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Polymorphism of The KCNJ11 E23K (rs5219) Gene and HbA1c Concentration in First Derivatives of Diabetes Mellitus Ethnic Lembak, Bengkulu

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Abstract

Background & Objectives: The KCNJ11 E23K (rs5219) polymorphism in Lembak ethnicity may be a basic predisposing factor for DM heritability. Diagnosing of DM and grouping people at risk of DM confirmed by examining HbA1c. The research to identify the polymorphism of KCNJ11 E23K (rs5219) gene and its relationship with the concentration of HbA1c in the first generation of Lembak ethnicity in Bengkulu. **Methods**: The cross sectional study of 54 samples. The detection of single nucleotide polymorphism (SNPs) achieved through the Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) technique. The measurement of HbA1c concentrations performed using the boronate affinity method. **Results**: Identification frequencies of KCNJ11 E23K polymorphism in first generation of DM showed that genotypes EE, EK, and KK were respectively 55.6%, 29.6% and 14.8%. In the non-DM first generation showed that genotypes EE, EK and KK were respectively 44.4%, 25.9%, 29.6%. There was no statistically significant disparity observed in the KCNJ11 E23K genotype across the groups ($p = 0.420$). Meanwhile, there was no statistically significant relationship observed between the KCNJ11 E23K polymorphism and HbA1c concentration in both the first generation DM ($p=0.681$) and non DM ($p=0,882$) groups. **Conclusions**: This study showed no significant difference in genotype allocation of the KCNJ11 E23K (rs5219) polymorphism between the first generation of DM and non-DM in Lembak Ethnicity. This study also showed no linked between the KCNJ11 E23K (rs5219) polymorphism and HbA1c concentrations in the first generation of Lembak Ethnicity, Bengkulu.

Keyword: DM - Single Nuclotide Polymorphism (SNP) - KCNJ11 - HbA1c - Lembak Ethnicity

Introduction

Diabetes mellitus (DM) is a persistent metabolic condition distinguished by elevated levels of blood glucose resulting from a deficiency in insulin, dysfunction of β-cells, insulin resistance, or a combination of these factors[1]. DM represents approximately 1.5 million deaths from non-communicable diseases[2] and Indonesia ranks seventh among a group of ten countries with the highest prevalence of DM, with approximately 10.7 million individuals affected by the condition[3]. The prevalence of DM cases in 2018 in all age groups was 0.91% in Bengkulu Province with the highest prevalence being in Bengkulu City at 1.28%[4].

The increased prevalence of DM can be caused by may a rise from a complex relationship between several genes and environmental factors[5]. SNPs may increase DM risk. KCNJ11 is linked to DM susceptibility. This gene affects ATP-sensitive potassium channels, inhibiting insulin production[6,7]. Genome-wide association studies have identified genetic predisposition to type 2 DM, which may increase diabetes risk when paired with environmental variables. Diet can also affect gene expression[8]. Preliminary observations of the Lembak ethnicity found a habit called "Neron," which is the hereditary habit of consuming tea/coffee with added sugar and complementary foods like cakes up to three times a day, while the recommendation for daily sugar consumption based on Regulation of The Minister of Health No. 30 in 2013 is only 50 grams, which is equivalent to 5-9 teaspoons or 4 tablespoons[9]. This may predispose the KCNJ11 gene polymorphism to elevated blood sugar or type 2 DM.

HbA1c, a biochemical marker, can be used to diagnose and classify diabetes mellitus[10]. HbA1c is a blood glucose test that measures erythrocyte glycated hemoglobin levels over a period of 2-3 months[11]. The HbA1c number is unaffected by daily blood sugar levels and long-term lifestyle factors like diet, drink, and exercise[12]. Souza et al. (2017) found that type 1 and 2 DM patients had higher HbA1c levels than normal controls in the Euro-Brazilian KCNJ11

rs5219 gene polymorphism analysis. Still, insulin usage may cause poor glycemic control and elevated HbA1c levels[13].

A study examined the relationship between the KCNJ11 E23K gene polymorphism and HbA1c levels to determine its function in type 2 diabetes mellitus. Thus, researchers want to detect the KCNJ11 E23K (rs5219) gene polymorphism and HbA1c levels in the first generation of Lembak DM patients in Bengkulu City.

