using red gel, so that later can be seen as a gel placed over Gel Doc.

From these electrophoresis it can be seen that the ARMS-PCR reactions are performed can be used to detect SNPs in the gene TCF7L2 particular SNP rs12255372. But this method has its limitations include: the reaction may not be able to detect 100% of SNP in the gene TCF7L2. Nevertheless, the high specificity and sensitivity in detecting SNPs can be used as one factor why this method can be used. In addition, if compared with other SNP detection method, ARMS-PCR reaction has several advantages such as cheaper and easy to apply. Process/ shorter implementation time,
starting from the preparation of reagents, equipment, including the addition of genomic DNA (template), three-ARMS-PCR amplification and electrophoresis on agarose can be completed in one day. Its application is quick and easy method to detect SNP rs12255372 is a very important value for the prevention of type 2 diabetes [13]. Sequencing. Besides using three-ARMS-PCR method to know SNP rs12255372 TCF7L2 used sequencing method [14]. Six samples were sequenced to accuracy of three-ARMS-PCR method. Based on the sequencing results are conformity with three-ARMS-PCR method. While the results showed polymorphisms can be seen in Figure 3. While the results showed not happen polymorphisms can be seen in Figure 4. Sequencing can be seen in the following figure:

Figure 3. The results of sequencing the samples undergo polymorphisms at rs12255372 when the change in the base G into T.

Figure 4. The results of sequencing the samples did not experience polymorphisms at rs12255372 where no change of base G.

4. Conclusion

Based on the results of data analysis can be concluded that it has successfully constructed three primary pieces that RS12F forward primer, RS12C reverse primer and RS12R forward primer. The third primer capable of recognizing constructed the SNP rs12255372 TCF7L2 gene with ARMS-PCR method.

Acknowledgments

On this occasion, we would like to thank the director of DRPM Kemristek and Dikti Republik Indonesia, as research sponsors. So also all staff at Biomedical Laboratorium, Faculty of Medicine, Andalas University and laboratory of Genetics and Biotechnology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang that helped the success of this research.

References

[1] IDF, Diabetes Atlas, IDF, Editor 2017, IDF: Brussels Belgium.
[2] IDF, Diabetes Atlas, 2015, IDF: Brussels.
[3] Syamsurizal S 2017 Bioscience 1 (1) 1-7
[4] Grant S F, et al. 2006 Nature genetics 38 (3) 320-323.
[5] ADA 2010 Standards of Medical Care in Diabetes CareDiab 33 (1) 1-5
[6] Chen R, et al. 2012 Type 2 Diabetes Risk Alleles Demonstrate Extreme Directional Differentiation among Human Populations, Compared to Other Diseases. PLoS Genetics, 8 (4)
[7] Mohan R V V 2007 Indian J Med Res 12 (5) 259-274
[8] Nordman S 2008 Identification of susceptibility gene type-2 diabetes (Stockholm: Karolinski Institutet)

[9] Komar A 2009 Single Nucleotide Polymorphisms: Methods and Protocols (Cleveland, OH, USA: Humana Press)

[10] Kwok P 2003 Single nucleotide polymorphisms: Methods and Protocols (Totowa, NJ: Humana Press)

[11] Lyssenko V, et al. 2007 The Journal of clinical investigation 117 (8) 2155-2163

[12] Alsmadi O, et al. 2009 BMC Research Notes 2 (48) 1-7.

[13] Syamsurizal and Sardi A. 2015 Transcription factor 7-like 2 as type-2 diabetes mellitus diagnostic marker in ethnic Minangkabau Univ Med. 33 (1) 206-13.

[14] Abdullah N, et al. 2015 Characterizing the genetic risk for Type 2 diabetes in a Malaysian multi-ethnic cohort. Diabet. Med. 1(1)