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SNP (Single Nucleotide Polymorphism) at Adiponectin Gene in Type 2 Diabetes Mellitus (T2DM) Patients)

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Abstract

Type 2 diabetes (T2DM) is a complex metabolic disorder characterized by insulin resistance and glucose dysregulation. Single nucleotide polymorphisms (SNPs) within the adiponectin gene have been extensively studied in association with T2DM susceptibility and associated clinical manifestations. Adiponectin, an adipocyte-derived hormone, plays important role in insulin sensitivity and glucose homeostasis. This overview provides comprehensive overview of SNP mutations within the adiponectin gene and their impact on T2DM patients. A number of studies have identified specific SNPs such as rs2241766, rs1501299, rs266729, and rs822395 that are commonly associated with T2DM susceptibility. These SNPs contribute to changes in adiponectin expression, secretion, and function, thereby affecting insulin resistance and glucose metabolism. Reduced levels of adiponectin due to specific SNP variations may contribute to the development of insulin resistance and her T2DM. The aim of this research was to identify the presence of SNP 45 in patients with type 2 diabetes mellitus. Method of this research is descriptive exploration using type 2 diabetes mellitus patients in Gledug Village, Blitar Regency as specimen donors. Identification using DNA extraction, DNA Amplification, sequencing and bioinformatics analysis. The results of this project seven DNA extracted, the seven sequences have the same Qv20+ value as the sequence base pair, alignment analysis using blast, when compared with the adipoQ gene sequence with accession number NG_021140.1, it was found that the KT, SR and BP sequences had a 100% similarity level. Identification of candidate SNPs in the absence of the adenine nucleotide was found in the SR, KT, and ST sequences.

1. INTRODUCTION

Diabetes mellitus is a metabolic disease with a high prevalence in the world. According to the international diabetes federation there are around 537 million adults living with diabetes and it is estimated that this will increase to 643 million people by 2030. Carried out by the Sanankulon health center in the village of Gledug, according to Posyandu records in Gledug Village, around 50 people who came to the Posyandu suffered from diabetes mellitus. Metabolic diseases are mostly caused by unhealthy lifestyles, such as consumption of foods that are high in carbohydrates and fat greater than consumption of foods that contain lots of fiber and vitamins, the habit of staying up late, smoking and drinking alcoholic beverages [1]. In addition to an unbalanced lifestyle, both the imbalance in the number of calories consumed and calories expended also triggers the occurrence of metabolic diseases such as obesity, cardiovascular disease, hypertension and diabetes [2].

The type of diabetes that most people suffer from is type 2 diabetes. The cause of type 2 diabetes is resistance to the insulin hormone, when insulin does not work properly, blood glucose was increases, it will trigger continuous insulin secretion, which will trigger pancreatic damage and causes less insulin to be produced even though blood sugar continues to increase (hyperglycemia)[3]. The occurrence of insulin productivity resistance is not only triggered by diet, but also lifestyle. It was influenced by changes in the sequence of several genes involved in insulin production, for example the adiponectin gene [3}.

Adiponectin is a gene responsible for the synthesis of serum adipocytokines. Adipocytokine is a protein produced by pancreatic beta cell adipocytes, because adipocytokine synthesis influences insulin resistance. According to adipocytokine protein synthesis affects insulin resistance in the participants. If there is an abnormality in the adiponectin gene, it is the same case to triggering an abnormality in insulin synthesis. One of the abnormalities that have been identified in the adiponectin gene in Japanese and European communities is the presence of a SNP (single nucleotide polymorphism) that appears in the gene sequence [4].

SNP or single nucleotide polymorphism is the occurrence of polymorphism in only one nucleotide of a DNA sequence. Notably, a representative number of SNPs within this region have been selected to assess the relationship between ADIPOQ on T2D susceptibility, but the results are somehow inconsistent depending on the population studied [5]. Interestingly, there is decent evidence to suggest that a silent T to G substitution in exon 2 (rs2241766) and a G to T substitution in intron 2 (rs1501299) is associated with changes in plasma adiponectin, and indeed, more consistent association data are noted for these two SNPs, especially in Asian populations [6]. Therefore it is necessary to explore the SNPs in the adiponectin gene in type 2 diabetes mellitus.

