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Allele Frequency of a Common Variants and Two Common Loss-of-Function Variants in Organic Cation Transporter 1 (OCT1) among Balinese Diabetic Patients

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Metformin is the most common drug prescribed for patient with type 2 diabetes mellitus (T2DM). Although it is widely used as first line therapy for T2DM, there were huge variations in its clinical efficacy among population. It was postulated that genetic polymorphisms of metformin transporter, especially organic cation transporter 1 (OCT1) encoded by SLC22A1 gene, have a considerable effect on respon of metformin therapy. However, data for this polymorphism in Balinese population was not well established. The aim of this study was to identify genetic variation in OCT1, especially rs628031, rs122083571, and rs623442, in Balinese diabetic patients. It was a descriptive study to explore genetic variation in OCT1 encoded by SLC22A1 gene. A total of 133 diabetic patients were recruited from Departement of Internal Medicine at Sanjiwani Hospital Gianyar and Tabanan Hospital, Bali. DNA was extracted and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to assess the polymorphism rs628031. While, polymorphism rs122083571 and rs623442 were assessed by direct sequencing. The minor allele frequency (MAF) for polymorphism rs628031 in this population was 0.59 with genotype frequency of AA, AG, and GG accounted for 16.5%; 48.9%, and 34.6% respectively. Minor allele frequency for polymorphism rs623442 was 0.20 with genotype frequency of CC, CA, and AA 5.4%; 29.0%; and 65.6% respectively. Polymorphism rs122083571 was not found in this population (100% genotype CC). Genetic polymorphism of OCT1 rs628031 in this population was occurred in relatively high frequency, while polymorphism OCT1 rs623442 was occurred only in one fifth of studied population. Further studies are needed to address the effect of this polymorphism to therapeutic respons of metformin in Balinese population.

Keywords: OCT1 polymorphism; rs628031; rs623442; rs122083571; type 2 diabetes mellitus.

Metformin (1.1-dimethyl-biguanide), first line drug for type 2 diabetes mellitus, is used worldwide for over than 60 years.[1,2] This drug has good efficacy, well tolerated, and relative low in price.[3] Some studies also indicated that metformin has anti-inflammatory and anti-oxidative effect, antitumor effect, antiaging effect, cardiovascular protective effects, neuroprotective effects and an optional treatment for PCOS.[4-12] Although it has almost the ideal profile of the drug, its exact mechanism of action have not yet been fully understood.[1,13]

Metformin has a hydrophilic base therefore it needs transporter to facilitate its transport across

the membrane.[14] Organic cation transporter (OCT) has a predominant role in metformin transport. It is responsible for metformin transport across the enterocyte basolateral membrane, uptake of metformin into hepatocyte, and metformin excretion in the kidneys. Because of its inability to passively diffuse into intracellular space, therapeutic effectiveness of metformin may depend on the expression and various genetic forms of the membrane transporter OCT.[13]

OCT1 is expressed on the basolateral membrane and cytoplasm of the enterocytes. It may facilitate the transfer of metformin into the interstitial fluid. It is also expressed on the basolateral membrane of hepatocytes thus promote the hepatic uptake of metformin.[15,16] In human, OCT1 encoded by solute carrier 22A1 (SLC22A1) gene. This gene spans about 37 kb and consist of 13 exons that has been mapped to chromosome 6q25.3. Human OCT1 gene is highly polymorphic, and numerous polymorphisms have been described in various populations leading to differences in transporter function.[17]

A number of SLC22A1 polymorphisms have been associated with functional changes in protein activity, as well as drug disposition, response, and toxicity.[18] Genetic effect of OCT1 polymorphisms on metformin responses is ethnic specific. Many studies have identified genetic polymorphisms in the SLC22A1 gene among different populations groups but there are still contradictory reports on the effects of OCT1 polymorphisms on metformin-related therapeutic responses.[19-21]

The polymorphism rs628031 (1222A>G) was the most genotyped and its frequency has been found to range from 15% to 80%. Japanese population has the highest frequency (80%), while rs628031 is present only in 40% in Caucasians.[19] A study investigating rs628031 in 277 Han Chinese participants found a significant reduction in HbA1c levels ($p<0.02$) in individuals carrying the AA genotype compared to those with the heterozygous genotype (AG).[22] By contrast, investigation of rs628031 in Iranian and Japanese populations showed that there were no association between this polymorphism and metformin responses.[23-24]

