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Single nucleotide polymorphism analysis of the peroxisome proliferator-activated receptor gamma gene in type 2 diabetes mellitus patients at Ministry of Defense Hospital, RSPPN Soedirman Jakarta

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Abstract

Type 2 diabetes mellitus is a metabolic disorder influenced by genetic and environmental factors. The *Peroxisome Proliferator-Activated Receptor Gamma* gene is known to play a role in T2DM pathogenesis, but its polymorphism profile in Indonesian patients remains poorly characterized. This study aimed to analyze single nucleotide polymorphisms in the *PPAR-γ* gene of T2DM patients at Rumah Sakit Pusat Pertahanan Negara (RSPPN) Soedirman Jakarta using adaptive sampling with nanopore sequencing. Eighteen DNA samples that passed quality control were analyzed using UGENE v52.1. A total of 26 SNPs were identified in 8 subjects, including C161T, which has a reported protective effect in T2DM. The clinical significance of the remaining SNPs remains unclear. This study provides initial insights into genetic variation in T2DM, which may contribute to personalized medical strategies for military personnel and support the development of a more resilient national defense health system by improving disease prevention and management in high-risk populations.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood sugar levels and disturbances in the metabolism of carbohydrates, fats, and proteins (Sapra & Bhandari, 2023). This is due to decreased sensitivity of adipose tissue to insulin (Zheng et al., 2018). The recent increase in the prevalence of diabetes mellitus in a number of developing countries has attracted much attention, mainly due to the rising standard of living in these countries. The increase in per capita income and changes in lifestyle, especially in urban areas, have contributed to the high incidence of degenerative diseases such as diabetes mellitus. This situation has made diabetes a major challenge in the national health sector (Decroli, 2019). The two most common types are type 1 (T1DM) and type 2 (T2DM) diabetes mellitus (PE Indonesia, 2021). T1DM is caused by damage to pancreatic beta cells, while T2DM is caused by insulin resistance exacerbated by inadequate insulin production (Baynest, 2015).

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As a non-communicable disease, diabetes mellitus accounts for 72% of global deaths, nearly four times more than communicable diseases, maternal issues, perinatal issues, and nutritional issues combined (WHO, 2016). According to data from the International Diabetes Federation (IDF) in 2021, an estimated 537 million adults aged 20 to 79 will have diabetes mellitus worldwide. This number is projected to increase to 643 million by 2030 and 783 million by 2045. Indonesia ranks fifth, with an estimated 19.5 million people affected, accounting for 10.6% of the total population (IDF, 2021). Additionally, the 2023 Basic Health Research report shows that the prevalence of diabetes mellitus (DM) in Indonesia among the population aged 15 years and older is 11.7%, based on doctor diagnoses, and 10%, based on blood tests. The highest prevalence was recorded in the DKI Jakarta region (Kemenkes, 2023). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, accounting for approximately 90–95% of all cases (Kahn et al., 2014).

The *PPAR-γ* gene is an interesting candidate related to susceptibility to T2DM and other phenotypes, because *PPAR-γ* products play an important role in modulating insulin sensitivity, inflammation, and adipocyte differentiation and proliferation through the regulation of gene expression related to specific adipocyte development (Sarhangi et al., 2020). The presence of genetic variation in the *Peroxisome Proliferator-Activated Receptor Gamma* (*PPAR-γ*) gene reflects differences in DNA sequences between individuals and plays a crucial role in determining the genetic profile necessary to identify appropriate drug therapies. Polymorphisms can influence an individual's response to certain drugs (Irham et al., 2022).

The process of evaluating genetic activity is very important in order to identify phenotypes that arise as a result of genetic variation (De Graaff et al., 2013). Knowing that someone has a specific genetic polymorphism allows for adjustments to be made in the treatment therapy to increase its effectiveness and reduce the risk of side effects, making the treatment more personalized and precise. As a transcription factor, *PPAR-γ* plays an important role in regulating lipid metabolism, adipocyte differentiation, and glucose homeostasis (Choi et al., 2014). *PPAR-γ* affects insulin sensitivity; therefore, genetic variations in this receptor can cause diabetes (Galicia-Garcia et al., 2020). To date, there is limited data on *PPAR-γ* gene polymorphisms in patients with type 2 diabetes mellitus (T2DM), especially in Indonesia.