Material & Methods

Subjects

This study was Conducted in January-February 2023 at Bengkulu University and Kimia Farma Laboratory. Cross-sectional study of 54 Lembak subjects under 40 who used "neron" daily were studied. The case group had 27 biological children of parents with DM from Primary Health Facilities were tested. The control group had 27 non-DM parents. This study excluded individuals with DMT1 or DMT2 (HbA1c value >6.5%), smoking, obesity according to WHO criteria in Asian populations (BMI < 25 kg/m2), and hypertension according to the Joint National Committee (JNC) 8 criteria (TDS < 140 mmHg and TDD < 90 mmHg). This ethical study approved from the Faculty of Medicine and Health Sciences, University of Bengkulu Ethics Committee with reference No. 208/UN30.14.9/LT/2022 and informed consent from all research subjects.

Biochemical assays

Random Plasma Glucose (RPG) Examination uses the hexokinase method with the Indiko (Finland) tool from venous blood. HbA1c examination using the boronate affinity chromatography method with an Epitod 616 (Korea). The HbA1c examination stage uses 5 μL of blood sample which is put into a capillary tube and incubated in reagent (R1) from Epitod

616. Then 25 µL of Ephitod 616 Cartridge is added and 25 µL is added washing buffer (W1) from Epitod 616.

Molecular genotyping

A DNA isolation kit (Promega, USA) isolated DNA from fresh blood. For PCR, DNA was kept at 40°C. RLFP genotyped KCNJ11 rs5219 gene polymorphism. An Agilent Technologies SureCycler 8800 performed PCR reactions. GoTaq Green PCR with a primer (Thermo Scientific, USA) amplified a specific area of the KCNJ11 gene. The 210-base-pair amplicon resulted. Primer sequences used in this study were forward (5'GACTCTGCAGTGAGGCCCTA 3') and reverse (5' ACGTTGCAGTTGCCTTTCTT 3') primers.

The 35-cycle PCR amplification followed PCR reaction. These circumstances included a 5-minute denaturation at 95^{o}C and a 30-second denaturation at 94^{o}C. After that, 30 seconds of annealing at 60^{o}C, and the extension step was done at 71^{o}C. Final step was a 5-minute 72^{o}C extension phase. Incubation At 37°C for 16 hours, the BanII enzyme (NEB, UK) digested amplified DNA fragments of 5 µl of PCR product, 1 µl of 10X Tango buffer, 0.5 µl of BanII enzyme, and 3.5 µl of NFW. Electrophoresis on a 2% agarose gel at 75 volts for 60 minutes followed to visualize restriction process. After that, sequences of the sample was obtained using Sanger sequencing conducted by Macrogen Laboratory. Sequences blasted in NCBI DNA database.

Statistical analysis

IBM IPSS Statistic 26 analyzed data. Descriptive analysis with nominal data will show KCNJ11 gene genotype frequencies and percentages. The Chi-Square Test and Contingency Coefficient test were used to evaluate genotype-DM relationship and genotype-HbA1c connection. The Mann-Whitney test compared group means.

RESULTS

Characteristics of Research Subjects

This study employed 54 blood samples from 26 male and 28 female respondents ranging in age from 15 to 35 years, BMI from 18.5-22.9 kg/m2, and systolic/diastolic blood pressure from 120-139/80-90 mmHg (Table 1).

Table 1 Characteristics of Research Subjects

Characteristic	Frequency F1 DM		Frequency F1 Non-DM		Total		P-*value*
Data	N	%	n	%	N	%	
Age (Years)							
< 20	2	7,4 %	3	11,1 %	5	9,3 %	0,639
20-40	25	92,6 %	24	88,9 %	49	90,7 %	
Gender							
Male	11	40,7 %	15	55,6 %	26	48,1 %	0,276
Female	16	59,3 %	12	44,4 %	28	51,9 %	
Parent's Disease History							
DM	27	50%	0	0%	27	50%	
Non-DM	0	0%	27	50%	27	50%	
Body Mass Index (BMI)							
<18,5 kg/m^2	0	0	0	0	0	0	
18,5-22,9 kg/m^2	27	50%	27	50%	54	100%	
23-24,9 kg/m^2	0	0	0	0	0	0	
Systolic Blood Pressure / Diastolic Blood Pressure							
<120 mmHG/ <80mmHG	9	33,3%	15	55,6%	24	44,4	0,100
120-139 mmHG/80-90 mmHG	18	66,7%	12	44,4%	30	55,6	

Description: F1DM: The first generation of DM patients; F1non-DM: The first generation of non-DM; P-value: from the Chi-Square Test, significant p<0.05.