On the other hand, one of common loss-of-function variants in the OCT1 gene (SLC22A1) R61C (rs122083571), contributes to decrease in OCT1-mediated uptake by more than 70% for all substrates tested, including metformin.[25,26] The 181C>T polymorphism at rs122083571 consisting of an amino acid substitution from arginine to cysteine at position 61 (Arg61Cys). Numerous studies reported the deleterious effect of rs122083571 polymorphism.[27-29]

Indonesia, an island nation, hosts a sizeable proportion of global human diversity including genetic diversity. Limited study has been published related to OCT1 polymorphisms among different ethnic population in Indonesia. The most recent study conducted by Ningrum et al (2017) reported the allele frequency of OCT1 polymorphism rs628031 is 60.47% in Javanese population.[30] There is no data about this polymorphism in Balinese population. This study aims to investigate the OCT1 polymorphism rs628031, rs623442, and rs12208357 in Balinese population to set ethnicity-specific reference for OCT1 polymorphisms.

MATERIAL AND METHODS

Clinical Settings and Protocol

This study involved 133 T2DM patients that were predominantly Balinese ethnicity. Patients were recruited from Departement of Internal Medicine at Sanjiwani Hospital Gianyar and Tabanan Hospital, Bali. The study protocol was approved by ethical committees of Udayana University/Sanglah Hospital Denpasar. Written informed consent was obtained from all subject. Each subject was evaluated for their demographic characteristics, medical history, physical examination and standard laboratory tests for T2DM.

Genotyping of OCT1 polymorphism rs628031

A 2 mL of blood sample was collected in EDTA-containing tube and stored at 40^0C for genotyping. Purelink Genomic DNA Mini Kit (Promega) was used to extracted the genomic DNA from the archived sample. Genotyping for the OCT1 polymorphism rs628031 was performed using a previously published and validated polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.[24] PCR amplification was carried out using forward primer F5'-CTAAACCCAGTGATTCATGCTCTTT-3' and reverse primer R5'-TTTGTTCTCATTC

CAGAGGCTTATC-3'. The PCR was carried out for each sample in a final volume 25µL. The thermal stages with their temperatures are given in Table 1.

Amplification products from each sample (422bp) were cleaved by MscI restriction enzyme (Thermofisher Scientific) after overnight incubation at 37^0C and resulted in 154 and 268-bp fragments which were subjected to electrophoresis on 1.5% agarose gel.

Genotyping of OCT1 polymorphism rs623442 and rs12208357

For studying genetic variations of polymorphism rs6223442 and rs12208357 (R61C), direct sequencing was carried out. PCR was performed to amplify a 216 bp region of polymorphism rs6223442 and a 210 bp region of polymorphism rs12208357. PCR amplification for OCT1 polymorphism rs12208357 was carried out using forward primer F5'-AGCCTTCCTCATCTTATG- 3' and reverse primer R5'-CCAGTCCACTTCATAGCA -3', while forward primer F5'-CAG AGA GAA TCA GTG AGC TGT G-3' and reverse primer R5'-CCC AGG CTG GTC TTT TTA AG-3' were used to amplify OCT1 polymorphism rs622342.[31]

The presence of band as a marker for successful PCR reaction was visualized in 2% Agarose gel. Samples were then purified using the Wizard ® Genomic DNA Purification Kit (Promega, USA) for direct sequencing analysis. Samples were sent to the Macrogen Laboratories for sequencing analysis services.

Table 1. PCR condition for amplification OCT1 allele

Stages	Temperature	Time
Initial denaturation	95^0C	5'
Denaturation	95^0C	30"
Annealing	64^0C	35"
Extension	72^0C	1'
Final extension	72^0C	5'

Data Analysis

Patients demographics were summarized using descriptive statistics. Percentages were used to describe categorical data, whereas continuous data was reported as means ± standard deviations. The data were compiled according to the genotype and allele frequencies which were compared using a ÷2 test. The Hardy-Weinberg equilibrium was determined by comparing the genotype frequencies with the expected value using a contingency table ÷2 test.

RESULTS AND DISCUSSION

Subjects Characteristics

A total of 133 samples (78 males, 55 females) gave successful results for SLC22A1 rs628031 polymorphism analysis, while only 93 samples (from 96 samples) and 95 samples (from 96 samples) gave successful results for SLC22A1 rs622342 polymorphism and SLC22A1 rs12208357 polymorphism direct sequencing analysis, respectively. The allele and genotype frequency distribution along with expected Hardy-Weinberg distribution for SLC22A1 rs628031 polymorphism and SLC22A1 rs622342 polymorphism. Clinical characteristics of patients including age, height, body weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, and fasting blood glucose levels are summarized in Table 2.

