Allele frequency and genotype distribution of a common variant in the 3´-untranslated region of the SLC22A3 gene in patients with type 2 diabetes: Association with response to metformin

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Background: Organic cation transporter 3 (OCT3) is an excellent transporter for metformin, which is used as first-line therapy for type 2 diabetes (T2D). OCT3 genetic variants may influence the clinical response to metformin. This study aimed to determine the genotype and allele frequency of OCT3-564G>A (rs3088442) variant and its role in the glycemic response to metformin in patients with newly diagnosed T2D. **Materials and Methods:** Based on the response to metformin, 150 patients were classified into two groups: Sixty-nine responders (decrease in glycated hemoglobin [HbA1c] values by more than 1% from the baseline) and 81 nonresponders (decrease in HbA1c values <1% from the baseline). HbA1c levels were determined by chromatography. The variant OCT3-564G>A was genotyped using polymerase chain reaction - based restriction fragment length polymorphism. **Results:** The genotypes frequencies were 51.3% GG, 36% AG, and 12.7% AA. Allele frequency of major allele (G) and minor allele (A) in OCT3-564G>A variant was found to be 0.69 and 0.31, respectively. Fasting glucose, HbA1c, body mass index, and lipid profile in both GG genotypes and GA + AA group decreased significantly after 3 months of metformin therapy compared with baseline ($P < 0.05$). In both responders and nonresponders, HbA1c and fasting glucose levels were lower in patients with the GA + AA genotype than in those with the GG genotype; however, the differences were not statistically significant ($P > 0.05$). **Conclusion:** The A allele frequency (which may be a protective allele against coronary heart disease) in the Iranian diabetic patients was lower than Iranian, Caucasian and Japanese healthy populations. Metformin is useful in improving the lipid profile, in addition to its impacts in glycemic control, and these effects are regardless of OCT3-564G>A variant.

Key words: Genetic variation, glycated hemoglobin, metformin, organic cation transporter 3, type 2 diabetes

INTRODUCTION

Diabetes is a major threat to public health worldwide, and its prevalence has rapidly increased and even has influenced the younger population.[1-3] The World Health Organization estimates that about 366 million people worldwide will develop diabetes by 2030, the majority of who develop type 2 diabetes (T2D).[4] Lifestyle negative changes such as obesity, weight gain, reduced physical activity, high-calorie diet and smoking contribute to the increased prevalence of the disease.[5,6]

Metformin is the most widely used antidiabetic medication among different medications administered to treat the disease, and it is considered as the first-line oral treatment for T2D.[7,8] In general, the mechanisms

of metformin action include reduction of hepatic glucose production, increased glucose uptake in skeletal tissue, suppression of the intestinal absorption of glucose, and improvement of insulin sensitivity.[9,10] Metformin reduces triglyceride (TG) levels, free fatty acids, and low-density lipoprotein (LDL) in plasma and increases high-density lipoprotein (HDL).[11] Due to slight hydrophobic property, metformin needs a transporter to cross plasma membrane into the cell.[12] A family of transporters called organic cation transporters (OCTs) is among transporters with an important role in bioavailability, clearance, and pharmacodynamics of metformin.[12] OCTs have three members: OCT1-3. OCT3 is coded by SLC22A3 gene located on chromosome 6q26-q27.[13] OCT3 has a high-capacity for binding to the substrate and an extensive tissue distribution in the body, particularly in muscles and adipose tissue.[14,15] This transporter contributes to the transfer of endogenous physiological amino compounds such as catecholamines. [16] Like two other members of OCT family, OCT3 is an excellent transporter for metformin.[12] Various factors such as nature of the disease and organ function affect drug response, but estimates show that genetics can dictate 20–95% of variability in different responses to the same medication.[17] Hence, OCT3 genetic variants may influence the clinical response to metformin. OCT3-564G>A is a noncoding variant in 3′-untranslated region (3′-UTR) of OCT3.[16,18] The displacement G >A creates a binding site for microRNA called miR-147. The presence of miR-147 leads to negative expression of OCT3 gene. In other words, this variant contributes to the gene expression of OCT3 and affects the final production of mRNA.[19]

So far, few studies have been conducted on the relationship between OCT3 and metformin response. Therefore, similar studies on various variants of OCT3 should be conducted in different ethnic populations. Hence, in this study, we evaluated whether the variant OCT3-564G>A influences metformin response and also the distribution of genotypes of this variant in Iranian patients with T2D.