Random Plasma Glucose (RPG) and HbA1c

The mean difference in RPG yield between the DM first derivative and the Non-DM first derivative was found to be 82.704 mg/dL. The findings suggest that the F1 DM group exhibits a propensity for elevated blood glucose levels in comparison to the non-DM F1 group (Figure 1).

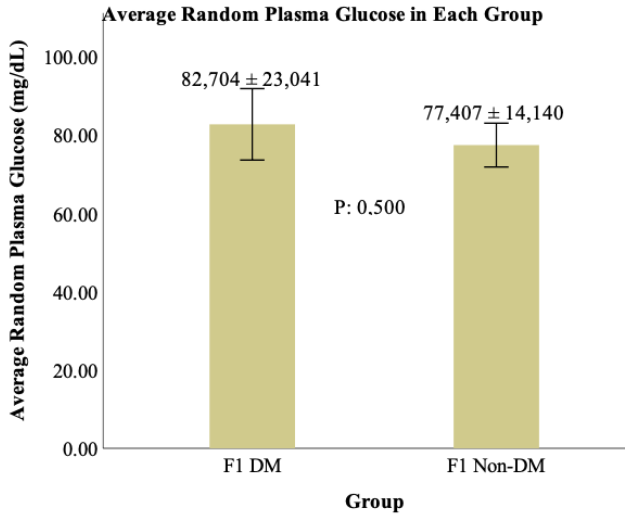

Figure 1 RPG concentration on F1 DM and non-DM; no significant different between groups

The average HbA1c concentration of the initial cohort of individuals with diabetes mellitus (DM) was recorded as 4.903%, a slightly elevated value compared to the HbA1c level of the initial cohort of individuals without DM from the Lembak ethnic group, which measured at 4.892% (refer to Figure 2). This finding indicates that individuals belonging to the first generation with DM have a greater propensity for elevated HbA1c levels compared to individuals from the first generation without DM.

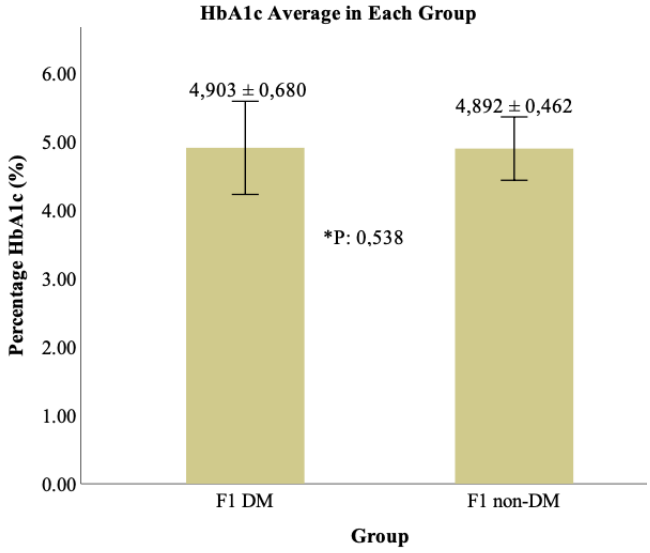

Figure 2 HbA1c concentration in F1 DM and F1 non-DM groups

Genotype KCNJ11 E23K (rs5219) gene

All samples averaged 1.81 ± 0.017 and 321.28 µg/mL ± 41.67 DNA purity and concentration. The amplified PCR reaction produced a 210-bp band at 210 bp. Enzymatic digestion with BanII yielded a single 150 base pair band for the wild type homozygote EE genotype, a single 178 base pair band for the KK mutant homozygous genotype, and two distinct bands for the EK mutant heterozygous genotype (Figure 3).

