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GENETIC STUDY OF HEPATOCYTE NUCLEAR FACTOR-1 ALPHA (HNF-1A) MUTATIONS IN DIABETES MELLITUS PATIENTS

Studi Genetik Mutasi Hepatocyte Nuclear Factor-1 Alpha (Hnf-1α) Pada Pasien Diabetes Mellitus

Fusvita Merdekawati[1*], Ira Gustira Rahayu[1], Aditya Juliastuti[1], Citra Amaniah Anhar[2], Zulfikar Ali Hasan[3], Larasati Putri Maynar[1]

[1*] Jurusan Teknologi Laboratorium Medis, Poltekkes Kemenkes Bandung, Cimahi Indonesia, *Email: fusvitamerdekawati@gmail.com

[2] Jurusan Teknologi Laboratorium Medis, Poltekkes Kemenkes Jakarta III, Email: citrania.anhar@gmail.com

[3] Jurusan Teknologi Laboratorium Medis, Poltekkes Kemenkes Makasar, Email: fikaroxy@poltekkes-mks.ac.id

ABSTRAK

Maturity-Onset Diabetes of the Young (MODY) merupakan bentuk diabetes monogenik yang disebabkan oleh mutasi genetik, salah satunya pada gen HNF1A, dan sering salah didiagnosis sebagai diabetes tipe 1 atau tipe 2. Penelitian ini bertujuan untuk mendeteksi mutasi gen HNF1A pada pasien yang diduga mengalami MODY menggunakan metode Sanger sequencing *guna menunjang diagnosis yang lebih spesifik. Metode yang digunakan meliputi isolasi DNA dari dua sampel pasien, dilanjutkan dengan amplifikasi sepuluh ekson gen HNF1A menggunakan PCR konvensional, analisis hasil amplifikasi menggunakkan elektroforesis gel agarose, dan sequencing metode Sanger. Hasil sequencing dianalisis menggunakan perangkat lunak BioEdit dan ClustalW untuk mengidentifikasi mutasi terhadap sekuens referensi gen HNF1A (NM_001306179.2). Ditemukan enam mutasi titik pada gen HNF1A yang tersebar di ekson 7, 9, dan 10, terdiri atas satu mutasi silent (p.Leu459Leu), empat mutasi missense (p.Gln460His, p.Ser486Asn, p.Ser581Gly, dan p.Val705Leu), serta satu mutasi nonsense (p.Trp785*) yang menyebabkan terminasi translasi dini. Mutasi yang ditemukan berpotensi memengaruhi struktur dan fungsi protein HNF1A, termasuk domain transaktivasi yang penting dalam regulasi ekspresi gen target. Penelitian ini menunjukkan bahwa deteksi mutasi genetik, khususnya pada HNF1A, penting dalam mendiagnosis MODY secara akurat dan dapat menjadi dasar pemilihan terapi yang lebih tepat, seperti penggunaan sulfonilurea sebagai alternatif insulin.*

Kata kunci: *MODY, HNF1A, mutasi gen, PCR, Sanger sequencing, diabetes monogenik*

ABSTRACT

Maturity-Onset Diabetes of the Young (MODY) is a monogenic form of diabetes caused by genetic mutations, including those in the HNF1A gene, and is often misdiagnosed as type 1 or type 2 diabetes. This study aims to detect HNF1A gene mutations in patients suspected of having MODY using Sanger sequencing to support a more specific diagnosis. The research methods include DNA isolation from two patient samples, followed by amplification of ten exons of the HNF1A gene using conventional PCR, analysis of amplification results using agarose gel electrophoresis, and and sequence analysis of Sanger sequencing results. Sequencing results were analyzed using BioEdit and ClustalW software to identify mutations relative to the HNF1A gene reference sequence (NM_001306179.2). Six point mutations were identified in the HNF1A gene,

distributed across exons 7, 9, and 10, consisting of one silent mutation (p.Leu459Leu), four missense mutations (p. Gln460His, p.Ser486Asn, p.Ser581Gly, and p.Val705Leu), and one nonsense mutation (p.Trp785*) causing premature translation termination. The identified mutations have the potential to affect the structure and function of the HNF1A protein, including the transcription activation domain, which is crucial in regulating the expression of target genes. This study demonstrates that the detection of genetic mutations, especially in HNF1A, is important in accurately diagnosing MODY as well as determining appropriate theraphy approaches, such as the use of sulfonylureas as an alternative to insulin.
Keywords: MODY, HNF1A, gene mutation, PCR, Sanger sequencing, monogenic diabetes