Allele and Genotype Frequency Distributions

The frequency of A and G alleles in this study were 41% and 51%, respectively. AA

Table 2. Subject characteristics

Variabel	Male (n=78)	Female (55)	*p*
Age (years)	60.68 ± 9.61	58.53 ± 8.63	0.19
Body weight (kg)	65 (50-92)	62 (39-80)	0.11
Height (cm)	165 (150-180)	157 (130-170)	0.00
Body mass index (kg/m^2)	23.38 (17.93-35.94)	25.14 (17.11-36.23)	0.17
Systolic blood pressure (mmHg)	130 (100-199)	130 (92-220)	0.64
Diastolic blood pressure (mmHg)	80 (52-104)	80 (60-100)	0.88
Fasting blood glucose (mg/dL)	146 (80-396)	141 (85-245)	0.92

genotype (wild type) was accounted for 16.5% of total subjects, while AG (heterozygous mutant) and GG genotypes (mutant homozygotes) were accounted for 48.9% and 34.6%, respectively. Allele and genotype frequency for OCT1 polymorphism rs628031 in this study sumarized in Table 3.

OCT1 polymorphism rs628031, occurs in the form of single nucleotide variations (1222A>G), causes a missense mutation in exon 7. This mutation consists of an amino acid substitution of methionine to valine at position 408 (Met408Val) in the OCT1 protein.[32]

Its frequency has been found to range from 30% to 80%. Asian population has higher frequency than other population (Table 4). The highest frequency has been found in Japanese population (72-85%), followed by Korean and Han Chinese population (74% and 72%, respectively). It is only present around 30% in Iranian and about 40% in Caucasian population. In Indonesia, this polymorphism was found in frequency 59% in Balinese (recent study) and 60% in Javanese population.[30]

Met408Val tends to lower OCT1 mRNA expression in enterocytes leading to decreased intestinal metformin uptake and hence its accumulation. The minor allele of rs628031 (Met408Val), which is in strong linkage disequilibrium with rs36056065 (8 bp

Table 3. Allele and genotype distbution of OCT1 polymorphism rs628031

Gene	SNP	Allele	n	%	MAF	Genotype	n	%	P-value HWE*
SLC22A1	rs628031	A	109	40.98	0.41	AA	22	16.5	0.91
		G	157	59.02	0.59	AG	65	48.9	
						GG	46	34.6	
	rs12208357	C	170	100	1.00	CC	95	100	NA
	(R61C)	T	0	0	0.00	CT	0	0	
						TT	0	0	
	rs622342	C	37	19.89	0.20	CC	5	5.4	0.39
		A	149	80.11	0.80	CA	27	29.0	
						AA	61	65.6	

*HWE: Hardy-Weinberg equilibrium

Table 4. Minor allele frequency (MAF) of OCT1 polymorphism rs628031 in various populations

OCT1 polymorphism	Population	Total sample	MAF	Reference
rs 628031	Balinese (Indonesian)	133	0.59	Present study
	Javanese (Indonesian)	86	0.60	Ningrum et al[30]
	Japanese	66	0.85	Chen et al[31]
	Japanese	24/9	0.81/0.72	Shikata et al[23]
	Caucasian	120	0.40	Becker et al[32]
	Iranian	140 (77/63)	0.32/0.33	Shokri et al[24]
	Han Chinese	137	0.72	Zhou et al[22]
	Korean	150	0.74	Kang et al[33]
rs 12208357	Balinese (Indonesian)	95	0	Present study
	Xhosa population	148	0	Jacobs et al[34]
rs 622342	Balinese (Indonesian)	93	0.20	Present study
	Xhosa population	148	0.22	Jacobs et al[34]
	Egyptian	127	0.19	Ebid et al[35]
	Jordanian	212	0.23	Al-Eitan et al[36]

insertion), was significantly associated with the presence of gastrointestinal side effects. The local increase of drug concentration in the intestinal tissue is proposed as a mechanism of metformin intolerance.[37]

Association of rs628031 with the glycemic response to metformin was assessed in various studies. A study investigating rs628031 in 137 participant found that rs628031 GG genotype exhibited greater reductions in fasting plasma glucose (FPG), and those with rs628031 AA genotype exhibited greater reductions in HbA1c, compared to those with different genotypes of these SNPs.[22] While, investigation of rs628031 in Caucasian and Japanese populations showed that there were no association between this polymorphism and metformin responses.[23,31,32]

A systematic review conducted by Mato et al (2018) reported that the potential role of OCT1 polymorphisms in metformin therapeutic responses is population specific.[19] Some of them exhibited positive effects on metformin efficacy, while the others reported no effects. The controversial findings related to these polymorphisms may be attributable to differences in the frequency of associated genetic variants and/or population differences that could be genetic or environmental.