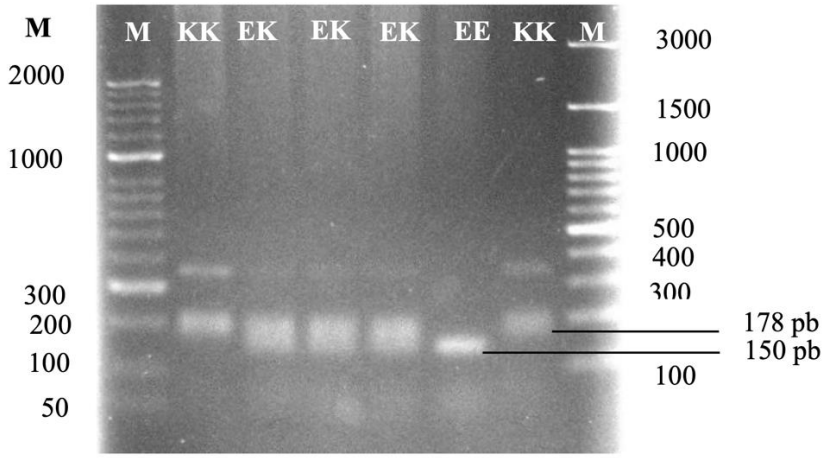

Figure 3 Genotypes some samples of the KCNJ11 E23K rs5219 gene

The genotype frequency distribution for the KCNJ11 E23K rs5219 gene diverged from Hardy-Weinberg Equilibrium (HWE)/Hardy-Weiberg with a value of X2: 8.708 and p= 0.013 (P <0.05). 36.1% of the population had the K allele of SNP E23K rs5219. The genotype distribution of the KCNJ11 E23K rs5219 gene polymorphism in first-generation DM and non-DM from Lembak ethnicity of Bengkulu City was determined from 27 DM and 27 non-DM (Table 2). Genotypic identification of the KCNJ11 E23K rs5219 gene polymorphism showed that 15 first-generation DM patients (55.6%) had the EE (Wild Type) genotype, compared to 12 non-DM first-generations (44.4%). Still, p= 0.414 showed no statistically significant difference. DM first-generationers had a higher E allele frequency (70.4%) than non-DM first-generationers (57.40%). Both groups had similar allele frequencies (p= 0.161). This study explored the effects of the KCNJ11 E23K rs5219 gene polymorphism on first-generation DM patients and controls. Two genotype models were evaluated (Table 2).:

• Dominant Model (EK+KK vs KK): OR: 1.563 (95% CI 0.534-4.571, P= 0.414)

• Recessive Model (KK vs EE+EK): OR: 2.421 (95% C1 0.61-9.295, P= 0.190)

Table 2 Genotyping of the KCNJ11 E23K rs5219 gene

Variable	F1 DM (n=27)	F1 Non DM (n=27)	*P-value*
Genotype			
E/E (Wild Type)	15 (55,6%)	12 (44,4%)	0,414
E/K (Hetrozigot Mutan)	8 (29,6%)	7 (25,9%)	0,761
K/K (Homozigot Mutan)	4 (14,8%)	8 (29,6%)	0,190
Dominant Model			
E/E	15 (55,6%)	12 (44,4%)	
E/K + K/K	12 (44,4%)	15 (55,6%)	0,414
Recessive Model			
E/E + E/K	23 (85,2%)	19 (70,4%)	0,190
K/K	4 (14,8%)	8 (29,6%)	
Alleles			
E	38 (70,4%)	31 (57,40%)	0,161
K	16 (29,6%)	23 (42,6%)	

Sequencing Result

The sequence of nitrogen base sequences obtained following sequences.

GGTCCTCCGATGGGGGAGCGCTCCCTGGGGGTCACCGGAGCCATGCTGTCCCGC

AAGGGCATCATCCCCGAGGAATACGTGCTGACACGCCTGGCAGAGGACCCTGCC

GAGCCCAGGTACCGTGCCCGCCAGCGGAGGGCCCGCTTTGTGTCCAAGAAAGGC

AACTAGCAA

Figure 4 shown that sequence results from the Blast process were 97% identical with human KCNJ11 gene in database (https://blast.ncbi.nlm.nih.gov/Blast.cgi#1219731502).

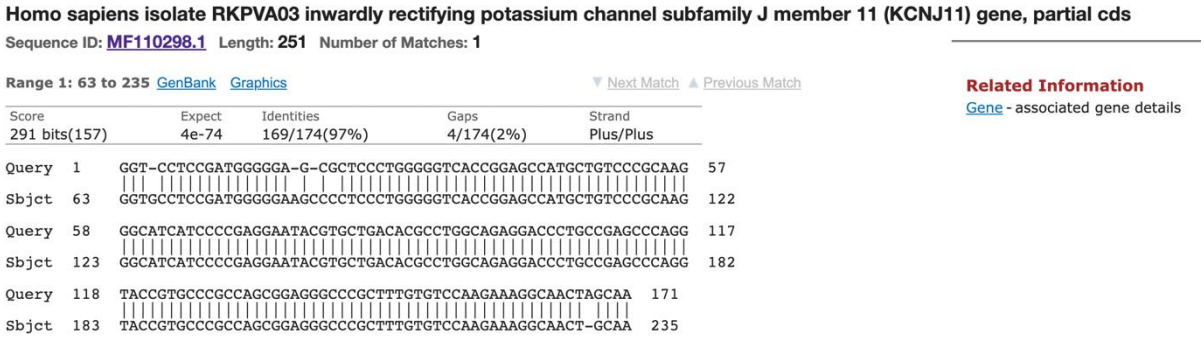

Figure 4 Blast Result from KCNJ11 sequences

Relationship of KCNJ11 Gene Genotype with HbA1c

After the correlation test, the results were carried out using the Contingency Coefficient which tested the correlation of genotypes and HbA1c in both groups in the dominant form and showed no significant p-values (Table 3).