The adaptive sampling method using nanopore sequencing is a highly effective molecular technique for identifying all genetic variations in the target gene (Anderson et al., 2023). This technology's main feature is its ability to directly read the nucleotide or base sequence in DNA molecules through nanopores, achieving real-time sequencing (Chen & Xu, 2023). The read length of DNA generated by Oxford Nanopore Technologies (ONT) continues to increase, with the maximum reported read length reaching 1 million base pairs (Miga et al., 2020). In this study, sequencing methods identified variations in the *PPAR-γ* gene suspected to be associated with DMT2 risk (Lin et al., 2021). The adaptive sampling technique was applied to the sequencing process to obtain more precise and accurate data for detecting genetic variations (Wetterstrand, 2024). This study aims to identify variations in the *PPAR-γ* gene in patients with T2DM, which could lay the groundwork for personalized diabetes management strategies based on individual genetic profiles.

METHOD

Research Design

This study is a descriptive observational study using a cross-sectional design, aimed at identifying genetic variants in patients with type 2 diabetes mellitus (T2DM). The study was conducted in three main phases: (1) sample selection and DNA quality assessment, (2) sequencing using adaptive sampling via the Oxford Nanopore PromethION 24 platform, and (3) bioinformatics analysis to identify single nucleotide polymorphisms (SNPs) in the *Peroxisome Proliferator-Activated Receptor Gamma* (*PPAR-γ*) gene.

An overview of the study workflow is as follows:
Sample collection → DNA quality control → Library preparation → Adaptive sampling sequencing → SNP analysis and visualization

Population and Research Sample

The study involved stored DNA isolates from T2DM patients. A purposive sampling method was used, including all available DNA isolates that met the inclusion and exclusion criteria. A total of 35 DNA isolates had been preserved for approximately one year at -80°C in the Laboratory of Pharmacology, Faculty of Military Pharmacy, Republic of Indonesia Defense University. After quality control, 18 isolates were eligible for sequencing.

Tools and Materials

Equipment used in the study included: Gel Electrophoresis (blueGel™ QP-1500-01, Taiwan), GelDoc UV Transilluminator (LabTron, UK), PromethION 24 device, Hula mixer (gentle rotator mixer), Microfuge, Magnetic rack, Vortex mixer, Thermal cycler, Heating block for 1.5ml tubes set to 37°C, P1000 pipette and tips, P200 pipette and tips, P100 pipette and tips, P20 pipette and tips, P10 pipette and tips, P2 pipette and tips, Ice bucket with ice, Timer, Eppendorf 5424 centrifuge, Qubit Fluorometer 3.0, Ice Box.

Materials included: T2DM patient DNA isolates, stored for approximately one year at -80°C. The materials for electrophoresis are Taq Master Mix (Thermo Scientific™, USA), Gel Green Strain (blueGel™, Taiwan), TAE 1X (VIVANTIS™), Loading Dye (Thermo Scientific™, USA), DNA ladder 100 Bp (Thermo Scientific™, USA), sterile Aquadest. The sequencing materials are the Native Barcoding Kit 24 V14 (SQK-NBD114.24), ProNex® beads (Promega), NEBNext FFPE DNA Repair Mix (NEB, M6630), NEBNext Ultra II End repair/dA-tailing Module (NEB, cat # E7546), NEB Blunt/TA Ligase Master Mix (NEB, cat # M0367), NEBNext Quick Ligation Module (NEB, cat # E6056), 1.5 ml Eppendorf DNA LoBind tubes, 0.2 ml thin-walled PCR tubes, Nuclease-free water (e.g. ThermoFisher, cat # AM9937), Freshly prepared 80% ethanol in nuclease-free water, Qubit™ Assay Tubes (Invitrogen, Q32856), Qubit dsDNA HS Assay Kit (ThermoFisher, cat # Q32851), PromethION R10.4.1 ow cells (FLO-PRO114M), Sequencing Auxiliary Vials V14 (EXP-AUX003), Flow Cell Wash Kit (EXP-WSH004).

Ethical Consideration

This study involved the use of human-derived biological material. Therefore, ethical approval was obtained from the Health Ethics Committee of the National Research and Innovation Agency (BRIN), under approval number **005/KE.03/SK/01/2025**, dated January 15, 2025. All procedures were conducted in accordance with ethical standards and guidelines for research involving human subjects.