INTRODUCTION

Diabetes Mellitus (DM) is a disorder of the endocrine system. According to data from the International Diabetes Federation, in 2021, 537 million adults were living with diabetes, and this number is expected to increase to 643 million in 2030 and 783 million in 2045 [1], [2], [3], [4].

Maturity-onset Diabetes of the Young (MODY) is a monogenic form of diabetes that is often confused with type 1 and type 2 diabetes mellitus. The pathophysiology of MODY involves genetic mutations in nuclear transcription factors and glucokinase, resulting in pancreatic ß-cell dysfunction. In patients with MODY, insulin is produced by beta cells, but failure in insulin secretion causes hyperglycemia. MODY is a genetically heterogeneous group of diabetes characterized by non-insulin dependence, contributing to 1%–2% of all diabetes cases.

MODY is often misdiagnosed as type 1 or type 2 diabetes. This misdiagnosis often leads to inappropriate treatment and adverse outcomes. Therefore, molecular diagnosis of MODY is necessary to distinguish it from other types of diabetes [5], [6], [7], [8]. Several biomarkers play a crucial role in distinguishing MODY subtypes from type 1 and type 2 diabetes. These biomarkers include C-peptide, high-sensitivity C-reactive protein (hs-CRP), cystatin C, apolipoprotein M (ApoM), 1,5-anhydroglucitol (1,5-AG), transthyretin (TTR), complement 5 (C5), and complement 8 (C8), d-glycan, islet autoantibodies, high-density lipoprotein (HDL-C), triglycerides, and sensitivity to sulfonylurea. However, genetic testing is highly recommended for diagnosis confirmation and better prognosis [9]. Molecular diagnostic testing can identify genetic mutations causing MODY, improve diagnostic accuracy, and support precision medicine—that is, to help determine the appropriate method of blood glucose monitoring and to prevent unnecessary insulin use [10], [11].

There are several subtypes of MODY that can be clearly identified using molecular genetic testing. Hepatocyte Nuclear Factor 1 homeobox A (HNF1A) MODY3 is the most common form of MODY, caused by a mutation in the HNF1A gene on chromosome 12 (12q24.31), found primarily in the liver and kidney and encoding a protein containing 631 amino acids. HNF1A has three domains: a dimerization domain, a DNA-binding domain (DBD), and a transactivation domain. HNF1A interacts with other transcription factors such as HNF1B and HNF4A through the dimerization domain to regulate gene expression. To date, more than 450 different mutations, including missense, frameshift, insertion, and complete or partial exon deletions, have been reported in the HNF1A gene [12], [13], [14]. The National Center for Biotechnology Information (NCBI) notes that the HNF1A reference genome has 10 exons organized into two different transcript variants (variant 1 and 2), which serve as the reference sequence for genetic analysis.

Mutations in the HNF1A gene cause a progressive decrease in insulin secretion, eventually requiring patients to undergo insulin replacement therapy and leading to

vascular complications, heart damage, kidney complications, and eye complications. Untreated MODY3 patients who do not receive proper medical treatment will eventually experience the devastating effects of diabetes, including ketoacidosis. Based on this background, the authors were interested in conducting a genetic study to detect base changes in DNA by analyzing sequencing results against reference database sequences, with the aim of supporting specific and accurate DM diagnosis and enabling appropriate treatment for patients [15], [16].

METHODS

This study is a descriptive study. The first stage is the collection of specimens from patients with diabetes mellitus, followed by DNA extraction and DNA amplification using the PCR method with 10 pairs of primers for all exons of the HNF1A gene. Next, nucleotide sequencing was performed using the Sanger method. The sequencing results were then analyzed to determine the presence or absence of mutations in the MODY3 diabetes mellitus variant. The DNA isolation and PCR processes were carried out at the Molecular Biology Laboratory of the Medical Laboratory Technology Department of the Bandung Ministry of Health Polytechnic, while the sequencing process was sent to Macrogen, China.

