Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Feb 16; 10(A):273-277.
https://doi.org/10.3889/oamjms.2022.8473
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation

Elly Usman*, Yusticia Katar

Department of Pharmacology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

Edited by: Sinisa Stojanoski
Citation: Usman E, Katar Y. A Metformin Pharmacogenetic Study of Patients with Type 2 Diabetes Mellitus and SLC22A1 Gene Mutation. Open Access Maced J Med Sci. 2022 Feb 16; 10(A):273-277. https://doi.org/10.3889/oamjms.2022.8473
Keywords: Fasting blood glucose; Metformin; SLC22A1; T2DM; Pharmacogenetic
***Correspondence:** Elly Usman, Department of Pharmacology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia. E-mail: ellyusman@med.unand.ac.id
Received: 01-Jan-2022
Revised: 05-Feb-2022
Accepted: 06-Feb-2022

Funding: This study was supported by Universitas Andalas
Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: The purpose of this study was to determine the profiles of patients with Type 2 diabetes mellitus (T2DM) and an SLC22A1 gene mutation to evaluate the effect of metformin pharmacogenetics.

METHODS: To assess the effect of pharmacogenetics, a mutation of the SLC22A1 gene in T2DM patients receiving metformin was investigated. Blood samples were taken from 50 diabetics of Minangkabau ethnicity who met the inclusion criteria and SNP genotyping and blood glucose levels were determined. DNA is extracted and purified from blood samples using DNAzol® Genomic DNA Kits (Thermofischer Scientific) reagents. The Chi-square test and independent sample T test were used to analyze the data. A statistically significant association was defined as $p < 0.05$. Finally, the GraphPad Prism 7.00 program was used to gather and analyze data.

RESULTS: The adjusted odds ratio for inadequate fasting blood glucose was 1.48 (95% confidence interval [CI] 1.18–1.95) in this study, while the adjusted odds ratio for diet discipline was 1.23 (95% CI 1.18–1.95). The adjusted odds ratio for low physical activity was 1.18 (95% CI 1.05–1.81). According to the sequencing data, the proportion of mutants is high at exon 2 rs683369 (G>C), while the percentage of wildtype and heterozygous mutants is the same at introns rs4646272 (T>G).

CONCLUSION: Obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM patients. Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants.

Introduction

Metformin, a medicine recommended for its relative absence of adverse effects and great patient tolerance, is the first line of the treatment for Type 2 diabetes mellitus (T2DM) [1], [2]. Metformin, on the other hand, does not operate similarly or ideally in all patients due to variances in individual genetic profiles, resulting in a decrease in the drug's effectiveness and safety [3], [4]. As a result, determining the genetic component behind metformin response variability is critical, particularly in areas with a high prevalence of T2DM [5], [6]. The previous research has found that the genetically unique Arab, Chechen, and Circassian groups have varied clinical features of diabetes, necessitating specialized diabetes management and treatment strategies for each [7], [8].

Metformin works by lowering hepatic glucose synthesis while boosting glucose absorption in the peripheral tissues [9]. Metformin is unusual in that it does not need to be broken down by the body to effect blood glucose management [10], [11]. Metformin, on the other hand, requires membrane transport proteins produced by solute carrier (SLC) genes to enter cells and reduce hepatic glucose synthesis [12], [13]. The OCT1 and OCT3 proteins, which are predominantly important for hepatic and intestinal metformin absorption, are encoded by the SLC family 22 member 1 (SLC22A1) and 3 (SLC22A3) genes, respectively [14], [15]. Several single-nucleotide polymorphisms (SNPs) in the SLC22A1 gene have been shown to impact metformin pharmacodynamics and pharmacokinetics, and hence, patient responses to the medicine [16], [17].

Despite accounting for a significant amount of Jordan's disease burden, there have been little investigations on T2DM's hereditary component and the impact of the latter on metformin response. The aim of this study was to determine the profiles of patients with T2DM and an SLC22A1 gene mutation to evaluate the effect of metformin pharmacogenetics.

Materials and Methods

Study design

To assess the influence of pharmacogenetics, profiles of patients with T2DM and SLC22A1 gene mutation were compared between patients using metformin. The Ethics Committee of the Faculty

of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia gave the study ethical permission (No: 95/KEP/FK/2018). The Declaration of Helsinki was followed in all of the procedures used in this study. All recruited individuals provided written informed permission after being told of the study's aim and assured of patient confidentiality.