Data Analysis

Sequencing data in FASTQ format were analyzed using Epi2me for quality control and initial SNP calling. Identified SNPs were further visualized and validated using UGENE software (version 52.1) (Okonechnikov et al., 2012), enabling the graphical localization of variations within the PPAR-γ gene. Descriptive analysis was conducted to interpret the frequency and distribution of observed SNPs.

RESULTS AND DISCUSSION

For this study, 35 DNA samples were obtained from patients with type 2 diabetes mellitus (T2DM) who were undergoing routine checkups at the Internal Medicine Polyclinic of RSPPN Soedirman Jakarta. The DNA isolates were obtained through a whole blood DNA isolation process carried out by previous researchers.

Gel Electrophoresis Results

Visualization of the research sample gel electrophoresis was documented using a GelDoc UV transilluminator, resulting in the appearance of DNA bands, as shown in Figure 1. Figures 1.(a), 1.(b), and 1.(c) show clearly visible and horizontally aligned DNA bands, indicating that the DNA is intact and detectable. Figure 1.(d) also shows a detectable DNA band; however, the gel area appears shrunken, possibly due to insufficient soaking in TAE 1X buffer. Despite this, the DNA remains suitable for downstream analysis.

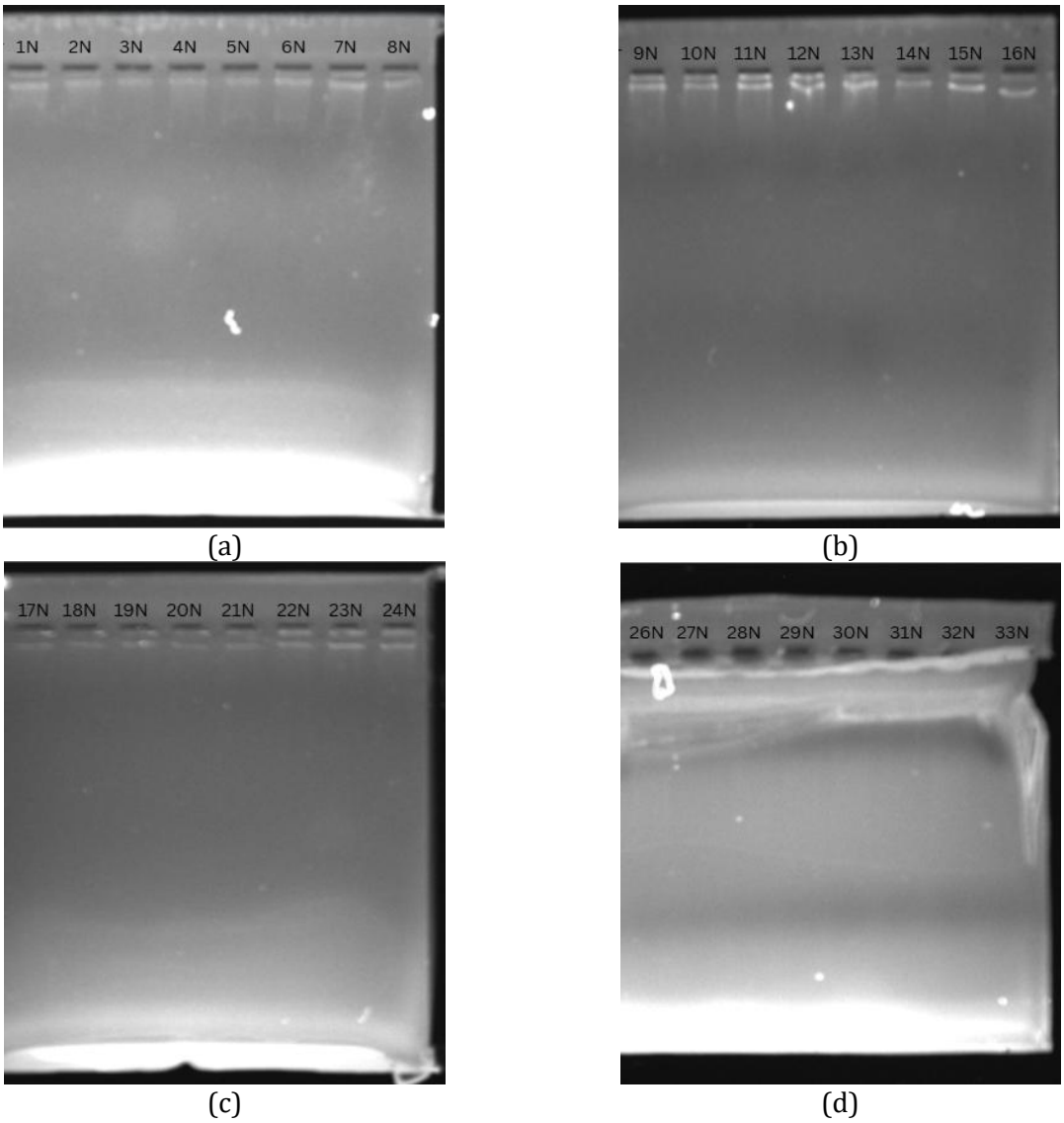

Figure 1. Gel Electrophoresis Visualization

The quality analysis of DNA isolates was conducted using the agarose gel electrophoresis method, which aims to detect the presence and integrity of DNA bands resulting from the isolation process. The appearance of DNA bands in the electrophoresis results indicate that the DNA isolate is in relatively good condition, while the absence of bands may indicate DNA degradation or a DNA concentration that is too low to be visualized. In the obtained visualization results, all DNA samples showed the appearance of bands with a uniform distribution in a single horizontal line, indicating that the DNA quality is quite stable and does not show significant degradation between samples. But there is damage to the part of the gel containing samples 26N to 33N, due to the agarose shrinking because of insufficient immersion in the 1X TAE solution. Nevertheless, the appearance of bands in all samples still indicates that, in general, the DNA isolates stored for one year are still in a condition suitable for further sequencing analysis.

Measurement of DNA Isolate Concentration

