Rs10830963 Polymorphism of the MTNR1B Gene in Type 2 Diabetes Mellitus Patients with Dyslipidemia and Non-Dyslipidemia

Yanuarita Tursinawati[1(✉)] and Ari Yuniastuti[2]

[1] Faculty of Medicine, University Muhammadiyah Semarang, Semarang, Indonesia
yanuarita_tursina@unimus.ac.id
[2] Faculty of Mathematics and Natural Sciences, Semarang State University, Semarang, Indonesia

Abstract. Background: Insulin resistance in Diabetes mellitus is found to be the contributor to atherogenic dyslipidemia and results in diabetic dyslipidemia. Rs10830963 polymorphism of the MTNR1B gene is proven to be significant for obesity in T2DM which is usually accompanied by lipid metabolism disorder.

Aim: This research aims to identify the correlation between rs10830963 polymorphism of the MTNR1B gene with the risk of dyslipidemia in Javanese T2DM patients.

Methods: This study used cross-sectional study which involved 107 patients with T2DM treatment at some primary health care centre in Semarang. Gene polymorphism was analyzed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The polymorphism of rs10830963 MTNR1B was presented in allele frequency and genotype frequency, then analyzed using the x^2 test with $p < 0.05$.

Results: Most of the respondents were found to have a C allele in both groups. On the other hand, the CG genotype was the most found in both groups. There was no significant difference between groups in terms of allele frequency ($p = 0.767$) and genotype frequency ($p = 0.507$). The significant difference was only found in the microalbuminuria parameter of the dyslipidemia group ($p = 0.028$) and BMI of the non-dyslipidemia group ($p = 0.018$).

Conclusion: The polymorphism rs10830963 MTNR1B does not play a role in the risk of dyslipidemia in Javanese T2DM patients. Meanwhile, there is significant difference in the microalbuminuria parameter of the dyslipidemia group and BMI of the non-dyslipidemia group.

Keywords: Polymorphism · rs10830963 MTNR1B of Type 2 Diabetes Mellitus · Dyslipidemia

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1 Introduction

Type 2 diabetes is an impairment in the way the body cell responds the insulin hormones. This condition is commonly mentioned as "insulin resistant". Insulin resistance is found to be the etiology of the atherogenic dyslipidemia such as the increase in total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and the lowering of high-density lipoprotein cholesterol (HDL-C) [1, 2]. It is caused by the lowered insulin effect in the fat tissue, so the lipogenesis process is lowered and lipolysis increased. The process contributes to glucotoxicity accompanied by lipotoxicity which further results in an increase in LDL cholesterol level. In the case of high blood glucose levels (hyperglycemia), LDL oxidation is faster due to the chronic increase in plasma glucose [1]. Based on a study in 2018, it was found that out of 9,285 respondents with T2DM, 59.28% of it was diagnosed with dyslipidemia [3]. Other studies mentioned that 97.8% of diabetic female respondents and 85.5% of diabetic male respondents have dyslipidemia [4].

Pathogenesis of diabetic dyslipidemia which involves lipoprotein metabolism is also affected by melatonin hormones which regulate biological rhythm and are responsible for the level of total cholesterol, triglycerides, LDL, and HDL in patients with diabetes. The mechanism of diabetic dyslipidemia involves the increase of inflammatory cytokines such as TNF α. TNF α contributes to insulin resistance, down-regulates Apo a-I and the production of HDL, also improving the hypertriglyceridemia enzymes activity [5]. The study conducted by Denis et al., 2019 showed that there was a significant increase in the TNF α level of the experimental animal models for diabetes. In the group with melatonin intervention, TNF α plasma was lowered and contributed to the improvement of lipid profile [6, 7]. The performance of melatonin bind to the receptors of which one of the receptors was coded by the MTNR1B gene. Rs10830963 polymorphism of the MTNR1B gene was proven to be significant to obesity in T2DM which is usually accompanied by lipid metabolism impairment [8]. The polymorphism is also correlated to VLDL and triglycerides levels [9]. Nevertheless, there is no research on the involvement. Rs10830963 polymorphism of the MTNR1B gene in T2DM patients based on the case of dyslipidemia. So, this research aims to identify the correlation between rs10830963 polymorphism of the MTNR1B gene with the risk of dyslipidemia in Javanese T2DM patients as Javanese is the largest ethnic group in Indonesia.