MATERIALS AND METHODS

Study subjects
This observational study conducted on 150 newly diagnosed patients with T2D (according to the WHO criteria) treated with metformin from March 2014 to August 2015. The study was performed in Northern province of Iran, Mazandaran. The patients were followed for 3 months and during this period, they received 1000 mg of metformin (Aria pharmaceutical company, Iran) twice a day. None of the patients were receiving antidiabetic medication prior to their diabetes diagnosis. Information about medical history, personal habits, demographic parameters, and medication use was obtained through a questionnaire. Patients were divided into two groups based on the response to metformin:[10] (1) Responder group with a reduction in HbA1c values by more than 1% compared to before taking metformin and (2) nonresponder group with a reduction in HbA1c values <1% compared to before taking metformin. Subjects with type 1 diabetes, and previous history of renal failure, chronic diseases, autoimmune, and liver diseases was not included in the study. The study protocol was approved by the Ethical Committee at Mazandaran University of Medical Sciences (ethnic number: 93-1303) and informed consent was obtained from all participants.

Sampling and laboratory assays
Blood samples were taken from subjects after an overnight fast (for 10-12 h). Sera were isolated by low-speed centrifugation, and the serum aliquots were stored at −70°C until use. The HbA1c levels were determined by boronate affinity technique (Axis-Shield PoC AS, Oslo, Norway; accuracy, failure <5%). HDL-cholesterol (HDL-C), total cholesterol (TC), triglycerids (TGs), fasting blood glucose (FBG), alanine aminotransferase (ALT) levels were measured using an auto-analyzer (Prestige, Japan). Levels of LDL-cholesterol (LDL-C) were determined by the Friedewald formula.[20] All of the tests were performed before and after 3 months of metformin therapy.

Genotyping of OCT3-564G>A variant
The OCT3-564G>A variant was genotyped using the restricted fragment length polymorphism analysis after polymerase chain reaction amplification. The designed primers used for this variant were: F 5′-AGATTGCATGGAGGATGAAC-3′ and R 5′-TGTTACAGAGGAGGTGGACG-3′. DNA was amplified with an initial melting temperature of 93°C for 3 min, followed by 35 cycles of 50 s at 93°C, 40 s at 55°C, and 40 s at 72°C, with a final extension step of 5 min at 72°C. Amplification products from each sample (171 bp) were digested with the restriction enzyme AciI (thermoscientific) at 37°C for 16 h, and resulted in 67 and 104 bp fragments, which were subjected to electrophoresis on a 2.5% agarose gel. Wild-type (GG) patients were identified by the presence of 67 and 104 bp fragments. Heterozygous (GA) patients were identified by the presence of 171, 104, and 67 bp fragments, while the presence of an undigested fragment (171 bp) was the basis for the identification of mutants (AA).

Statistics
The distribution normality of the variables was checked by the Kolmogorov–Smirnov test. To analyze the differences between the parametric variables, we used independent *t*-test or paired *t*-test. Mann–Whitney U-test used to analyze the nonparametric variables. The Chi-square test was used

for checking Hardy–Weinberg equilibrium. Deviation from the equilibrium may be indicative of problematic assays. A $P < 0.05$ was accepted as statistically significant. Statistical analyses were performed by SPSS (version 16.0) software (SPSS Inc, Chicago, IL).

RESULTS

Table 1 indicates the changes in study parameters after 3 months of metformin therapy. As can be seen in the table, most of these parameters including systolic and diastolic blood pressure ($P < 0.001$), body mass index (BMI) ($P < 0.001$), fasting glucose ($P < 0.001$), and HbA1c ($P < 0.001$) were significantly reduced after 3 months of treatment. Compared with baseline levels before treatment, TG ($P < 0.001$), TC ($P = 0.001$), and LDL-C ($P < 0.001$) were improved following 3 months of treatment. HDL-C levels were increased ($P = 0.101$) after 3 months of treatment compared with baseline levels.

OCT3-564G>A genotypes were in Hardy–Weinberg equilibrium in the study patients. The frequency of GG, AG, and AA genotypes were 51.3%, 36%, and 12.7%, respectively. The allele frequency of G and A was found to be 0.69 and 0.31, respectively.