To assess the samples suitability for further analysis, we performed DNA concentration measurements using a Qubit Fluorometer 3.0. These measurements determine whether the DNA isolates meet the minimum quality criteria for use in gene sequencing. The eligibility criterion is 20 ng/µL. DNA concentration measurements for 35 samples in this study are shown in Table 1.

Table 1. Results of DNA Concentration Measurement

No	Sample Code	Concentration (ng/µl)	No	Sample Code	Concentration (ng/µl)
1	1N	68,2	19	19N	9,04
2	2N	41,4	20	20N	9,56
3	3N	3,22	21	21N	7,86
4	4N	12,9	22	22N	10,8
5	5N	4,5	23	23N	38,2
6	6N	18,1	24	24N	68
7	7N	33,8	25	26N	50,6
8	8N	11,1	26	27N	54,8
9	9N	29,2	27	28N	20,2
10	10N	11,7	28	29N	45,4
11	11N	26,8	29	30N	58,4
12	12N	6,66	30	31N	84,6
13	13N	17,8	31	32N	44,2
14	14N	5,96	32	33N	80,4
15	15N	5,74	33	34N	66,4
16	16N	4,66	34	35N	171
17	17N	8,04	35	36N	68,8
18	18N	15			

The concentration values of DNA isolates obtained from T2DM patients, measured using a Qubit Fluorometer 3.0. Only DNA samples with concentrations exceeding the minimum quality threshold are eligible for further processing in downstream nanopore sequencing. Despite being stored for approximately one year, the DNA isolate used in this study is of sufficient quality for genetic analysis of the *PPAR-γ* gene.

The quality and concentration of isolated DNA are crucial parameters that determine the success of downstream sequencing processes, particularly for platforms like nanopore sequencing which require high-quality input material. In this study, 35 samples concentrations were measured using a Qubit Fluorometer 3.0. According to the standard criteria for nanopore sequencing, a DNA concentration above 20 ng/µl is considered the minimum threshold for passing Quality Control (QC). Based on this requirement, only 18 out of the 35 DNA samples met the QC criteria, indicating that just over half of the samples were suitable for further sequencing analysis. This minimum concentration serves as a reference point because excessively low DNA levels can affect the accuracy of the sequencing results.

The remaining samples with concentrations below the threshold were excluded to prevent sequencing failures or poor-quality data output. This may be caused by several factors, including the initial sample concentration that has decreased due to the storage mass reaching approximately one year or DNA degradation during storage. This statement is in line with the research by Huang et al. (2017), which states that long storage duration does not cause a decrease in DNA quality, but it can lead to a decrease in DNA concentration. This selection process ensures that only high-quality DNA is used for nanopore sequencing to obtain reliable and interpretable genetic information.