Patients recruitment

The participation of eligible subjects in this study were patients with T2DM in Padang, West Sumatera, Indonesia. The population sampled satisfies the following criteria: Age 25–50 years, fasting blood sugar levels <100 mg/dL, and blood sugar levels 2 h after providing glucose 75 g <140 mg/dL. If a sample's liver or kidney function is affected, it will be discarded. A total of 5 mL of blood is collected from the sample and kept at-80° C until needed.

Data collection

Direct interviews were used to obtain information in Padang City in 2018. Clinical data were gathered using a study-specific questionnaire that was filled out by health-care professionals interviewing the individuals. Demographic information was obtained as part of the clinical data. During the interview, a phlebotomist took blood samples to determine fasting blood glucose levels using ethylenediamine tetraacetic acid (EDTA) collection tubes.

Isolation and purification of genomic DNA

DNA is extracted and purified from blood samples using DNAzol® Genomic DNA Kits (Thermofischer Scientific) reagents. The extraction of genomic DNA from whole blood is done according to the provider's instructions. Chloroform is used to extract DNA from homogenates, resulting in aqueous, interphase, and organic layers. With the addition of 100% ethanol and Trizol reagent, DNA was deposited from the interphase and organic layer. Furthermore, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and 75% ethanol in a sequential order. After resensing the dry pellets in 8 mM NaOH, DNA can be kept at-20° C in HEPES buffer pH 7–8 with 1 mM EDTA.

PCR and SLC22A1 gene sequencing

Primer Blast (NCBI) software was used to create PCR primers and sequencing. HPLC was used to purify the produced primer. The PCR technique was used to reproduce the DNA fragments. The PCR procedure was carried out with the Gotaq TM PCR Core System kit (Promega) and a total volume of 50 L for each reaction. DNA samples were amplified for 35 cycles, and the amplicon was kept at 4° C once the procedure was done. Electrophoresis of the application's results in a 2% agarose gel with Gelred and DNA ladder separated the results. Amplicon DNA was extracted and produced in quantities up to 500 ng for sequencing using Illumina's next generation sequencing technique.

Data analysis

Mean ± SD, median, and percentage were used to capture the quantitative data. The Chi-square test and the independent sample T test were used to analyze the data. Statistical significance was defined as a two-tailed $p < 0.05$. GraphPad Prism 7.00 was used to gather and analyze data.

Results

Profiles of patients with T2DM (Table 1).

Table 1: Profiles of patients with T2DM

Variables	Adequate fasting blood glucose (n=25) (%)	Inadequate fasting blood glucose[a] (n=25)	p-value
Sex			0.986[b]
Male	7 (28.0)	8 (32.0)	
Female	18 (72.0)	17 (68.0)	
Age (Years)			0.086[b]
19–29	0	1 (4.0)	
30–49	5 (20.0)	4 (16.0)	
50–64	19 (76.0)	17 (68.0)	
≥65	2 (8.0)	3 (12.0)	
Ethnicity			0.023*[b]
Minangnese	23 (92.0)	21 (84.0)	
Bataknese	1 (4.0)	2 (8.0)	
Others	1 (4.0)	2 (8.0)	
Occupational			0.783[b]
Working	21 (84.0)	20 (80.0)	
Not working	4 (16.0)	5 (20.0)	
Physical activity			0.048*[b]
Low	13 (52.0)	5 (20.0)	
High	12 (48.0)	20 (80.0)	
Diet discipline			0.041*[b]
Not good	14 (56.0)	11 (44.0)	
Good	11 (44.0)	14 (56.0)	
Regularly check blood sugar			0.871*[b]
Regular	21 (84.0)	20 (80.0)	
Not regular	4 (16.0)	5 (20.0)	
Age at diagnosis T2DM (years)	49.93±8.30	51.12±9.62	0.531[c]
Basal Mass Index (BMI) (kg/m^2)	23.71±6.42	25.42±5.20	0.047*[c]

*p-value < 0.05 is considered significant; [a], defined as fasting blood glucose level ≥ 126 mg/dL according to the American diabetic association (ADA) guidelines; [b], Chi-square test; [c], Independent sample T test, T2DM: Type 2 Diabetes Mellitus.

Table 1 shows that ethnicity, physical activity, diet discipline, and body mass index are all associated with fasting blood glucose levels ($p < 0.05$). However, no significant relationship was seen between sex, age, occupation, and age at diagnosis of T2DM and fasting blood glucose ($p > 0.05$).

The unadjusted (univariate) and adjusted (multivariate) odds ratios and 95% CIs for inadequate fasting blood glucose (Table 2).