2 Methods

Design and subject of the study

It used cross-sectional study involving 107 patients with T2DM treatment at some primary health care centre in Semarang, Central Java, Indonesia. The subject has signed the informed consent as the agreement. The inclusion criteria of this research are the Javanese T2DM patients, 30 – 70 years old, with the exclusion criteria of the sample such as a history of cardiovascular diseases such as stroke, heart failure, and acute myocardial infarction.

Genotyping the rs10830963 polymorphism of the MTNR1B by PCR RFLP
The gene polymorphism used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The DNA was extracted from the whole blood by using GeneJETTM Genomic DNA Purification Kit. The MTNR1B gene amplification used PCR Mix 21 μl (2.5 μl PCR buffer with MgCl2, dNTP 0.25 μl, Primer forward 5'-ATG CTA AGA ATT CAC ACC AGC T-3', reverse 5'-CAC AGT GCA GAC TGT TTT CTA ATC-3' 1.5 μl each DNA Taq polymerase 0.25 μl, DNA 1 μl, H2O 14 μl, DNA 1 μl). The PCR Protocol is described as follow: pre-denaturation 95 °C, 7 min, 33 cycles: Denaturation 95 °C, 30 s, annealing 60 °C, 30 s, elongation 72 °C, 1 min with final extension 72 °C, 3 min. RFLP with 100 μl final volume (PCR product 10 μl, PvuII 5 μl, enzyme buffer (10×) 10 μl, H2O 75 μl, with BSA 10 μl) then dissolve the PCR product with enzyme mix (10 μl: 10 μl) then incubated at 370C, for an hour. Product digestion was visualized at 4% agarose gel, 120 V, 30 min. The C allele (125 bp) and the G allele (105 bp and 20 bp) in the electrophoresis result.

Clinical laboratory test
The sample of respondents' venous blood was taken after 8 h of fasting for the Fasting Plasma Glucose (FPG) test by using an enzymatic method (GOD PAP), total cholesterol testing by using CHOD-PAP method, triglycerides testing by using GPO-PAP method, HDL and LDL testing by using a direct enzymatic method, urea testing by using Urease – GLDH, creatinine testing by using Jaffe method, and urine microalbumin testing by using immunoturbidimetry. The dyslipidemia criteria based on total cholesterol level of >200 mg/dL, LDL cholesterol levels >130 mg/dL or triglyceride levels >200 mg/dL [10, 11].

Data analysis
The data about MTNR1B rs10830963 is presented in a form of allele frequency and genotype frequency for then being analyzed using the x^2 test with $p < 0.05$. The difference between variable characteristic numbers among groups was determined using an independent T-test.

3 Results

Out of 107 respondents, 46 are dyslipidemia and 61 are non-dyslipidemia. The average FPG, Systolic Blood Pressure (SBP), cholesterol, triglycerides, ratio chol/HDL, and HbA1c in dyslipidemia respondents are higher than in the non-dyslipidemia group as described in Table 1. The significant difference can be seen in their age, FPG, cholesterol, triglycerides, HDL, LDL ratio chol/HDL, and HbA1c parameters. Most respondents identified as C allele (wild type allele) in dyslipidemia (62%) and non-dyslipidemia (63.9%). On the other hand, the CG genotype is the most found in both groups with a frequency of 71.7% in the dyslipidemia group and 72.1% in the non-dyslipidemia group. There is no significant difference between both groups in terms of allele frequency and genotype frequency as described in Table 2.