2. MATERIALS AND METHODS

Study subjects

The donor of the specimen was a patient with type 2 diabetes mellitus in Gledug Village, Sanankulon District, Blitar Regency . The sample age range used is 18-55 years old. Specifically for the age range, no specific age reference is used due to limited willingness to be a respondent or sample donor. Data on type 2 diabetes mellitus patients were obtained from the village posyandu with permission from the local polindes. Before agreeing to become a specimen donor, the respondent must sign a letter of consent as a respondent, and obtain a clear explanation of the benefits of the study, as well as the risks that may be accepted if they agree to become a donor (informed consent). The specimens used were cells of the inner oral mucosa of patients with type 2 diabetes

mellitus. The specimens were collected using a buccal swap. Specimens that have been taken will be stored in a vtm (virus transport medium) tube and then brought to the laboratory.

DNA Extraction and Purification

Identification of SNP 45 was carried out through several stages, the first being DNA extraction. DNA extraction was carried out using the NEx kit. The DNA that had been obtained was then amplified using PCR Master Mix Nexpro using primers SNP 45 Forward(5' GGCTCAGGATGCTGTTGCTGG3') and SNP 45 Reverse (5' GCTTTGCTTTCTCCC). The amplification results were then identified using gel electrophoresis and continued with sequencing.

DNA Sequencing

DNA Sequencing use sanger method, Its ingredients are similar to those needed for DNA replication in an organism, or for polymerase chain reaction (PCR), which copies DNA in vitro. They include: A DNA polymerase enzyme A primer, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase The four DNA nucleotides (dATP, dTTP, dCTP, dGTP) The template DNA to be sequenced. However, a Sanger sequencing reaction also contains a unique ingredient: Dideoxy, or chain-terminating, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye. The result of DNA sequencing than examine using Qv20+.

SNP Identification

SNP identification start with alignment result sequence with Adiponectin sequence which already registered on NCBI with accession number NG_021140.1, and find the single nucleotide polymorphism on those BLAST result.

3. RESULTS

Based on Gledug village posyandu data, DNA samples were taken from 8 people in the sub-district of Sanankulon who had type 2 diabetes mellitus. Based on the work schedule of the Health Office, type 2 mellitus patients are certainly being monitored for and data collecting on is being done in the Blitar Regency area, one of which is for residents of Gledug Village. Only 8 of the 20 individuals who tested positive for type 2 diabetes mellitus agreed to sign a letter of concern, and only samples of partial epithelium in the cheek wall in these 8 patients may be collected using a buccal swab.

Use the DNA extraction procedure to identify polymorphisms, followed by PCR, purification with gel electrophoresis, sequencing, and identification of sequence results using NCBI blasting (figure 1). (table 1.)

Utilizing kitNEX, DNA specimens were extracted. Using a primer designed specifically for cloning a particular portion of the adipoQ gene, extracted DNA was first amplified using a Master Mix Nexpro PCR equipment. SNP 45 Forward (5' GGCTCAGGATGCTGTTGCTGG3') and SNP 45 Reverse (5' GCTTTGCTTTCCCCC TGTGTCT 3') are the primers used in this test. After determining that the target gene was amplified, the amplification result is determined by its purity using gel electrophoresis (Figure 1). The DNA sample is subsequently sequenced using the 1stBASE sequencing service Laboratories Sdn Bhd.

Only seven of the eight samples were sequenced since only seven of the samples matched the length of the control sequence, and one sample could not be interpreted as "smear" (figure 1).

DNA smearing on agarose gels after restriction digestion can occur for a variety of

reasons. After restriction digestion, DNA smears may appear on agarose gels for one or more of the following reasons.

Nuclease contamination in the digestion reaction, running buffer issues in the gel box, or restriction enzymes with high affinity for DNA nuclease contamination.

After incubation at a given digestion temperature, each of these components can be tested individually with appropriate controls. Running buffers stored at room temperature for long periods of time can eventually expire and adversely affect electrophoresis. For best results, if the buffer in the gel box is cloudy and the gel run is showing erratic results, rinse the gel box and use a new gel before loading the digest. . Addition of a 0.1–0.5% SDS solution after digestion facilitates the dissociation of the enzyme from the DNA so that the correct banding pattern is displayed when the samples are tested on a gel if DNA binding is suspected.