The data obtained was recorded, collected, processed, and presented in the form of narrative tables and curves.

RESULT

This study aims to conduct a genetic study of the HNF1A gene mutation (HNF1A gene graph in Appendix 1 and HNF1A gene exon reference sequence in Appendix 2) as one of approaches in the initial detection of diabetes mellitus (DM) patients suspected of having Maturity-Onset Diabetes of the Young (MODY). The detection process is carried out in several stages.

In this study, two DM patient samples were used, each coded A and B. Patient A is a 17-year-old female, while patient B is a 45-year-old male. These two patients were selected as samples based on inclusion criteria (clinically confirmed DM patients aged 10–40 years with a family history of diabetes in at least two generations) that pointed to suspected MODY and had been clinically confirmed as DM patients.

From these two patients, DNA was isolated from whole blood specimens to obtain pure DNA. The isolated DNA was then amplified using specific primers targeting 10 exons of the HNF1A gene using conventional PCR methods. The amplification results, in the form of DNA templates, were analyzed based on their size and electrical charge to estimate the length of the DNA fragments in base pairs (bp) using agarose gel electrophoresis. Furthermore, the DNA templates were prepared along with diluted forward and reverse primers to be sent to PT. Genetika Science Indonesia for sequencing.

DNA Isolation Results

The DNA isolation process in this study was carried out using whole blood specimens from two DM patients suspected of having MODY. Isolation was performed using the Promega Wizard® Genomic DNA Purification Kit, which is based on four main stages of DNA purification. The isolation process began with cell and nucleus lysis, in which red blood cells were lysed using Cell Lysis Solution, followed by the lysis of white blood cells and their nuclei using Nuclei Lysis Solution. This stage aimed to release the genomic DNA content from within the cell nucleus. After that, protein precipitation was carried out using a salt solution to precipitate the protein. The protein will separate as a precipitate, while the DNA remains in the solution phase. The final stage of this process is DNA

precipitation with isopropanol, which serves to concentrate genomic DNA and remove residual salt. The resulting DNA was dissolved in a solvent solution for use in the PCR stage. The results of this procedure show that DNA isolation was successful, as indicated by the visualization of DNA bands appearing on the agarose gel.

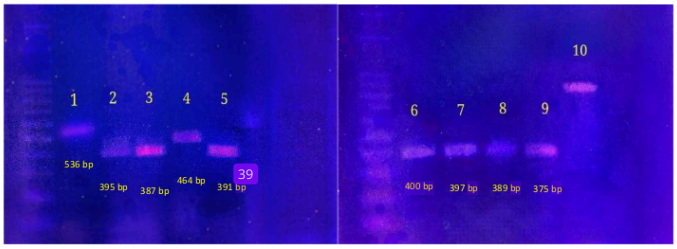
Results of HNF1A Target Gene Amplification

After obtaining pure genomic DNA from the isolation, the following step is to amplify the HNF1A gene using the conventional PCR method with specific primers targeting all 10 exons of the gene.

The PCR cycle was carried out in five stages, starting with enzyme activation at 95°C for 2 minutes, followed by a denaturation stage at 95°C for 15 seconds, which lasted for 35 cycles. Next, the annealing process was carried out at a temperature adjusted for each primer pair, based on the optimization temperature of each exon, and lasted for 1 minute in 35 cycles. The next stage is extension at 60°C for 1 minute, also lasting for 35 cycles. After the PCR cycle is complete, the reaction is closed with a cooling stage at 35°C for 30 seconds. The result is an amplicon or DNA template that is ready for analysis using agarose gel electrophoresis and sent to PT. Genetika Science Indonesia for sequencing.

Agarose Gel Electrophoresis Visualization Results

In this study, agarose gel electrophoresis was used to confirm the presence and size of HNF1A gene DNA fragments before sequencing. The agarose gel electrophoresis results showed the formation of DNA bands in each sample, indicating that the HNF1A gene amplification process was successful. The DNA fragments appeared to migrate according to the expected size based on comparison with the DNA ladder as a size marker. Smaller fragments migrated faster than larger fragments, in accordance with the principles of electrophoresis. Visualization of the DNA bands was performed using a UV transilluminator.