As shown in Table 2, the majority of the studied parameters such as HbA1c, fasting glucose, BMI, and lipid profile in both GG genotypes and GA + AA group decreased significantly after 3 months of metformin therapy compared with baseline. Although there was an increase in HDL-C levels in both patients with the GA + AA genotype and GG genotype after 3 months of treatment with metformin, statistically significant differences were not found.

In the current study, subjects were divided into two groups: Metformin responders (n = 69) and metformin nonresponders (n = 81). There were no statistically significant differences between responders and nonresponders with respect to the study parameters, except HbA1c levels before treatment (results not shown). When we analyzed the study parameters with respect to metformin response according to OCT3-564G>A genotypes, our findings showed no statistically significant differences between responders and nonresponders with respect to the majority of the parameters of this study [Tables 3 and 4]. In both nonresponders [Table 3] and responders [Table 4], fasting glucose levels were lower in patients with the GA + AA genotype than in those with the GG genotype; however, the differences were not statistically significant. Furthermore, our results showed that in GA + AA group compared with GG group, reduction in average HbA1c values was higher in responders than in nonresponders [Figure 1], although were not found the statistically significant differences among the groups.

Table 1: Change in the study variables from baseline to 3 months of metformin treatment (*n*=150)

Parameter	Baseline	After 3 months	*P*
Age (years)	52.7±10.7	-	-
SBP (mmHg)	130.37±15.52	125.38±16.66	<0.001
DBP (mmHg)	80.3±9.7	76.4±9.59	<0.001
BMI (kg/m²)	31.18±5.2	30.6±5.23	<0.001
FBG (mmol/L)	7.87±1.5	7.16±1.83	<0.001
HbA1c (%)	7.65±0.81	7±1.15	<0.001
ALT (µkat/L)	0.42±0.17	0.41±0.17	0.042
TG (mmol/L)	2.11±0.9	1.85±0.69	<0.001
TC (mmol/L)	4.9±1.05	4.54±0.85	0.001
HDL-C (mmol/L)	1.21±0.39	1.26±0.37	0.101
LDL-C (mmol/L)	2.7±0.89	2.34±0.7	<0.001
LDL-C/HDL-C	2.46±1.09	2±0.77	<0.001

Data are means±SD. SBP = Systolic blood pressure; DBP = Diastolic blood pressure; BMI = Body mass index; FBG = Fasting blood glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; TC = Total cholesterol; HDL-C = High-density lipoprotein-cholesterol; LDL-C = Low-density lipoprotein-cholesterol; SD = Standard deviation

Table 2: Change in the study parameters from baseline to 3 months of metformin therapy according to the genotypes of organic cation transporter 3-564G>A

Parameter	GG (*n*=77)			GA + AA (*n*=73)		
	Baseline	After 3 months	*P*	Baseline	After 3 months	*P*
SBP (mmHg)	130.3±14.9	124.73±12.3	<0.001	131.3±16.3	124.1±20.7	<0.001
DBP (mmHg)	80.5±9.07	76.4±9.9	<0.001	80.4±10.5	77.07±9.8	0.001
BMI (kg/m²)	30.5±5.7	29.9±5.7	<0.001	31.4±4.6	30.8±4.7	<0.001
FBG (mmol/L)	7.85±1.6	7.37±2.09	0.005	7.93±1.44	7.05±1.61	<0.001
HbA1c (%)	7.7±0.8	7.09±1.23	<0.001	7.6±0.81	6.9±1.10	<0.001
ALT (µkat/L)	0.43±0.16	0.43±0.2	0.296	0.43±0.19	0.39±0.15	0.047
TG (mmol/L)	2.11±0.91	1.8±0.66	0.001	2.16±0.94	1.87±0.72	0.008
TC (mmol/L)	4.91±1.18	4.36±0.77	0.001	4.92±0.92	4.72±0.9	0.056
HDL-C (mmol/L)	1.19±0.39	1.23±0.41	0.238	1.24±0.4	1.3±0.34	0.143
LDL-C (mmol/L)	2.73±0.96	2.26±0.58	0.002	2.7±0.84	2.45±0.84	0.036
LDL-C/HDL-C	2.6±1.2	1.9±0.69	0.001	2.37±0.98	2.03±0.91	0.004