Table 2 shows that obesity was associated with a higher risk of low fasting blood glucose, with an

Table 2: The unadjusted (univariate) and adjusted (multivariate) odds ratios and 95% confidence intervals for inadequate fasting blood glucose

Variables	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sex		
Male	0.88 (0.34–2.25)	0.85 (0.29–2.01)
Female	0.92 (0.36–2.89)	0.89 (0.31–2.11)
Age (Years)		
19–29	Ref	Ref
30–49	Ref	Ref
50–64	0.98 (0.31–3.22)	0.95 (0.29–3.15)
≥65	0.93 (0.28–2.99)	0.89 (0.26–2.94)
Ethnicity		
Minangnese	0.93 (0.27–1.43)	0.90 (0.21–1.32)
Bataknese	0.88 (0.21–1.12)	0.79 (0.19–0.98)
Others	0.73 (0.19–1.09)	0.69 (0.17–0.88)
Occupational		
Working	Ref	Ref
Not working	1.11 (0.37–3.32)	0.98 (0.32–3.01)
Physical activity		
High	Ref	Ref
Low	1.20 (1.11–1.89)*	1.18 (1.05–1.81)*
Diet discipline		
Good	Ref	Ref
Not good	1.25 (1.16–1.91)*	1.23 (1.09–1.71)*
Regularly check blood sugar		
Regular	Ref	Ref
Not regular	0.71 (0.22–0.85)	0.68 (0.21–0.81)
Age at diagnosis T2DM (years)		
<50	Ref	Ref
≥50	0.98 (0.34–0.93)	0.93 (0.32–0.89)
Body Mass Index (BMI)		
Normal	Ref	Ref
Overweight	0.97 (0.37–1.92)	0.95 (0.31–1.88)
Obesity	1.51 (1.21–2.01)*	1.48 (1.18–1.95)*

Ref, reference; *p<0,05, significance was considered, OR: Odds ratios, CI: Confidence intervals, T2DM: Type 2 diabetes mellitus.

unadjusted odds ratio of 1.51 (95% CI 1.21–2.01) and an adjusted odds ratio of 1.48 (95% CI 1.18–1.95). Diet discipline was also significantly risk of inadequate fasting blood glucose; the unadjusted odds ratio was 1.25 (95% CI 1.16–1.91) and the adjusted odds ratio was 1.23 (95% CI 1.09–1.71). Low physical activity the unadjusted odds ratio was 1.20 (95% CI 1.11–1.89) and the adjusted odds ratio was 1.18 (95% CI 1.05–1.81).

Gene sequencing results in T2DM patients receiving metformin (Table 3).

Table 3: Gene sequencing results in T2DM patients receiving metformin

Exon/Intron	SNPs	Genotyped	f	%
Exon 1	rs200710420 (G>A)	GG	47	94.0
		GA	3	6.0
		AA	0	0
	rs1867351 (T/C)	TT	28	56.0
		TC	17	34.0
		CC	5	10.0
Intron	rs4646272 (T>G)	TT	21	42.0
		TG	21	42.0
		GG	8	16.0
	rs74795793 (T>C)	TT	47	94.0
		TC	2	4.0
		CC	1	2.0
Exon 2	rs683369 (G>C)	GG	2	4.0
		GC	13	26.0
		CC	35	70.0
	rs201942835 (G>T)	GG	49	98.0
		GT	1	2.0
		TT	0	0
Intron	rs4646273 (G>A)	GG	29	58.0
		GA	18	36.0
		AA	3	6.0

T2DM: Type 2 diabetes mellitus.

Table 3 shows that rs200710420 (G>A), which is present in Exon 1, has a greater GG (wildtype) genotype (94.0%) than mutants and no homozygous mutants. Wildtype (TT) was likewise shown to be greater (56.0%) than mutants in rs1867351 (T/C). Heterozygous (TC) mutants were detected in 34% of the cases, whereas homozygous mutants were found in 10.0% of the cases. There was rs4646272 (T>G) in the intron, with the same proportion of wildtype and heterozygous mutants (42.0%). Wildtype (TT) was reported to be greater (94.0%) than mutants in rs74795793 (T>C). Heterozygous mutants accounted for 4.0% of the total, whereas homozygous mutants accounted for 2.0%.