Table 3 Correlation Analysis of the KCNJ11 Gene Genotype (Dominant Model) Against HbA1c Concentration

Hereditary History	KCNJ11 E23K Gene Polymorphism (rs5219)	HbA1c						P-*Value*
		Normal		Prediabetes		Total	Total	
		n	%	n	%	n	%	
F1 DM	EE	13	86,7	2	13,3	22	100	0,681
	EK and KK	11	91,7	1	8,3	5	100	
	Total	24	88,9	3	11,1	27	100	
F1 non-DM	EE	7	87,5	1	12,5	8	100	0,882
	EK and KK	17	89,5	2	10,5		100	
	Total	24		3		27		

Discussion

Analysis of Research Subject Characteristics

The present study was carried out on a sample of 54 participants belonging to the Lembak ethnic group residing in Bengkulu City. The participants were divided into two distinct groups: the first group consisted of individuals from the DM first generation, while the second group comprised individuals from the non-DM first generation. The characteristics of the research participants encompassed factors such as age, gender, parental history of diabetes mellitus (DM), systolic blood pressure, diastolic blood pressure, and body mass index (BMI). The prevalence of diabetes mellitus (DM) tends to rise in correlation with advancing age, frequently manifesting between the ages of 55 and 64 years.[3]. The increased risk of DM is caused by a degenerative process due to a progressive decrease in pancreatic β cells, resulting in diminished production of the hormone insulin. It causes an increase in glucose levels which leading to glucose intolerance blood stream[14].

These findings revealed that within the DM first-generation was a higher representation of women compared to men in terms of gender. This can be caused by the sampling technique that uses the consecutive sampling method based on those who meet the criteria without considering the ratio of male and female respondents. Research before, reported that a higher prevalence of DM in the female sex group compared to the male sex group, with a prevalence of around 1.78% and 1.21%[4]. Menopause lowers estrogen levels, causing DM. This hormonal decrease affects pancreatic beta cell insulin output and target organ and tissue insulin sensitivity. BMI rises greater in women[15,16].

Both DM and non-DM parents had 27 respondents. DM risk factors include family history[3]. If one parent has DM, the first generation has a 15% risk of DM, according to research. The same study found that if both parents had DM, 75% of their first generation will have it[17]. This study's participants had a BMI range of 18.5-22.9 kg/m2, which is good because BMI is linked to DM etiology[18]. Mild obesity doubles DM risk[19]. Obesity alters metabolism, releasing fatty acids, glycerol, hormones, and pro-inflammatory cytokines from adipose tissue. These compounds cause insulin resistance. Thus, insulin resistance and beta cell malfunction can cause uncontrolled blood glucose levels[20]. Systolic blood pressure is 120-139 mmHg, and diastolic blood pressure is 80-90 mmHg. Prehypertension, grade 1 hypertension, and grade 2 hypertension increase diabetes risk by 23%, 26%, and 60%, respectively[21].

Analysis of RPG and HbA1c Concentration

DM first-generation had greater trend RPG and HbA1c than non-DM first-generation. Perkeni 2021 defined normal HbA1c as <5.7% and prediabetes as 5.7–6.4%. This study found no statistically significant difference in mean HbA1c values between DM and non-DM first-generation patients. A family history of diabetes mellitus (DM) raises HbA1c levels in both diabetics and non-diabetics[22,23]. Due to inheritable SNPs, those with first-degree relatives with

diabetes mellitus (DM) are more likely to get DM. These DM-related genes can disrupt insulin sensitivity, β-cell functioning and proliferation, and obesity[24].

ATP-binding transporter subfamily C gene 8 (ABCC8), KCNJ11, and Peroxisome Proliferator-Activated Receptor-Gamma (PPARG) genes are involved in DM etiology. Most of these genes affect insulin activity, glucose metabolism, pancreatic beta-cell function, energy intake and expenditure, and lipid metabolism. Mutations in ABCC8 and KCNJ11 genes can disrupt KATP channels, causing DM. The PPARG gene helps adipogenesis and insulin resistance[7].