Data are means±SD. SBP = Systolic blood pressure; DBP = Diastolic blood pressure; BMI = Body mass index; FBG = Fasting blood glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; TC = Total cholesterol; HDL-C = High-density lipoprotein-cholesterol; LDL-C = Low-density lipoprotein-cholesterol; SD = Standard deviation

Table 3: Change in the study variables after 3 months of metformin therapy in metformin nonresponders according to the genotypes of organic cation transporter 3-564G>A

Parameter	GG	GA + AA	P
SBP (mmHg)	128.81±11.83	125.42±13.69	0.229
DBP (mmHg)	77.02±11.27	77.95±8.66	0.762
BMI (kg/m^2)	30.11±5.47	30.3±4.65	0.552
FBG (mmol/L)	7.93±2.53	7.57±1.68	0.627
ALT (μkat/L)	0.47±0.23	0.4±0.17	0.157
TG (mmol/L)	1.9±0.69	1.92±0.78	0.836
TC (mmol/L)	4.3±0.74	4.9±0.98	0.013
HDL-C (mmol/L)	1.18±0.37	1.3±0.32	0.009
LDL-C (mmol/L)	2.21±0.49	2.58±0.92	0.102
LDL-C/HDL-C	2±0.62	2.1±0.93	0.749

Data are means±SD. SBP = Systolic blood pressure; DBP = Diastolic blood pressure; BMI = Body mass index; FBG = Fasting blood glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; TC = Total cholesterol; HDL-C = High-density lipoprotein-cholesterol; LDL-C = Low-density lipoprotein-cholesterol; SD = Standard deviation
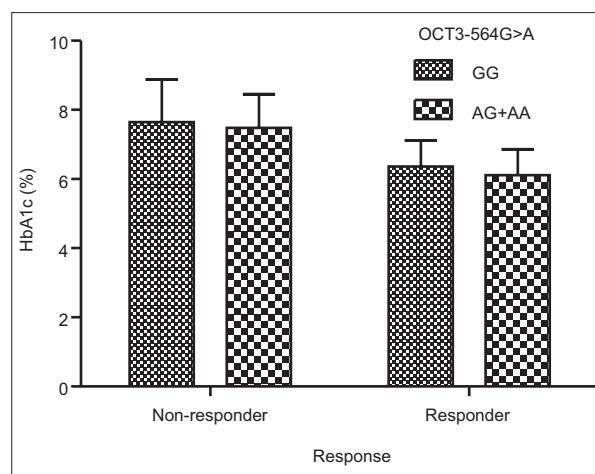
Table 4: Change in the study variables after 3 months of metformin therapy in metformin responders according to the genotypes of organic cation transporter 3-564G>A

Parameter	GG	GA + AA	P
SBP (mmHg)	119.38±10.91	121.81±26.8	0.328
DBP (mmHg)	75.47±7.76	75.4±10.73	0.922
BMI (kg/m^2)	29.64±6.01	31.32±4.7	0.149
FBG (mmol/L)	6.63±0.97	6.42±1.31	0.5
ALT (μkat/L)	0.37±0.13	0.37±0.12	0.567
TG (mmol/L)	1.68±0.62	1.81±0.67	0.493
TC (mmol/L)	4.45±0.83	4.47±0.74	0.697
HDL-C (mmol/L)	1.3±0.46	1.31±0.38	0.685
LDL-C (mmol/L)	2.33±0.69	2.26±0.71	0.721
LDL-C/HDL-C	1.97±0.78	1.91±0.88	0.64

Data are means±SD. SBP = Systolic blood pressure; DBP = Diastolic blood pressure; BMI = Body mass index; FBG = Fasting blood glucose; HbA1c = Glycated hemoglobin; TG = Triglyceride; TC = Total cholesterol; HDL-C = High-density lipoprotein-cholesterol; LDL-C = Low-density lipoprotein-cholesterol; SD = Standard deviation

Figure 1: Change in glycated hemoglobin levels after 3 months of metformin therapy in metformin responders and nonresponders based on the genotypes of OCT3-564G>A