(a) (b)

Figure 1. Agarose Gel Electrophoresis Visualization

In Figure 1, clear DNA bands are visible in lanes 1 to 10, showing bands measuring 536 bp, 395 bp, 387 bp, 464 bp, 391 bp, 400 bp, 397 bp, 389 bp, 375 bp, and 800 bp. All bands appear single and clear, indicating that the amplification was successful.

Sequencing Results Analysis

The DNA template that had been analyzed through electrophoresis was then packaged along with each specific primer for 10 exons of the HNF1A gene, which had been diluted to a concentration of 10 µM in a volume of 10 µL. All samples were then sent to PT. Genetika Science Indonesia for sequencing analysis to detect possible mutations. Mutations were analyzed using the Sanger sequencing method. The sequencing results were obtained in the form of .ab1 files, which were then analyzed using BioEdit software to obtain FASTA format and view the resulting chromatogram

graphs. The chromatograms for samples A and B showed good signals and could be analyzed.

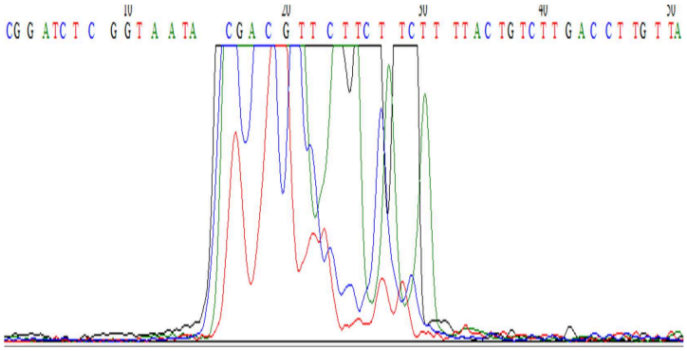


Figure 1. Example of chromatogram results for exon 10 of sample A

Further analysis was performed using ClustalW software to compare the nucleotide sequences of the samples with reference sequences from NCBI (to identify possible mutations).

Table 1. Results of nucleotide base mapping of sample A

No.	Exon	Reference Nucleotide (NM_001306179.2)	Sample Nucleotides (Sample A)	Base Point	Type of Mutation	Description
1.	7	C	T	1375	Substitution (Transition)	Similar base changes.
2.	7	G	T	1380	Substitution (Transversion)	Dissimilar base changes.
3.	7	G	A	1460	Substitution (Transition)	Similar base changes.
4.	9	A	G	1741	Substitution (Transition)	Similar base changes.
5.	10	G	T	2114	Substitution (Transversion)	Dissimilar base changes.

Based on the results of DNA sequencing analysis of the target gene compared to the reference sequence NM_001306179.2, five mutations were found in sample A spread across three different exons, namely exons 7, 9, and 10, as shown in Table 2. These mutations consisted of five substitution mutations.

In exon 7, three substitution mutations were identified. A transition substitution mutation occurred at position 1375 (C>T), indicating a change from a pyrimidine base (cytosine) to a pyrimidine base (thymine). A transversion substitution mutation occurred at position 1380 (G>T), indicating a change from a purine base (guanine) to a pyrimidine base (thymine). Another transition mutation occurred at position 1460 (G>A), indicating a change from a purine base (guanine) to a purine base (adenine). In exon 9, one substitution mutation was identified at position 1741 (A>G), which was a transition mutation involving a change from a purine base (adenine) to a purine base (guanine). In exon 10, one substitution mutation was identified at position 2114 (G>T), which is a

transversion mutation involving a change from a purine base (guanine) to a pyrimidine base (thymine).

Table 2. Results of mapping changes in the DNA and protein levels of sample A

No.	Exon	Changes at the DNA Level	Codon	Changes at the Protein Level	Types of Mutation	Description
1.	7	c.1375C>T	CTG>TTG	p.Leu459Leu	Silent	No amino acid change
2.	7	c.1380G>T	CAG>CAT	p.Gln460His	Missense	Amino acid change occurs
3.	7	c.1460G>A	AGC>AAC	p.Ser486Asn	Missense	Amino acid change occurs
4.	9	c.1741A>G	AGC>GGC	p.Ser581Gly	Missense	Amino acid change occurs
5.	10	c.2114G>T	GTG>TTG	p.Val705Leu	Missense	Amino acid change occurs

Analysis of the HNF1A gene sequencing results in sample A showed five protein changes spread across several exons, namely exons 7, 9, and 10, as shown in Table 3. Of these five changes, four were missense mutations, which cause amino acid changes, and one mutation was classified as a silent mutation, which is a base change that does not cause changes in amino acids.