Exon 2 yielded a larger proportion of mutants than wildtype for rs683369 (G>C). Homozygous (CC) mutants accounted for 70.0% of the total, heterozygous (GC) mutants 26.0%, and wildtype 4.0%. Wildtype (GG) mutants were detected in more than 49.0% of rs201942835 (G>T) mutants, heterozygous mutants were found in 2.0%, and homozygous mutants were not found. Wildtype (GG) was detected in a larger percentage (58.0%) than mutants in the intron rs4646273 (G>A). Heterozygous (GA) mutants were detected in 36.0% of the cases, whereas homozygous (AA) mutants were found in 6.0% of the cases.

According to the sequencing data, the proportion of mutants is high at Exon 2 rs683369 (G>C), while the percentage of wildtype and heterozygous mutants is the same at introns rs4646272 (T>G).

Discussion

Recent breakthroughs in identifying common T2DM variations highlighted their association with the disease's pathogenesis, which assists in the assessment of individual risk and treatment effectiveness [18], [19]. Despite its rising prevalence in Indonesia, T2DM has not been adequately investigated pharmacogenically in the Indonesian population. As a result, the present study is extremely important since it gives information on the relationship between metformin metabolism and Indonesian genetic profiles.

This study found that Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants. The extent to which T2DM-predisposing polymorphisms in the SLC22A1 genes are associated with good glycemic control was investigated in this study. The OCT proteins, which are organic cation transporters that play crucial roles in the regulation of essential metabolic processes, are encoded by the aforementioned genes, which are highly relevant to the field of drug transport [20].

This study revealed that obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM

patients. Obesity is a cause of diabetes and insulin resistance. Adipose tissue releases more non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines in obese people, which might contribute to the development of insulin resistance [21], [22].

The significant rise in the incidence of diabetes in emerging nations is due to dietary choices and a sedentary lifestyle. Recently, increased HbA1c levels in Type 2 diabetics have been identified as one of the primary risk factors for microvascular and macrovascular problems. Diet management can help patients with their increased HbA1c levels, preventing them from acquiring diabetic complications [23].

The majority of the advantages of physical exercise for diabetes control come from changes in insulin action, which may be achieved with both aerobic and resistance training. Physical training advantages are reviewed, as well as advice for various activities, physical activity-related blood glucose control, diabetes prevention, gestational diabetes mellitus, and safe and effective techniques for physical activity with diabetes-related problems [24].

The genetic connection of these SNPs with fasting blood glucose levels in the management of diabetes is also influenced by the age at which diabetes is diagnosed. When treating diabetic patients, several covariate variables should be taken into account. It is also crucial to understand the influence of these variables on T2DM patients' genetic connections with fasting blood glucose levels. One possible weakness of the present study is that the length of the illness was not taken into account, and people who had the condition for a longer period may have had lower endogenous insulin production, implying that endogenous insulin levels were varied among the subjects.

This research suggests that increased awareness of diabetes complications leads to improvements in dietary knowledge and physical activity. In addition, maintaining a healthy body mass index helps to keep the condition under control. Stakeholders (health-care practitioners, health-care institutions, diabetes-care organizations, and so on) should assist patients to recognize the relevance of nutrition in disease management, adequate self-care, and improved quality of life.

Conclusion

Obesity, diet discipline, and low physical activity were all found to increase the likelihood of insufficient fasting blood glucose in T2DM patients. Exon 2 rs683369 (G>C) has a high proportion of mutants, but introns rs4646272 (T>G) have the same percentage of wildtype and heterozygous mutants.

Acknowledgments

The authors would like to thank Universitas Andalas for their grant research and all of the participants in this study.

References

1. Arimany-Nardi C, Koepsell H, Pastor-Anglada M. Role of SLC22A1 polymorphic variants in drug disposition, therapeutic responses, and drug-drug interactions. Pharmacogenomics J. 2015;15(6):473-87. https://doi.org/10.1038/tpj.2015.78
   PMid:26526073
2. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: Association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics. 2005;115(3):e290-6. https://doi.org/10.1542/peds.2004-1808
   PMid:15741354
3. Ministry of Health Republic of Indonesia. Basic Health Research. Ministry of Health Republic of Indonesia. Jakarta: Ministry of Health Republic of Indonesia; 2013.
4. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease?: A meta-analysis of prospective studies. Arch Intern Med. 2004;164(19):2147-55. https://doi.org/10.1001/archinte.164.19.2147
   PMid:15505129
5. Shu Y, Brown C, Castro R, Shi R, Lin E, Owen R, *et al*. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther. 2008;83(2):273-80. https://doi.org/10.1038/sj.clpt.6100275
   PMid:17609683
6. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, *et al*. Management of hyperglycemia in Type 2 diabetes: A patient-centered approach position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2012;35(6):1364-79. https://doi.org/10.2337/dc12-0413
   PMid:22517736
7. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of Type 2 diabetes: Perspectives on the past, present, and future. Lancet. 2014;383(9922):1068-83. https://doi.org/10.1016/S0140-6736(13)62154-6
   PMid:24315620
8. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333(9):541-9. https://doi.org/10.1056/NEJM199508313330902
   PMid:7623902
9. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BHC. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. Pharmacogenom J. 2009;9:242-7. https://doi.org/10.1038/tpj.2009.15
   PMid:19381165
10. Ajlouni K, Khader YS, Batieha A, Ajlouni H, El-Khateeb M. An increase in prevalence of diabetes mellitus in Jordan over 10 years. J. Diabetes Complicat. 2008;22(5):317-24. https://doi.org/10.1016/j.jdiacomp.2007.01.004
    PMid:18413210