The seventh successfully purified DNA samples are then sequenced to determine whether the SNP is present in the DNA. Sequencing was performed using the sequencing service of 1stBASE Laboratories Sdn Bhd. Seven SNP45 gene isolates were successfully sequenced as indicated by Qv20+ values. This is equivalent to the base pair length of the sequencing results.

Qv20+ is the total number of bases across lanes with a base caller quality score >=20. Security measures for base calling and consensus calling algorithms. A high quality value that corresponds to a low probability of algorithmic error. The trace quality value is the quality value for each criterion of the trace. Consensus Quality Score is the quality score per consensus (more suitable for variant analysis engine and variant reporter).

After reliably analyzing the length of the sequence using Sanger sequencing data analysis (Qv20+), the accuracy of the sequence was confirmed by matching the analyzed sequence with the ADIPOQ gene sequence registered with NCBI by the BLAST method. (table.3).

Based on Table 3, the lowest sequence similarity is the sequence of the AST1 code, 97.67%, and the highest similarity is 100% for the sample codes SR, KT, BP. The degree of similarity indicates how similar the base sequence of the nucleotides that make up the analysis sequence is to the ADIPOQ gene sequence registered with NCBI. A similarity score of 100% means that there is a high probability that no polymorphism will be found in the target sequence, and a similarity score of less than 100% will likely find a nucleotide change in the target sequence. Results are not accurate. as a sequence index (gene AdipOQ with accession code NCBI NG_021140.1).

Table 1. Sample Prescriptions

No.	Code	Gender (Male (M)/Female (F)	Age (years)	Blood Sugar Level (mg/dl)	Prescription	Diet
1.	SR	F	55	160	Metformin	Without Rice
2.	ASR	M	35	140	Metformin	With Rice
3.	KT	F	52	190	-	Without Rice
4.	AKT	F	26	140	Amaryl	With Rice
5.	BY	M	55	140	-	Without Rice
6.	ABY	F	31	180	-	With Rice
7.	ST	F	35	170	Metformin	With Rice

8.	AST	M	18	140	-	With Rice

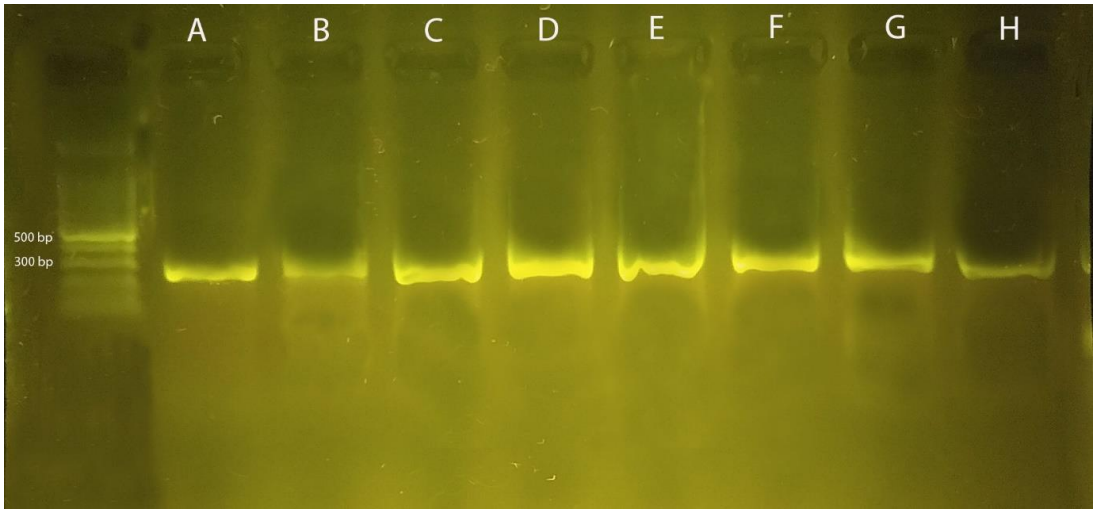

Figure 2. Electrophoresis of PCR results. A: ABY, B: AKT, C: AST, D: SR, E: ST, F: KT, G: BY, H:ASR