A mutation was found in exon 7 with a c.1375C>T change, which causes the CTG codon to change to TTG, but both codons code for leucine (Leu), so there is no amino acid change (p.Leu459Leu) and it is classified as a silent mutation. The other four mutations in sample A showed amino acid changes and were classified as missense mutations. The mutation in exon 7 (c.1380G>T) caused a change from glutamine (Gln) to histidine (His) at position 460 (p.Gln460His). The mutation in exon 7 (c.1460G>A) changes serine (Ser) to asparagine (Asn) at position 486 (p.Ser486Asn). A mutation in exon 9 (c.1714A>G) causes a change from serine (Ser) to glycine (Gly) at position 581 (p.Ser581Gly). The mutation in exon 10, c.2114G>T, causes a substitution of valine (Val) to leucine (Leu) at position 705 (p.Val705Leu).

Table 3. Results of nucleotide base mapping of sample B

No.	Exon	Reference Nucleotide (NM_001306179.2)	Sample Nucleotides (Sample A)	Base Point	Type of Mutation	Description
1.	7	G	T	1380	Substitution (Transversion)	Dissimilar base changes.
2.	7	G	A	1460	Substitution (Transition)	Similar base changes.
3.	9	A	G	1741	Substitution (Transition)	Similar base changes.
4.	10	G	T	2114	Substitution (Transversion)	Dissimilar base changes.
5.	10	T	A	2355	Substitution (Transversion)	Dissimilar base changes.

Table 4. Results of mapping changes in the DNA and protein levels of sample B

No.	Exon	Changes at the DNA Level	Codon	Changes at the Protein Level	Types of Mutation	Description
1.	7	c.1380G>T	CAG>CAT	p.Gln460His	Missense	Amino acid change occurs
2.	7	c.1460G>A	AGC>AAC	p.Ser486Asn	Missense	Amino acid change occurs
3.	9	c.1741A>G	AGC>GGC	p.Ser581Gly	Missense	Amino acid change occurs
4.	10	c.2114G>T	GTG>TTG	p.Val705Leu	Missense	Amino acid change occurs
5.	10	c.2355T>A	TTG>TAG	p.Trp785*	Nonsense	A change to a stop codon occurs

Based on the results of DNA sequencing analysis of the target gene compared to the reference sequence NM_001306179.2, five point mutations were found in sample B, spread across three exons, namely exons 7, 9, and 10, as shown in Table 4. These mutations consisted of five substitution mutations. The analysis of sample B showed a similar mutation pattern to sample A, particularly at positions 1380 and 1460 of exon 7, position 1741 of exon 9, and position 2114 of exon 9. A different mutation was found at position 2355 of exon 10.

Most of the mutations found in sample A were also found in sample B, namely the missense mutations p.Gln460His, p.Ser486Asn, p.Ser581Gly, and p.Val705Leu. This indicates a similarity in the mutation profiles between the two patients, suggesting the possibility of consistent recurrent mutations between individuals and the possibility of a characteristic mutation pattern in MODY3 DM patients in the studied population.

The difference found between the two samples was the presence of a nonsense mutation at position 785 (p.Trp785*), which was only found in sample B. This mutation changes the amino acid tryptophan (Trp) to a stop codon (TAG), causing the appearance of a premature stop codon, which is very likely to result in truncated proteins that lose their normal function.