In Table 3, it can be seen that the ratio of blood chemical parameters based on genotype rs10830963 polymorphism of MTNR1B among dyslipidemia and non-dyslipidemia shows a significant difference only on a microalbumin parameter in the dyslipidemia

Table 1. Characteristic Data of Type 2 DM Patients in Obese and Non-Obese Groups

	Subjects (n = 107)		p*
	Dyslipidemia n = 46	Non-Dyslipidemia n = 61	
Age	56.91 ± 5.86	59.70 ± 7.35	0.037**
BMI	23.92 ± 3.01	24.61 ± 3.52	0.291
FPG (mg/dl)	179.09 ± 88.56	146.25 ± 63.82	0.028**
SBP (mmHg)	139.41 ± 20.72	137.67 ± 17.81	0.642
DBP (mmHg)	78.57 ± 9.11	81.89 ± 8.61	0.057
Cholesterol	232.15 ± 22.38	143.98 ± 39.06	0.00**
Triglyceride	236.33 ± 198.02	182.33 ± 62.28	0.048**
HDL	55.93 ± 16.79	63.72 ± 16.44	0.018**
LDL	184.46 ± 44.33	209.13 ± 60.77	0.022**
Chol/HDL	5.35 ± 1.40	4.45 ± 1.22	0.001**
HbA1c	8.37 ± 2.04	7.47 ± 1.84	0.019**
Urea	28.61 ± 11.98	30.62 ± 11.22	0.374
Creatinine	0.86 ± 0.44	0.98 ± 0.48	0.199
Microalbumin	251.97 ± 383.57	274.31 ± 1043.31	0.890

* $p < 0.05$ as a significant result used Independent t-test. ** significant result

Table 2. Allele and Genotype distribution of rs10830963 polymorphisms of MTNR1B gene

		Subjects (n = 107)		
		Dyslipidemia N = 46	Non-Dyslipidemia N = 61	p*
rs10830963 MTNR1B				
Allele	C	57 (62%)	78 (63.9%)	0.767
	G	35 (38%)	44 (36.1%)	
Genotype	CC	12 (26.1%)	17 (27.9%)	0.507
	CG	33 (71.7%)	44 (72.1%)	
	GG	1 (2.2%)	0 (0%)	

* Chi-Square test, $p < 0.05$ as a significant result

group and BMI in non- dyslipidemia group. However, if we take a look at the level, the value of CG + GG genotype for all parameters such as FPG, SBP, DBP, HbA1c, urea, creatinine, and microalbumin of the dyslipidemia group is higher.

Table 3. The Comparison of Clinical and Biochemical Profile Based on the Genotype of rs10830963 MTNR1B Polymorphism

Variable	Dyslipidemia (N = 46)		p*	Non-Dyslipidemia (N = 61)		p*
	CC	CG + GG		CC	CG + GG	
BMI	23.13 ± 1.59	24.21 ± 3.35	0.556	22.88 ± 2.97	25.28 ± 3.51	0.018**
FPG (mg/dl)	152.58 ± 64.77	188.44 ± 94.60	0.361	133.18 ± 66.09	151.30 ± 62.97	0.159
SBP (mmHg)	132.50 ± 13.91	141.85 ± 22.30	0.161	136.88 ± 13.37	137.98 ± 19.38	0.994
DBP (mmHg)	76.67 ± 7.13	79.24 ± 9.72	0.381	82.94 ± 7.11	81.48 ± 9.17	0.468
HbA1c	7.93 ± 1.95	8.53 ± 2.07	0.402	6.70 ± 0.94	7.77 ± 2.02	0.20
Urea	23.58 ± 7.24	30.38 ± 12.87	0.059	29.24 ± 11.62	31.16 ± 11.16	0.430
Creatinine	0.72 ± 0.13	0.911 ± 0.51	0.127	1.06 ± 0.59	0.95 ± 0.44	0.618
Microalbumin	111.042 ± 291.53	301.71 ± 403.15	0.028**	108 ± 107.39	338.57 ± 1224.49	0.955