SNP Analysis Results

This study focuses on identifying genetic polymorphisms in the exonic regions of the *PPAR-γ* gene. The *PPAR-γ* gene consists of eight exons that play an important role in regulating the expression of functional proteins. Since the analysis is conducted on mRNA sequences that encode the protein, the detected variations could directly affect the structure and function of the *PPAR-γ* protein. This approach is relevant because changes in exonic regions can lead to changes in codons, including synonymous, missense, and nonsense variants, each of which can impact the pathophysiology of DMT2. SNP data can be found in **Table 2**.

Table 2. Results of SNP sequencing of the PPAR-γ gene from T2DM patient DNA samples using nanopore sequencing.

Sample Code	Exon	SNP	Amino Acid Substitution	Clinical Implications	SNP (Literature)	ID variant	Reference
1N	7	T348C	Gly359Gly	NA	NA	NA	dbSNP, ClinVar
		T354A	Phe361Leu	NA	T/C	rs748541732	dbSNP, ClinVar
		G358T	Glu363Stop	NA	NA	NA	dbSNP, ClinVar
2N	8	T206G	Ser462Arg	NA	T/G	rs914519822	dbSNP, ClinVar
		T209C	Leu463Leu	NA	T/C	rs2051774353	dbSNP, ClinVar
		C210G	His464Asp	NA	NA	NA	dbSNP, ClinVar
27N	6	T106G	Leu212Arg	NA	NA	NA	dbSNP, ClinVar
		A111G	Lys214Gln	NA	NA	NA	dbSNP, ClinVar
	7	G79C	Glu270Gln	NA	G/C	rs1324719941	dbSNP, ClinVar
		G179A	Gly303Asp	NA	NA	NA	dbSNP, ClinVar
		T180C		NA	NA	NA	dbSNP, ClinVar
28N	7	T319A	Phe350Ile	NA	NA	NA	dbSNP, ClinVar
		T319A	Phe350Ile	NA	NA	NA	dbSNP, ClinVar
	5	C61T	Arg151Trp	NA	C/A/G	rs753857970	dbSNP, ClinVar
29N	8	C161T	His447His	- Glioma susceptibility 1 - PPARG-related familial partial lipodystrophy - Obesity - Insulin Resistance, Digenic	C/T	rs3856806	dbSNP, ClinVar
33N	7	G198C	Leu309Phe	NA	G/A	rs776983909	dbSNP, ClinVar
		G397A	Glu376Lys	NA	G/A/C	rs1400424764	dbSNP, ClinVar
		T400A	Leu377Ile	NA	NA	NA	dbSNP, ClinVar
		A43G	Gln408Arg	NA	NA	NA	dbSNP, ClinVar
	8	C161T	His447His	- Glioma susceptibility 1 - PPARG-related familial partial lipodystrophy - Obesity - Insulin Resistance, Digenic	C/T	rs3856806	dbSNP, ClinVar
34N	3	A196C	Tyr63Ser	NA	A/T	rs1183297709	dbSNP, ClinVar
	6	C82G	Pro204Arg	NA	NA	NA	dbSNP, ClinVar
		C95T	Asp208Asp	NA	NA	NA	dbSNP, ClinVar
		C99G	Arg210Gly	NA	C/T	rs745376706	dbSNP, ClinVar
		C127G	Ser219Stop	NA	C/G/T	rs768538430	dbSNP, ClinVar
35N	5	C69A	His153Gln	NA	NA	NA	dbSNP, ClinVar
		A71T	Lys154Ile	NA	NA	NA	dbSNP, ClinVar
		A73C	Lys155Gln	NA	NA	NA	dbSNP, ClinVar
Total 8 Subjek		26 SNPs	25 variants				