DISSCUSION

All samples that have undergone isolation and produced pure DNA were then amplified using specific primers from ten exons of the HNF1A gene. The amplification results in the form of DNA template amplicons were then analyzed using agarose gel electrophoresis to ensure the success of the amplification process and estimate the fragment size. The process of DNA migration in agarose gel electrophoresis is influenced by several important factors, namely the electric current used, the size of the DNA molecule, the concentration of agarose gel, the conformation of DNA, the presence of dyes in the gel and buffer solution, the type of agarose, and the type and concentration of the buffer solution used [17]. In this study, a 1.5% agarose gel was used because this concentration is ideal for separating DNA fragments ranging in size from 80 bp to 4,000 bp [17]. This is in accordance with the size of the target fragments in the study, which are around 300 to 800 bp. Therefore, selecting the appropriate agarose gel concentration is an important factor in obtaining optimal gel resolution tailored to the size of the DNA fragments to be analyzed [18].

The electrophoresis results of the PCR products from the ten exons of the HNF1A gene showed that DNA amplification was successful in all exons, both for sample A and sample B. This was indicated by the appearance of clear DNA bands and the estimated

DNA fragments for exons 1 to 10. The higher the DNA concentration, the sharper the intensity of the resulting bands [19].

In this study, BioEdit software was used as a tool in the sequencing data analysis stage, specifically to review the quality of the sequencing chromatograms from each exon to see the nucleotide sequence [20]. One of the main features utilized in this study was BioEdit's ability to open and display sequencing result files in .ab1 format, obtained from the Sanger sequencing method. DNA [21]. In addition, this software was also used to obtain files in FASTA format to be aligned with the HNF1A gene reference sequence (RefSeq: NM_001306179.2).

The chromatogram readings from the Sanger sequencing process show that most exons, namely exons 1 to 10 in samples A and B, produce neat and sharp peaks. Successful sequencing reactions are indicated by chromatograms that show clear and regular signal reading patterns. Good chromatogram characteristics include the appearance of well-defined single peaks, clear peaks, and a strong signal-to-noise ratio [22].

Next, analysis was performed using ClustalW as a bioinformatics tool to perform multiple sequence alignment (MSA) between the DNA sequences resulting from HNF1A gene sequencing from patient samples and the reference sequences obtained from the GenBank database. This alignment aimed to identify nucleotide variations, such as point mutations, deletions, or insertions, which could affect the structure and function of the protein encoded by the gene. This process was carried out to detect nucleotide differences in each exon. Based on the alignment results, it was found that there were several mutations with similar mutation patterns in samples A and B that occurred in exons 7, 9, and 10. Meanwhile, the nucleotide sequences in exons 1, 2, 3, 4, 5, 6, and 8 in both samples A and B were identical to the reference sequence, and no variations or base changes were found.

The results of the HNF1A gene sequence analysis of the samples studied showed six point mutations scattered across exons 7, 9, and 10. DNA molecules are double-stranded. If a mutation occurs and one base is replaced by another base, the DNA molecule will temporarily contain mismatched bases. When DNA undergoes replication, complementary base pairs will be synthesized opposite to the mismatched base pairs. As a result, one wild-type (normal) DNA molecule and one DNA molecule containing a mutation will be formed [23].

The six mutations identified consist of one silent mutation, four missense mutations, and one nonsense mutation. One mutation found in exon 7 is a silent mutation, namely the c.1375C>T nucleotide change that produces a new TTG codon from the original CTG codon, but both still encode the amino acid leucine (Leu). Therefore, this mutation does not alter the amino acid sequence at position 459 (p.Leu459Leu). Silent mutations generally do not affect protein structure or function because they do not alter the polypeptide chain [24].

Furthermore, four mutations were found that fall into the missense category, which are mutations that occur when a change in the base sequence alters a codon so that one amino acid in the protein is replaced by another amino acid. The severity of missense mutations depends on the location of the change and the nature of the replaced amino acid [23]. Two missense mutations were found in exon 7, namely the c.1380G>T;p.Gln460His mutation and the c.1460G>A;p.Ser486Asn mutation. The Ser486Asn amino acid is located in the C-terminal transactivation domain of HNF1A in a specific region involved in the recruitment of specific target genes and interaction with transcription co-activators, so this mutation will affect the transcription function of the HNF1A gene [25]. In exon 9, a missense mutation c.1741A>G;p.Ser581Gly was also found. Among the various mutations found in genes associated with diabetes, the HNF1A gene is the most promising candidate. In the patients studied, four non-

synonymous mutations were found, namely Ile27Leu (rs1169288), Ser487Asn (rs2464196), Leu551Ser (rs116930[illegible]), and Ser581Gly (rs587778398). Based on previous reports, mutations occurring in exons 8-10, which are only found in the longest isoform of the HNF1A gene, are associated with a later onset of MODY. Therefore, the Leu551Ser and Ser581Gly mutations are thought to play an important role in the observed clinical phenotype [26].