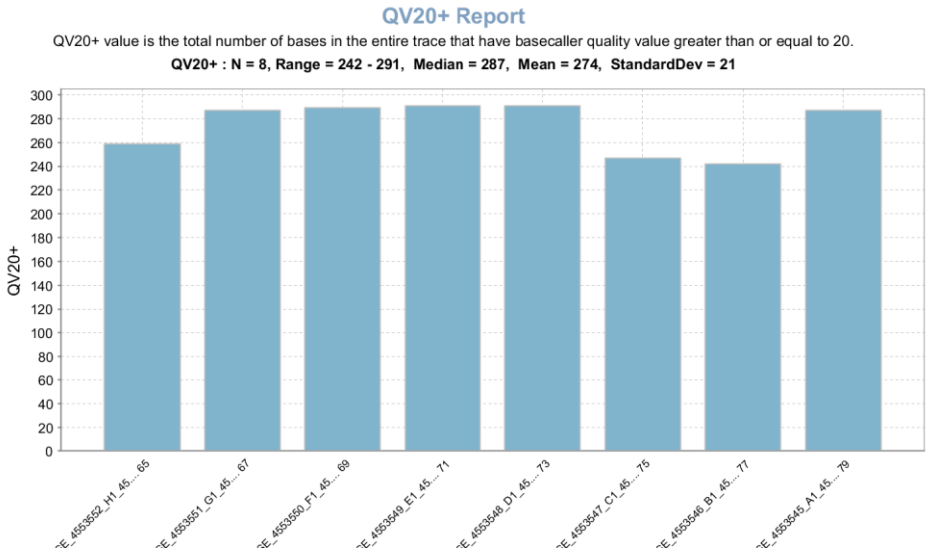

Figure 2. Sequencing analysis result using sanger sequencing data analysis.

Table 2. Sequences Analisis using Blast of NCBI

No	Sampel	Identifikasi	%	Seq ID
A	ABY	ADIPOQ	99.32	NG_021140.1
B	AKT	-	-	-
C	AST	ADIPOQ	97.67	NG_021140.1
D	SR	ADIPOQ	100	NG_021140.1
E	ST	ADIPOQ	99.32	NG_021140.1
F	KT	ADIPOQ	100	NG_021140.1
G	BY	ADIPOQ	100	NG_021140.1
H	ASR	ADIPOQ	98.21	NG_021140.1

4. Discussion

Identification of SNPs is often done by genetically searching for the presence of anomalies that cause a particular disease, or are caused by changes in a particular disease. Nucleotide base of this gene. This study seeks to find out if there are abnormalities in a person's genes people suffering from type 2 diabetes (T2DM). Type 2 diabetes is caused by disruption of the synthesis of the hormone insulin. Hormone Insulin synthesis is influenced by several genes. One of them is his ADIPOQ or APM1 gene, also known as adiponectin.

HMW adiponectin, which mediates insulin sensitivity in peripheral tissues, is the most physiologically active form in terms of glucose homeostasis [9]. The ratio of HMW plasma adiponectin levels to total adiponectin levels (HMWR) is more effective in monitoring increased insulin sensitivity in patients with thiazolidinedione-responsive type 2 diabetes [10].

Mapping data analysis by BLAST revealed that there were several probes that could be found as SNP candidates, namely ABY, AST, ST and ASR sequences with similarity indices greater than 100% with ADIPOQ sequences . A similarity index that is not 100% means that there are several different base nucleotides in the sequence when compared to the ADIPOQ sequence indexed by SEQ ID NO: NG_021140.1. According to the HapMap database (https://hapmap.ncbi.nlm) has over 100 SNPs that map and form the two major haplotype blocks. Adiponectin locus [11].

These polymorphisms include an aberrant variant with low allele frequency (MAF < 5 > 0.8). NCBI database (https:http://www.ncbi.nlm), there are 29 SNPs in the adiponectin coding region, 20 of which are missense mutations [12]. Various SNPs associated with adiponectin levels and/or diabetes have been reported in ADIPOQ with conflicting results [3].

5. CONCLUSION

From 8 oral mucosal cell samples, their DNA was successfully extracted, and after the purification process, 7 DNA samples were obtained and ready for sequencing. Based on the sequencing results, seven sequences have the same Qv20+ value with the sequence base pairs referred to as 241-291 base pairs. Alignment analysis using Blast also revealed that the KT, SR, and BP sequences had 100% similarity when compared with the adipoQ gene sequence of accession number NG_021140.1. Identification of candidate SNPs was ABY, AST, ST, and ASR sequences with similarity indices less than 100% It‘s means there will be possibility for having polymorphism on those sequence.

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