From 35 DNA specimens collected from subjects, only 18 met the quality and quantity criteria for sequencing analysis using the adaptive sampling method. Of those 18 samples, only eight showed genetic variation in the form of single nucleotide polymorphisms (SNPs) in the *Peroxisome Proliferator-Activated Receptor Gamma* (*PPAR-γ*) gene. Analysis of these eight samples identified 26 different SNPs scattered across exons 3, 5, 6, 7, and 8. The distribution of SNPs was uneven, with the highest concentration in exon 7 (accounting for 38.5% of all variants identified in the eight samples). The identified SNPs are functionally classified into three main groups:

a. Synonymous variants amount to 4 variants (16%), which are mutations that do not cause changes in the amino acid sequence. Among them are Gly359Gly, Leu463Leu, His447His, Asp208Asp, which have the potential to affect gene expression through mechanisms such as changes in mRNA stability, translation efficiency, or mRNA secondary structure. One of these synonymous SNPs, His447His, has been recorded in the NCBI ClinVar database as a variant that acts as a protective factor in the management of DMT2 conditions.
b. Second, there are 19 missense variants (76%), which are variants that cause amino acid substitutions. Among them are Phe361Leu, Ser462Arg, His464Asp, Leu212Arg, Lys214Gln, Glu270Gln, Gly303Asp, Phe350Ile, Arg151Trp, Leu309Phe, Glu376Lys, Leu377Ile, Gln408Arg, Tyr63Ser, Pro204Arg, Arg210Gly, His153Gln, Lys154Ile, and Lys155Gln, which have the potential to affect the structure or function of the protein in the *PPAR-γ* gene.
c. Third, 2 nonsense variants (8%) were identified to produce premature stop codons, namely Glu363Stop and Ser219Stop, which can terminate protein translation early and potentially cause functional disturbances.

Most SNPs were found in only one subject (n = 1), indicating characteristics of rare variants. However, two variants, Phe350Ile and His447His, showed slightly higher frequencies. Each variant was found in two subjects (n = 2), warranting further attention in the context of their potential clinical and functional associations with the pathophysiology of DMT2.

Main Findings

The results of this study indicate a significant genetic variation in the *PPAR-γ* gene among subjects from RSPPN Soedirman Jakarta, with a total of 26 SNPs identified in the exonic regions. This variation has the potential to affect the structure of the *PPAR-γ* protein and indirectly impact its biological function, particularly in the regulation of glucose and lipid metabolism. These findings indicate that the *PPAR-γ* gene exhibits a considerable level of polymorphism in the subject population from RSPPN Soedirman Jakarta.

Exon 7 Clustering

Based on the sequencing analysis results, it was found that the most SNP variations were located in exon 7 of the *PPAR-γ* gene, accounting for 38.5% of the total identified variations. This phenomenon can be explained by several factors. Structurally, exon 7 is one of the longer exons compared to other exons in the *PPAR-γ* gene, making the likelihood of random mutations higher due to the length of its nucleotide sequence. Additionally, exon 7 encodes part of the ligand-binding domain (LBD) of the *PPAR-γ* protein, which is an important area in regulating the interaction of the protein with endogenous ligands and agonist drugs such as thiazolidinedione (TZD), commonly used in DMT2 therapy. Because of its important role in regulating glucose and lipid metabolism, this region is likely a mutation hotspot, where amino acid changes can directly impact the biological function of the protein. Furthermore, dbSNP NCBI reports that SNPs in exon 7 are associated with insulin resistance and metabolic disorders, where these variants can act as factors that worsen the condition or conversely serve as protective factors against DMT2.

SNP Data with Patient Clinical Characteristics

This study identified SNP variants in the *PPAR-γ* gene in parallel with collecting clinical data from each subject, including body mass index (BMI) and diabetes status. Combining genetic and clinical data is important for exploring the relationship between genetic mutations and phenotypic manifestations related to glucose metabolism and insulin resistance. This approach enables a more thorough correlation analysis, especially when evaluating the functional impact of SNPs on glycemic control, therapeutic response, and other metabolic parameters in subjects. Table 3 shows details of the SNP mutations found in each subject along with their respective clinical characteristics. In

addition, the baseline clinical characteristics of T2DM patients whose DNA samples were analyzed in this study. The variables include body mass index (BMI) and diabetes status. These data help in correlating specific SNP findings in the PPAR-γ gene with clinical profiles.