Genotype Analysis

In this study, a higher frequency of K alleles was observed in the control group than the test group. Eventhough, the other study shown that the patients in the Czech Republic have prevalence rates of EK (43.8%) and KK (25%) variants. However, it is suggested that there may be an effect on the secretory activity of pancreatic β cells[29]. Mutations in the KCNJ11 gene have been identified as disruptors of the functional capacity of the KATP channel, thereby leading to the manifestation of Permanent Neonatal Diabetes Mellitus[33]. A study was undertaken to investigate the association between the KCNJ11 gene and carbohydrate intake in obese and non-obese adolescents in Indonesia. The findings revealed that the presence of a polymorphism in the KCNJ11 gene was linked to refined carbohydrate consumption in both the obese and non-obese groups, specifically among individuals with the KK genotype. The SNP rs5219 KCNJ11 gene exhibits a greater propensity for consuming refined carbohydrates compared to other genotypes, namely EE and EK[35].

In this study, researchers suspected that the control group or first-generation non-DM (29.6%) had more K alleles and KK genotypes than the first-generation DM group (14.8%), possibly due to ethnic factors, geography, and a relationship with glucose or carbohydrate intake, which tends to be excessive in the population, such as neuronal frequency and other

carbohydrates[5,35]. The non-DM first generation group exhibited a mean frequency ratio of 1.6 ± 1.0, compared to 1.3 ± 0.5 for the DM first generation group. Huriyati et al.'s 2020 investigation on this gene polymorphism and carbohydrate consumption supported this finding.

This study only found a connection between genetic variation, carbohydrate intake, and obesity, not a causal association. The investigation was limited by its focus on a single meal[35]. Due to the significant allele distribution change in DM patients across several investigations, the SNP rs5219 remains a risk factor for DM. In this study, the odds ratio value assessed the effect of the KCNJ11 E23K rs5219 gene polymorphism on DM using several genotypic models.

Relationship Analysis of KCNJ11 Gene Genotype with HbA1c

This study found no significant correlation between the KCNJ11 E23K (rs5219) gene polymorphism and first derivative HbA1c levels in Lembak people with and without diabetes mellitus (DM) in Bengkulu City. Thu and Bhin (2022) examined the KCNJ11 polymorphism (rs5219) and hyperglycemia in Vietnamese people. Their investigation, which included several blood glucose measurements, found no statistically significant connection between the KCNJ11 polymorphism and hyperglycemia in Vietnamese people. EE, EK, and KK genotypes had similar FPG and OGTT findings. ($p>0.05$)[36].

This study differs with Aswathi et al. (2020) on the KCNJ11 gene polymorphism in South Indian diabetics. Aswathi et al. found a correlation between KCNJ11 gene polymorphism E23K (rs5219) and HbA1c. This study's average BMI was 28.65 ± 4.88, therefore more research is needed to determine if obesity confounded the results[37]. In obese people, greater BMI values are connected with higher HbA1c levels[38]. A Tunisian study found that type 2 DM patients with the EK genotype had higher FPG levels than those with the KK and EE genotypes[39].

KCNJ11 E23K (rs5219) gene polymorphism did not affect HbA1c levels in this investigation. The KK genotype is rarer than the EE and EK genotypes, and HbA1c prediabetes criteria are rare. The KCNJ11 rs5219 polymorphism does not significantly affect DM, suggesting that food may be a factor. Mutations vary KATP channel activity. By decreasing KATP channel ATP sensitivity, the K allele from this location may inhibit insulin secretion. This increases channel activity and insulin suppression. KK genotypes had a greater effect on insulin secretion than EE genotypes[36]. In contrast, the KK genotype was the rarest. Thus, the gene polymorphism KCNJ11 E23K (rs5219) indirectly affects HbA1c levels in the first generation of Lembak people in Bengkulu City.

Conclusion

The non-DM first-generation group had more KK genotypes, while the DM first-generation group had more EE and EK genotypes. The KCNJ11 E23K rs5219 gene genotype frequency did not differ significantly between the two groups. Lembak people had a higher risk of acquiring DM in the first generation due to their baseline HbA1c values. The KCNJ11 E23K (rs5219) gene polymorphism does not correlate with HbA1c in the first generation of Lembak people residing in Bengkulu City.

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Conflicts of Interest

This research has no conflicts of interest in this study.

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