Gen AdipoQ Polymorphism on Type 2 Diabetes Mellitus Patients

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Abstract. DNA polymorphisms is variation on DNA sequence that change amino acid and gene function through changes in splicing, one of this polymorphism was SNP which only one nucleotide that change on target sequence. A survey that comprised 44 single nucleotide polymorphism (SNP)s of 31 candidate genes related to the lipid metabolism such as SNP on Gene AdipoQ, which affect some metabolite disease such as type 2 diabetes mellitus (T2DM). To identified those we need identifying some patient T2DM gen for understanding this fenomenon. 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.

Keywords: ADIPOQ · SNPs · Type 2 Diabetes Mellitus · T2DM

1 Introduction

Gen polymorphism refers to the substantial individual variation in DNA genetic sequences. DNA variations refers to all of these changes as a group. When a DNA variation lacks obvious functional importance, it is referred to as a DNA polymorphism. A protein's amino acid can change due to certain DNA variations. If the altered amino acid does not affect how a protein behaves, it may still be categorized as a neutral variant or polymorphism. While certain variants do not alter an amino acid, they can nevertheless affect how a gene functions by altering how certain genes are spliced. A change in an amino acid is referred to be a mutation if it alters how a protein or gene functions. It can be challenging to determine if a variation is pathogenic or not in a number of situations.

Due to its high incidence (20–25% of the adult population worldwide) and association with more serious diseases, metabolic illness is a significant global public health issue

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and cause for worry [1]. Cardiovascular illnesses (CVDs) are three times more likely to affect people with metabolic disease, and type 2 diabetic mellitus (T2DM) is five times more likely [2]. According to the International Diabetes Federation (IDF) in 2006 [2], metabolic disease is a condition that develops when many risk factors, such as abdominal obesity, insulin resistance, hyperglycemia, hyperlipidemia, and hypertension, combine to form the condition.

The first-line treatment for metabolite illness is to alter one's lifestyle to reduce the underlying, changeable risk factors such obesity, inactivity, and atherogenic diet [3]. Effective lifestyle change reduces type 2 diabetes risk in high-risk patients by 30 to 70% [4]. If the absolute risk is high enough, pharmacological therapy can then be considered and implemented in accordance with the current recommendation [5]. These show that the metabolic disorder is likely reversible if treated early on, in contrast to severe type 2 diabetes and cardiovascular diseases, and long-term engagement in lifestyle adjustments may result in its resolution.

Genetic background, on the other hand, is a risk factor that cannot be changed. The development of metabolic disease is significantly influenced by genetic factors in a variety of ways, however the exact mechanisms are still unclear. Obesity [6], dyslipidemia [7], hyperglycemia [8], and high blood pressure [9] are the basic elements of metabolic illness. They who have been investigated candidate genes for genetic foundation. The analysis of 44 single nucleotide polymorphisms (SNPs) of 31 potential genes involved in lipid metabolism, including SNP on Gene AdipoQ, produced the results.

Adiponectin synthesis-specific gene called Gen adipoQ has developed. Adipose tissue is the only place where this gene is expressed. It encodes a protein that is related to complement factor C1q, collagens X and VIII, and both. The plasma-circulating encoded protein participates in metabolic and hormonal functions. Adiponectin insufficiency is linked to mutations in this gene. There are numerous alternatively spliced forms of the same protein that have been found. Important adipokine having direct anti-diabetic, anti-atherogenic, and anti-inflammatory properties that regulates insulin sensitivity and fat metabolism. Enhances skeletal muscle and liver AMPK phosphorylation and activation, which improves fatty acid combustion and glucose uptake.

TNF-alpha is inhibited through adversely regulating its expression in many tissues, including the liver and macrophages, as well as by blocking its effects. Inhibits NF-kappa-B activation in endothelial cells via a cAMP-dependent mechanism. By interacting with and sequestering numerous growth factors with varied binding affinities, depending on the type of complex, LMW, MMW, or HMW, they may contribute to cell proliferation, angiogenesis, and tissue remodeling. This description informs us that variation on adipoQ would influence beta pancreatic cell insulin output. Determining if type 2 diabetes mellitus patients may have polymorphisms in the ADIPOQ gene is therefore necessary.

2 Methods

This research method is a descriptive exploration using type 2 diabetes mellitus patients in Gledug Village, Blitar Regency as specimen donors.

2.1 Donor Selection

The donor of the specimen was a patient with type 2 diabetes mellitus in Gledug Village, Sanan Kulon District, Blitar Regency. 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).

2.2 Sampling

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.

2.3 SNP + 45 Identification

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. The results of the sequencing were then aligned to identify the presence of SNP 45 in the ADIPOQ gene.