A nonsense mutation was found in exon 10, namely c.2355T>A;p.Trp785*, which changes the tryptophan codon (UGG) to a stop codon (UAG). This mutation causes premature translation termination, resulting in a truncated HNF1A protein at position 785. When a nonsense mutation occurs, the codon that originally encoded an amino acid changes to a stop codon, causing the ribosome to stop translating the mRNA earlier than it should, so that the rest of the protein chain is not formed. This premature stop codon is recognized by a special protein called a release factor, which then releases the unfinished polypeptide chain [23].

MODY3 is caused by mutations in the HNF1A gene, which produces a protein expressed in the liver, kidneys, intestines, and pancreatic beta cells. The HNF1A protein functions in regulating insulin gene expression and plays a role in controlling the expression of glucose transport genes, such as GLUT2 [27]. One of the compensatory mechanisms regulated by HNF1A is the increase in transcription and expression of the Sodium-Glucose Cotransporter 2 (SGLT2) gene. This mechanism helps maintain relatively normal blood glucose levels (euglycemia) for a certain period of time, especially in the early stages of metabolic disorders. However, in individuals with mutations in HNF1A, SGLT2 expression is drastically reduced, which impacts the ability to reabsorb glucose in the proximal tubules of the kidneys. This causes a decrease in the efficiency of glucose recovery filtered by the kidneys, and ultimately contributes to an increase in blood glucose levels [28]. The mutations found in this study, such as silent, missense, and nonsense mutations, have the potential to cause disturbances in the structure and function of HNF1A. These mutations can inhibit the amino-terminal domain, which plays a role in dimerization and DNA binding, and can inhibit the transactivation domain, thereby disrupting the transcription of various target genes, including genes that are important in glucose uptake by cells, hepatic glucose metabolism, and insulin secretion by pancreatic β cells [29].

Thus, the mutations found in exons 7, 9, and 10 in this study not only alter the structure of the HNF1A protein but may also affect its function in regulating glucose metabolism. Disruption of the HNF1A gene function can cause disruption of the expression of important genes that play a role in the process of glucose uptake by cells and insulin production. As a result, there is a decrease in insulin secretion and the body's response to glucose becomes suboptimal. This condition may explain the onset of diabetes symptoms in patients suspected of having MODY.

One of the most important and characteristic features of MODY3 patients is their sensitivity to sulfonylurea drugs, which are the first-line therapy for MODY3 cases. This has significant clinical implications, especially for patients who were previously misdiagnosed with type 1 diabetes (T1D), as they can discontinue insulin therapy and switch to sulfonylurea treatment, even after undergoing long-term insulin therapy. Children who previously used oral hypoglycemic agents or low-dose insulin can also discontinue insulin therapy and switch directly to low-dose sulfonylurea. The dose can be titrated gradually to achieve optimal glycemic control. Meanwhile, for patients undergoing full-dose insulin therapy (replacement dose), it is recommended to reduce basal insulin by at least 50% and discontinue bolus insulin when starting sulfonylurea therapy [28].

SUMMARY

Based on the results of the research and analysis conducted, it can be concluded that there are mutations in the HNF1A gene in certain exons, namely exons 7 and 9, in both samples of patients with suspected MODY diabetes. Six substitution mutations were found, namely three transversion substitution mutations c.1380G>T, c.2114G>T, c.2355T>A and three transition mutations c.1375C>T, c.1460G>A, c.1741A>G. The mutations found were characterized as silent, missense, and nonsense mutations. One silent mutation was found in exon 7 (p.Leu459Leu). Four missense mutations were found, namely in exon 7 (p.Gln460His and p.Ser486Asn), exon 9 (p.Ser581Gly), and exon 10 (p.Val705Leu). One nonsense mutation was found in exon 10 (p.Trp785*).

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