Table 3. Clinical Characteristics of Patients

Sample Code	SNP	Amino Acid Substitution	Clinical Characteristics	
			BMI	Diabetes Status
1N	T348C	Gly359Gly	Normal	Not controlled
	T354A	Phe361Leu		
	G358T	Glu363Stop		
2N	T206G	Ser462Arg	Normal	Not controlled
	T209C	Leu463Leu		
	C210G	His464Asp		
27N	T106G	Leu212Arg	Overweight	Not controlled
	A111G	Lys214Gln		
	G79C	Glu270Gln		
	G179A	Gly303Asp		
	T180C			
28N	T319A	Phe350Ile	Overweight	Not controlled
	T319A	Phe350Ile		
29N	C61T	Arg151Trp	Obesity	Controlled
	C161T	His447His		
	G198C	Leu309Phe		
33N	G397A	Glu376Lys	Obesity	Controlled
	T400A	Leu377Ile		
	A43G	Gln408Arg		
	C161T	His447His		
34N	A196C	Tyr63Ser	Obesity	Controlled
	C82G	Pro204Arg		
	C95T	Asp208Asp		
	C99G	Arg210Gly		
	C127G	Ser219Stop		
35N	C69A	His153Gln	Normal	Not controlled
	A71T	Lys154Ile		
	A73C	Lys155Gln		

From the analysis of 18 specimen samples, only 8 samples showed genetic variation in the *PPAR-γ* gene. Research subjects can be grouped into three main categories based on BMI and HbA1c status, namely:

a. The group with normal BMI and uncontrolled HbA1c, namely 1N, 2N, and 35N.

b. The group with overweight BMI and uncontrolled HbA1c, namely 27N and 28N.

c. The group with obesity BMI and controlled HbA1c, namely 29N, 33N, and 34N.

The genetic mutations frequently found in this study are associated with the observed clinical outcome, in this case, uncontrolled HbA1c levels.

In groups 1 and 2, several significant genetic mutations were found. Based on the identified genetic mutations, there is a tendency for subjects with those SNP variations to have uncontrolled HbA1c levels. This indicates a possible relationship between the genetic polymorphism and glucose regulation disorders in the body. Although it cannot be concluded with certainty, these findings provide a basis indicating that the lack of control over HbA1c in the subjects may be contributed by the presence of the identified genetic mutations.

In group 2, the identified genetic mutations are not only associated with the pathogenesis of T2DM but are also known to play a role in the regulation of fat metabolism. This can be seen from the profile of subjects with an overweight BMI, which indicates that these genetic variations likely contribute to body fat accumulation. Conversely, in group 1, the detected genetic mutations do not show a clear association with fat metabolism, as subjects in this group maintain a normal BMI range. This difference suggests that not all genetic polymorphisms in *PPAR-γ* have the same impact on aspects of body metabolism.

In group 3, several significant genetic mutations were found. Based on the identified genetic mutations, there is a tendency that subjects with those SNP variations have controlled HbA1c levels. This indicates that these genetic mutations tend to act as protective factors in the regulation of body glucose. This is reflected in the subjects' ability to maintain good glycemic control despite having an obesity-classified BMI, which is generally correlated with metabolic disorders. Although it cannot be concluded definitively, these findings provide a basis indicating that the presence of the SNP may potentially offer protection against metabolic dysfunction.

Of course, it is important to understand that many other factors can also affect HbA1c levels, such as an unhealthy lifestyle, a high-sugar diet, non-compliance with medication, a family history of diabetes, inappropriate treatment choices, environmental conditions, as well as the subject's level of education and economic status. However, through this research, the authors hope to contribute to the development of evidence-based medicine, particularly in exploring the potential role of genetic polymorphisms as one of the factors influencing glycemic control in subjects.

Of all the identified SNP variations in this study, only the His447His (rs3856806) variant has been discussed in previous studies and has clinical information recorded in the ClinVar database. This polymorphism has been associated with increased insulin sensitivity and protective effects against metabolic disorders, making it one of the most researched variants in the context of DMT2.

The results of this study indicate that the SNP C161T (His447His) in the *PPAR-γ* gene was found in subjects with clinical conditions of obesity and controlled diabetes status (HbA1c levels <7%). Previous studies using a meta-analysis approach support that T allele carriers act as a protective factor against the risk of T2DM (Tiongco et al., 2023). This alignment is demonstrated by the clinical condition of the subjects in the study, where individuals carrying the T allele had controlled HbA1c levels.

In the aspect of lipid metabolism reflected through the BMI value, the findings in this study are not in line with the statement by Li et al. (2022), which states that this allele is a protective factor against dyslipidemia, a common lipid metabolism disorder in patients with metabolic syndrome. Meanwhile, in this study, the clinical condition of the subjects falls into the obesity category.