3 Results

DNA samples were obtained from 8 patients with type 2 diabetes mellitus in Gledug Village, Sanankulon District based on posyandu data in gledug village. Based on the work program from the Health Office, data collection and monitoring were carried out on the condition of people suffering from type 2 diabetes mellitus in the Blitar Regency area, one of which was for residents of Gledug Village. Of the 20 people who were recorded as positive for type 2 diabetes mellitus, only 8 people agreed to sign a letter of concern; from these 8 patients, only a specimen in the form of the inner epithelium of the cheek wall using a buccal swap could be taken. Data on the blood sugar concentration of patients with anonymity is provided in Table 1.

The 8 respondents who were recorded as having type 2 diabetes mellitus (T2D) when measuring blood sugar before taking a sample of the inner cheek wall cells showed that blood sugar levels were still not so high <200 mg/dL (Table 1). The respondents' low blood sugar levels could be due to improved diet and/or consumption of blood sugar-lowering drugs. For example, SR respondents, according to data from the health posyandu in the village of Gledug, were known to suffer from diabetes mellitus for quite a long time, but when the sample was taken, the person concerned gave a description that he had not eaten white rice, only consumed brown rice and major vegetables with smaller

Table 1. Sample Prescriptions

Code	Gender	Age	Level of Blood Sugar (mg/dl)	Prescription	Diet
SR	F	55	160	Metformin	No Carb
ASR	M	35	140	Metformin	Carb
KT	F	52	190	–	No Carb
AKT	F	26	140	Amaryl	Carb
BY	M	55	140	–	No Carb
ABY	F	31	180	–	Carb
ST	F	35	170	Metformin	Carb
AST	M	18	140	–	Carb

portions than before, even those concerned showed that he received a prescription from the puskesmas midwife in the form of "Metformin" (blood sugar lowering drug).

This respondent screening data was used for additional data in assessing the results of the polymorphism test on the epithelial cell sample of the inner cheek wall. Identification of polymorphisms using DNA extraction method followed by PCR, purification by gel electrophoresis, identification of gene ADIPOQ sequence and SNP sequence identification.

Alignment sequence samples and sequence control ADIPOQ gene with accession number NG_021140.1 (https://www.ncbi.nlm.nih.gov/) obtained three candidates for polymorphisms in one nucleotide sequence SR, KT, and ST. The candidate SNPs in the SR, KT, and ST sequences were all absent of one adenine (A) nucleotide in the three sequences. This process of nucleotide absence is also known as deletion (Fig. 1).

Fig. 1. The potential polymorphism on a single nucleotide of the sample sequence. **A** the alignment result of gen ADIPOQ and SNP sequence sample SR. **B** the alignment result of gen ADIPOQ and SNP sequence sample KT, **C** the alignment result of gen ADIPOQ and SNP sequence sample ST.

4 Discussion

The identification of SNPs is often by genetic searching for the cause of a particular disease, the presence of abnormalities caused by changes in one nucleotide base of that gene. This study seeks to identify whether there are abnormalities in the genes of someone who has suffered from type 2 diabetes mellitus (T2DM).

A problem with insulin production is what leads to type 2 diabetes mellitus. The ADIPOQ or APM1 gene, commonly known as adiponectin, is one of many genes that affect the hormone insulin production. The most physiologically active form of adiponectin for maintaining glucose homeostasis is HMW adiponectin, which regulates insulin sensitivity in peripheral tissues [9]. When type 2 diabetic patients respond favorably to thiazolidinediones, the ratio of HMW plasma adiponectin levels to total adiponectin levels (HMWR) is a far more reliable indicator of improved insulin sensitivity [10].

In the process, three candidate SNPs have been obtained (Fig. 1). Based on this process, it can be seen that the abnormality in the sample sequence compared to the control sequence is the absence of one adenine nucleotide base in the SR, KT, and ST sequences. According to the HapMap database (https://hapmap.ncbi.nlm), there are more than 100 SNPs that map and form the 2 main haplotype blocks within the adiponectin locus [11]. These polymorphisms were represented by 21 SNP markers (association with $r2 > 0.8$), one of which was a rare variant with a minor allele frequency (MAF) 5%. There are 29 SNPs in the adiponectin coding area, 20 of which are missense mutations, according to the NCBI database (https://www.ncbi.nlm) [12]. Adiponectin levels and/or diabetes were linked to many SNPs identified in the ADIPOQ, however the results were inconsistent [13].

5 Conclusion

From the eight specimens of oral mucosal cells, their DNA was successfully extracted and after the purification process, seven DNA samples were obtained that were ready for sequencing. Based on the results of the sequencing, the seven sequences have the same Qv20 + value as the sequence base pair, named 241–291 base pair. And based on the 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.

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