* $p < 0.05$ as a significant result used Mann Whitney U Test. ** significant result

4 Discussion

The result of genetic analysis in this study shows that most of the respondents are identified with the C allele (Wild type allele) either in T2DM patients with dyslipidemia or non-dyslipidemia. CG heterozygous genotype is the most found in both groups. The finding is different from the study about T2DM patients in China which analyze polymorphism to prove that the G allele is the most found allele content in the DM group compared to the healthy group ($p < 0.05$) [12]. It proves that genetic variation in form of MTNR1B polymorphism is correlated to T2DM susceptibility. The rs10830963 in the MTNR1B locus is a functional polymorphism locus that takes part in glucose metabolism and pancreas β cell function. Minor G allele in MTNR1B rs10830963 could inhibit glucose tolerance on the carrier [13]. The study conducted by Vejrazkova et al., 2022 proves that a sample with GG genotype has a higher level of basalt glycemic level compared to the CC group [13]. The study is different from the finding of this research as this research involved only the DM patients so there is no comparison with the healthy control group.

DM is usually accompanied by lipid metabolism impairment which is known as diabetic dyslipidemia which involves lipoprotein metabolism. Lipid metabolism includes plasma lipid concentration, lipid absorption in the intestines, lipid biosynthetic which is controlled by the circadian system, and melatonin hormone, the hormone that regulates human sleep [14, 15]. The treatment using melatonin for 3 months is significant for human lipid profile by lowering total cholesterol serum and LDL-C and increasing HDL-C [16]. The study designed to evaluate melatonin and zinc effect on lipid metabolism and function of renal's patients with T2DM which is not well controlled due to the use of metformin medicine shows that the use of melatonin and zinc could improve lipid metabolism and the lowering of microalbumin level, also the improve in the response toward metformin [17]. It means that rs10830963 polymorphism in the MTNR1B gene is not only responsible for the case of DM, but also dyslipidemia. The study in 2014 stated that there is a significant correlation between rs10830963 polymorphism in the MTNR1B gene with Very Low-Density Lipoprotein (VLDL) $p = 0.0015$ dan triglyceride $p = 0.0018$ [9]. A meta-analytic study from a cohort study proved that rs10830963 polymorphism in the MTNR1B gene significantly correlated to HDL ($P = 0.02$) [18]. It is in line with the finding of this research, with FPG and Hba1 c levels in the CG + GG group of dyslipidemia and non-dyslipidemia group are higher than in the CC group, although it is not statistically significant. The non-significant result might be contributed by a less deep analysis of the sample's diet. An intervention study by giving Monounsaturated Fatty Acid (MUFA) diet shows that after the weight loss using MUFA, the parameter of total cholesterol and LDL lowered in the subject without the G allele. It proves that particular genetic variations are correlated to the effectiveness of the intervention of someone's lifestyle and diet.

The result of this study also shows a significant difference between the CC group and CG + GG, especially in the microalbumin parameter of the dyslipidemia group ($p = 0.028$) and BMI in non-dyslipidemia. It is also in line with other research which compared between obese and non-obese. There is no significant difference in FPG, Hba1c, Ureum, and kreatinin [8]. Obese is marked by the increase of adipose tissue or the imbalance distribution between central and peripheral areas of the body which could result in insulin resistance, dyslipidemia, and a higher risk of T2DM [19]. Compared to

peripheral obesity, central obesity tends to improve the delivery of free fatty acids (FFA) to the liver which contribute to the production of liver glucose by the fatty acid. It causes a problem with liver insulin release and further causes insulin resistance which is the main cause of T2DM [19]. This research did not the identify diet pattern of the sample and the use of lipid-lowering medicines, so it could be improved in the next research.

5 Conclusion

The polymorphism rs10830963 MTNR1B does not play a role in the risk of dyslipidemia in Javanese T2DM patients. Meanwhile, there is significant difference in the microalbuminuria parameter of the dyslipidemia group and BMI of the non-dyslipidemia group.

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