DISCUSSION

To the best of our knowledge, there are few studies on the allele frequency of OCT3-564G>A in different populations. In our study, the major allele frequency of G and minor allele frequency of A were 0.69 and 0.31, respectively, in patients with T2D. Hengen *et al.* reported that G and A allele frequencies were 0.53 and 0.47, respectively, in healthy Caucasians in the United States.[21] Moreover, in the study of Aoyama *et al.*,[22] G and A allele frequencies were 0.51 and 0.49, respectively, in a healthy Japanese population. It should be noted that according to another study we are doing, the frequency of A allele in a healthy Iranian population (*n* = 152) was 0.38. It seems that minor allele frequency of A in Iranian patients with T2D and also in healthy Iranian population is lower than that of healthy Caucasian and Japanese populations. According to an investigation recently published by Li *et al.*,[19] AA genotype of OCT3-564G>A variant compared to GG

genotype significantly reduces the risk of coronary heart disease (CHD) in a Chinese population. In other words, they reported that this variant can contribute to reducing the risk of CHD through its supporting role against inflammatory responses. Thus, lower frequency of A allele in Iranian patients with T2D may contribute to the risk of CHD in these patients. It should be noted that mortality in diabetic patients is more due to cardiovascular complications.[23]

Interindividual variations are involved in drug disposition and response.[24] For example, a sufficient response is not observed in certain metformin users that is one of the most important predicaments about efficacy of the important antidiabetic drug.[25,26] One reason for this poor response is genetic variability in metformin transporters.[18] OCT3 such as OCT1 and OCT2 is an important transporter for metformin.[12] There are few studies on the effect of genetic variants of OCT3 on the pharmacokinetics of metformin. Chen *et al.* showed that some different variants in OCT3 such as rs68187715 and rs8187717 affect metformin uptake *in vitro*.[27] However, Tzvetkov *et al.*[28] investigated the effect of some variants of OCT3 such as rs3123634 and rs2292334 on pharmacokinetics in healthy volunteers, but found no statistically significant relationship between these variants and clearance of metformin.

OCT3-564G>A is one of the variants in 3′-UTR region of OCT3 which affect the expression of this transporter.[19] Li *et al.* showed that G >A displacement in the variant leads to binding miR-147 to mRNA resulting in the decreased expression of OCT3.[19] Moreover, Nies *et al.*[18] indicated that three variants in the noncoding region including OCT3-564G>A are associated with decreased expression of mRNA in OCT3 in the liver cells. In the present study, given the importance of OCT3-564G>A in OCT3 expression, we investigated whether this variant can affect the response

to metformin in patients with T2D. As expected, the results showed that fasting glucose and HbA1c levels were significantly decreased after 3 months of treatment with metformin. When we analyzed these factors according to OCT3-564G>A genotypes, it was found that fasting glucose and HbA1c levels were significantly decreased in both GG and GA + AA groups after 3 months of metformin therapy. In addition, our study are consistent with results obtained by other authors[29,30] showed that metformin is effective in improving the lipid profile. The results analysis according to the genotypes of OCT3-564G>A showed that the lipid profile is improved after 3 months of treatment with metformin in both patients with GG and GA + AA genotypes.

Further analyses according to responders and nonresponders to metformin revealed that fasting glucose and HbA1c do not change significantly in responders and nonresponders with respect to the genotypes of study variant. In other words, it seems that OCT3-564G>A is not effective in the glycemic response to metformin. However, it should be noted that metformin can be transported by two other members of the OCT family, i.e., OCT1 and OCT2. This issue is more important in the major organ of metformin activity, i.e., the liver in which OCT1 is the main transporter. Hence, for a more comprehensive review of the role of OCT3 variants in response to metformin, these variants should be studied with functional variants of two other members particularly OCT1.

The relatively small sample size should be considered as a limitation of the study, and thus further research in different populations with a larger sample size is needed to clarify the role of the OCT3-564G>A variant in metformin response.

In general, considering the different consequences of the variations in drug transporters, the effect of OCT3 variations as a high-capacity transporter widely expressed in various tissues cannot be ignored.

CONCLUSION

The frequency of A allele (which may be a protective allele against CHD) in the Iranian diabetic patients was lower compared with Caucasian, Japanese, and Iranian healthy populations. Metformin is useful in improving the lipid profile, in addition to its effects in glycemic control, and these impacts are regardless of the variant OCT3-564G>A.

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Conflicts of interest
There are no conflicts of interest.

AUTHORS' CONTRIBUTION

AM contributed in the conception of the work, conducting the study, writing the article, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work MG contributed in the conception of the work, study design, samples and data collection, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work MBH contributed in the conception of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work SRH contributed in the conception of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work AA contributed in the conception of the work, data analysis, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work SMN contributed in the conception of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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