11. Song I, Shin H, Shim E, Jung I, Kim W, Shon J, Shin J. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. Clin Pharmacol Ther. 2008;84:559-62.
12. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and *in-vivo* renal functional consequence: Studies with metformin and cimetidine. Pharmacogenet Genom. 2008;18(7):637-45. https://doi.org/10.1097/FPC.0b013e328302cd41
PMid:18551044
13. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, Sehrt D, *et al*. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther. 2009;86(3):299-306. https://doi.org/10.1038/clpt.2009.92
PMid:19536068
14. Mahrooz A, Alizadeh A, Hashemi-Soteh MB, Ghaffari-Cherati M, Hosseyni-Talei SR. The polymorphic variants rs3088442 and rs2292334 in the organic cation transporter 3 (OCT3) gene and susceptibility against Type 2 diabetes: Role of their interaction. Arch Med Res. 2017;48(2):162-8. https://doi.org/10.1016/j.arcmed.2017.03.010
PMid:28625319
15. Tuomi T, Santoro N, Caprio S, Cai M, Weng J. Groop, L. The many faces of diabetes: A disease with increasing heterogeneity. Lancet. 2014;383(9922):1084-94. https://doi.org/10.1016/S0140-6736(13)62219-9
PMid:24315621
16. Karalliedde J, Gnudi L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrol Dial Transplant. 2014;31(2):gfu405. https://doi.org/10.1093/ndt/gfu405
PMid:25550448
17. Lango H, Palmer CN, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, *et al*. Assessing the combined impact of 18 common genetic variants of modest effect sizes on Type 2 diabetes risk. Diabetes. 2008;57(11):3129-35. https://doi.org/10.2337/db08-0504
PMid:18591388
18. Nigam SK. The SLC22 transporter family: A Paradigm for the impact of drug transporters on metabolic pathways, signaling, and disease. Annu Rev Pharmacol Toxicol. 2018;58:663-87. https://doi.org/10.1146/annurev-pharmtox-010617-052713
PMid:29309257
19. Pochini L, Galluccio M, Scalise M, Console L, Indiveri C. OCTN: A small transporter subfamily with great relevance to human pathophysiology, drug discovery, and diagnostics. SLAS Discov. 2019;24(2):89-110. https://doi.org/10.1177/2472555218812821
PMid:30523710
20. Dujic T, Zhou K, Yee S, van Leeuwen N, de Keyser C, Javorský M, *et al*. Variants in pharmacokinetic transporters and glycemic response to metformin: A metgen meta-analysis. Clin Pharmacol Ther. 2017;101(6):763-72. https://doi.org/10.1002/cpt.567
PMid:27859023
21. Al-Eitan LN, Amomani BA, Nassar AM, Elsaqa BZ, Saadeh NA. Metformin pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 polymorphisms on glycemic control and HbA1c levels. J Pers Med. 2019;9(1):17. https://doi.org/10.3390/jpm9010017
PMid:30934600
22. Wondmkun YT. Obesity, insulin resistance, and Type 2 diabetes: Associations and therapeutic implications. Diabetes Metab Syndr Obes. 2020;13:3611-6. https://doi.org/10.2147/DMSO.S275898
PMid:33116712
23. Sami W, Ansari T, Butt NS, Hamid MR. Effect of diet on Type 2 diabetes mellitus: A review. Int J Health Sci (Qassim). 2017;11(2):65-71.
PMid:28539866
24. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, *et al*. Exercise and Type 2 diabetes: The American college of sports medicine and the American diabetes association: Joint position statement. Diabetes Care. 2010;33(12):e147-67. https://doi.org/10.2337/dc10-9990
PMid:21115758