CONCLUSSION

Genetic polymorphism of OCT1 rs628031 in Balinese population was occurred in relatively high frequency, while polymorphism OCT1 rs623442 was occurred only in one fifth of studied population. On the other hand, polymorphism OCT1 rs12208357 did not found in this population. Further studies on defined populations with relatively large sample sizes must be done to address the effect of this polymorphism to therapeutic respons of metformin in Balinese population.

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Conflict of Interest

We have no conflicts of interest to disclose.

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References

REFERENCES

1. Marshall SMJD. 60 years of metformin use: a glance at the past and a look to the future. *Diabetologia*.; **60**(9):1561-5 (2017).
2. Bailey CJ. Metformin: historical overview. *Diabetologia.;* **60**(9):1566-76 (2017).
3. Sanchez-Rangel E, Inzucchi SEJD. Metformin: clinical use in type 2 diabetes. *Diabetologia.*; **60**(9):1586-93 (2017).
4. Dehkordi AH, Abbaszadeh A, Mir S, Hasanvand A. Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. *J Renal Inj Prev*.; **8**(1):54-61 (2019).
5. Zaidi S, Gandhi J, Joshi G, Smith NL, Khan SA. The anticancer potential of metformin on prostate cancer. *Prostate Cancer Prostatic Dis*.; **22**(3): 351-61 (2019).
6. Safe S, Nair V, Karki K. Metformin-induced anticancer activities: recent insights. *Biol Chem.;* **399**(4):321-35 (2018).
7. Wu P, Tang Y, Fang X, Xie C, Zeng J, Wang W, et al. Metformin Suppresses Hypopharyngeal Cancer Growth by Epigenetically Silencing Long Non-coding RNA SNHG7 in FaDu Cells. *Front. Pharmacol.,* **10**(143) (2019).
8. Valencia WM, Palacio A, Tamariz L, Florez H. Metformin and ageing: improving ageing outcomes beyond glycaemic control. *Diabetologia*.; **60**(9): 1630-8 (2017).
9. Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: A systematic review and meta-analysis. *Ageing Res Rev.*; **40**:31-44 (2017).
10. Loi H, Boal F, Tronchere H, Cinato M, Kramar S, Oleshchuk O, et al. Metformin Protects the Heart Against Hypertrophic and Apoptotic Remodeling After Myocardial Infarction. *Front. Pharmacol.;* **10**:154 (2019).
11. Keshavarzi S, Kermanshahi S, Karami L, Motaghinejad M, Motevalian M, Sadr S. Protective role of metformin against methamphetamine induced anxiety, depression, cognition impairment and neurodegeneration in rat: The role of CREB/BDNF and Akt/GSK3