Other SNPs found in this study have not been specifically reported in scientific publications related to DMT2. Some of them have indeed been listed in dbSNP NCBI, but they have not yet been supplemented with clinical data or functional information recorded in ClinVar, indicating that their biological and clinical significance is still not definitively known.

The results of this study show the presence of genetic variation diversity in the *PPAR-γ* gene among subjects from RSPPN Soedirman Jakarta, with the identification of 26 different SNPs, including variants that have been associated with insulin sensitivity such as His447His (rs3856806). The presence of the His447His variant in subjects with obesity can be an indicator of genetic risk for the development of T2DM. The presence of this mutation may indicate a protective or adaptive role of the His447His variant against glucose metabolism disorders. This suggests that the His447His variant has the potential to play a compensatory or protective role in counterbalancing the metabolic risks posed by obesity, and underscores the importance of a genetic approach in understanding variations in clinical responses among individuals with similar metabolic conditions.

Most of the SNPs identified in this study do not yet have documented clinical impact information, either in scientific publications or in databases such as ClinVar on NCBI, although some of them have been recorded in dbSNP. These are most likely very rare variants or even newly discovered ones. Nevertheless, the existence of these variants cannot be overlooked, especially since some of them are missense and nonsense mutations that have the potential to alter the structure and function of the *PPAR-γ* protein, indicating a possible contribution to the pathophysiology of DMT2.

Additionally, the correlation between SNPs and clinical data of subjects provides an initial basis for exploring the use of certain SNPs as candidate biomarkers in risk stratification or personalization of DMT2 therapy, particularly concerning the use of thiazolidinedione (TZD) class therapeutic agents such as pioglitazone, which work through the activation of the *PPAR-γ* receptor. These findings open up opportunities for further researchers to evaluate the functional impact and clinical implications of the identified SNPs through experimental approaches or broader cohort studies. Further exploration of these variants is important to assess their potential contribution to insulin sensitivity, regulation of glucose and lipid metabolism, and response to therapy. Thus, in the long term, it can support the development of new genetic biomarkers that play a role in risk prediction and more

personalized treatment strategies for subjects, which are expected to improve the effectiveness and reduce the side effects of type 2 DM treatment.

Compared to previous studies that often focused on a limited number of targeted SNPs, our research utilized nanopore sequencing technology, which enables comprehensive, long-read sequencing of the *PPAR-γ* gene. This approach allows for the identification of the full spectrum of SNPs present in each individual, including both known and potentially novel variants. By integrating these complete genetic profiles with each patient's clinical characteristics, such as BMI, age, and disease duration, we provide a more holistic view of genotype-phenotype correlations in T2DM. This method enhances the efficiency of genetic analysis and supports the application of precision medicine, particularly in tailoring treatment strategies based on individual genetic and clinical backgrounds.

This study has several limitations that need to be acknowledged. The research design employed was descriptive and cross-sectional in nature, conducted at a single point in time without longitudinal follow-up of the subjects. As a result, the findings only reflect the conditions observed during the time of data collection and do not allow for the establishment of causal relationships or direct associations between the identified genetic variations (SNPs) and specific clinical manifestations. Consequently, while the observed SNP patterns and their potential correlation with clinical parameters such as BMI and diabetes status offer valuable preliminary insights, they remain indicative rather than conclusive. The absence of follow-up data limits the ability to track changes in clinical outcomes over time in relation to genetic profiles, thereby constraining the predictive power of these findings.

CONCLUSION

Based on the identification results in this study, 26 SNPs were successfully found. One of the SNPs, namely C161T (His447His), has been known to have a potential protective effect against metabolic risk and response in subjects. Meanwhile, other identified SNPs do not yet have clear clinical information in scientific publications or databases such as ClinVar, although some have been recorded in dbSNP. Thus, this research has successfully identified SNPs in the PPAR-γ gene that may correlate with DMT2.

Future research is recommended to adopt a longitudinal study design that involves continuous monitoring of patient's clinical conditions over time. This would allow researchers to observe how specific genetic variants influence disease progression, treatment response, and metabolic parameters across different stages of T2DM. Such an approach would further strengthen the clinical relevance of SNP identification and support the development of more effective and personalized interventions.

AUTHOR CONTRIBUTIONS

L.S., S.T., and A.H. conceived the original idea and provided overall supervision of the project. L.S. and Y.M. carried out the experiment and collected the data. L.S. performed data analysis and wrote the manuscript with support from S.T., A.H. and Y.M.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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