signaling pathways. *Neurotoxicology.*; **72**: 74-84 (2019).
12. Sam S, Ehrmann DAJD. Metformin therapy for the reproductive and metabolic consequences of polycystic ovary syndrome. *Diabetologia.*; **60**(9): 1656-61 (2017).
13. Rena G, Hardie DG, Pearson ERJD. The mechanisms of action of metformin. *Diabetologia.;* **60**(9):1577-85 (2017).
14. Janic M, Volcansek S, Lunder M, Janez A. Metformin: from mechanisms of action to advanced clinical use. *Zdrav Vestn.*; **86**: 138–57 (2017).
15. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics.*; **22**(11): 820-7 (2012).
16. Kinaan M, Ding H, Triggle CR. Metformin: An Old Drug for the Treatment of Diabetes but a New Drug for the Protection of the Endothelium. *Med Princ Pract.*; **24**(5): 401-15 (2015).
17. NCBI. SLC22A1 solute carrier family 22 member 1 [Homo sapiens (human)]. Gene ID: 6580, updated on 21-Dec-2019. Available at: https://www.ncbi.nlm.nih.gov/gene/6580.
18. Goswami S, Gong L, Giacomini K, Altman RB, Klein TE. PharmGKB summary: very important pharmacogene information for SLC22A1. *Pharmacogenet Genomics*.; **24**(6):324-8 (2014).
19. Mato EPM, Guewo-Fokeng M, Faadiel Essop M, Owira PMO. Genetic polymorphisms of organic cation transporters 1 (OCT1) and responses to metformin therapy in individuals with type 2 diabetes mellitus: a systematic review protocol. *Syst Rev.*; **7**(1):105 (2018).
20. Chan P, Shao L, Tomlinson B, Zhang Y, Liu ZM. Metformin transporter pharmacogenomics: insights into drug disposition-where are we now? *Expert Opin Drug Metab Toxicol.;* **14**(11):1149-59 (2018).
21. Dujic T, Zhou K, Yee SW, van Leeuwen N, de Keyser CE, Javorsky M, et al. Variants in Pharmacokinetic Transporters and Glycemic Response to Metformin: A Metgen Meta-Analysis. *Clin Pharmacol Ther.*; **101**(6):763-72 (2017).
22. Zhou Y, Ye W, Wang Y, Jiang Z, Meng X, Xiao Q, et al. Genetic variants of OCT1 influence glycemic response to metformin in Han Chinese patients with type-2 diabetes mellitus in Shanghai. *Int J Clin Exp Pathol.;* **8**(8):9533-42 (2015).
23. Shikata E, Yamamoto R, Takane H, Shigemasa C, Ikeda T, Otsubo K, et al. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *J Hum Genet.;* **52**(2):117-22 (2007).
24. Shokri F, Ghaedi H, Ghafouri Fard S, Movafagh A, Abediankenari S, Mahrooz A, et al. Impact of ATM and SLC22A1 Polymorphisms on Therapeutic Response to Metformin in Iranian Diabetic Patients. *Int J Mol Cell Med.;* **5**(1):1-7 (2016).
25. Seitz T, Stalmann R, Dalila N, Chen J, Pojar S, Dos Santos Pereira JN, et al. Global genetic analyses reveal strong inter-ethnic variability in the loss of activity of the organic cation transporter OCT1. *Genome Med;* **7**(1):56 (2015).
26. Shu Y, Leabman MK, Feng B, Mangravite LM, Huang CC, Stryke D, et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc Natl Acad Sci U S A.;* **100**(10):5902-7 (2003).
27. Dujic T, Causevic A, Bego T, Malenica M, Velija-Asimi Z, Pearson ER, et al. Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. *Diabet Med.;* **33**(4):511-4 (2016).
28. Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of Organic Cation Transporter 1 With Intolerance to Metformin in Type 2 Diabetes: A GoDARTS Study. *Diabetes.;* **64**(5):1786-93 (2015).
29. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther.;* **83**(2):273-80 (2008).
30. Ningrum VDA, Ikawati Z, Sadewa AH, Ikhsan MR. Allele Frequencies of Two Main Metformin Transporter Genes: SLC22A1 rs628031 A>G and SLC47A1 rs2289669 G>A among the Javanese Population in Indonesia. *Current Pharmacogenomics and Personalized Medicine.*; **15**(2):121-8 (2017).
31. Chen L, Takizawa M, Chen E, Schlessinger A, Segenthelar J, Choi JH, Sali A, Kubo M, Nakamura S, Iwamoto Y, Iwasaki N, and Giacomini KM. Genetic Polymorphisms in Organic Cation Transporter 1 (OCT1) in Chinese and Japanese Populations Exhibit Altered Function. *JPET.* **335**: 42–50 (2010).
32. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. *Diabetes.;* **58**(3):745-9 (2009).
33. Kang HJ, Song IS, Shin HJ, Kim WY, Lee CH, Shim JC, Zhou HH, Lee SS, Shin JG.

Identification and functional characterization of genetic variants of human organic cation transporters in a Korean population. *Drug Metab Dispos.;* **35**(4):667-75 (2007).

34. Jacobs C, Pearce B, du Plessis M, Hoosain N, Benjeddou M. Genetic polymorphisms and haplotypes of the organic cation transporter 1 gene (SLC22A1) in the Xhosa population of South Africa. *Genet. Mol. Biol.;* **37**(2):350-9 (2014).
35. Ebid AH, Ehab M, Ismail A, Soror S, Mahmoud MA. The influence of SLC22A1 rs622342 and ABCC8 rs757110 genetic variants on the efficacy of metformin and glimepiride combination therapy in Egyptian patients with type 2 diabetes. *Journal of Drug Assessment.;* **8**(1):115–21 (2019).
36. Al-Eitan LN, Almomani 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.*; **9**(17):1-11 (2019).
37. Semiz S, Dujic T, Causevic A. Pharmacogenetics and personalized treatment of type 2 diabetes. *Biochem Med.*; **23**(2):154–71 (2013).