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Association between the synonymous variant organic cation transporter 3 (OCT3)-1233G>A and the glycemic response following metformin therapy in patients with type 2 diabetes

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ABSTRACT

Objective(s): Organic cation transporter 3 (OCT3) as a high-capacity transporter contribute to the metabolism of metformin. The present study was conducted to determine the genotype frequencies of the variant OCT3-1233G>A (rs2292334) in patients with newly diagnosed type 2 diabetes (T2D) and its relationship with response to metformin.

Materials and Methods: This study included 150 patients with T2D who were classified into two groups following three months of metformin therapy: responders (by more than 1% reduction in HbA1c from baseline) and nonresponders (less than 1% reduction in HbA1c from baseline). PCR-based restriction fragment length polymorphism (RFLP) served to genotype OCT3-564G>A variant.

Results: The parameters such as HbA1c (P<0.001) and BMI (P<0.001) in both patients with GA + AA genotype and GG genotype decreased significantly following 3 months of metformin therapy compared with baseline. The mean reduction in HbA1c levels following 3 months was higher in patients with the A allele (0.77% reduction from baseline) than in those with the homozygous G allele (0.54% reduction from baseline). Also, in GA + AA genotypes compared with GG genotypes, the mean reduction in HbA1c values from baseline was 0.34% for responders and 0.14% for non-responders.

Conclusion: Considering the roles of genetic variations in the function of metformin transporters, the effect of variations such as 1233G>A in the OCT3, which is a high-capacity transporter widely expressed in various tissues cannot be ignored. Comparing the allele frequencies of OCT3-1233G>A variant in our study and different ethnic populations confirm that the variant is a highly polymorphic variant.

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Introduction

As the most common type of diabetes mellitus, type 2 diabetes (T2D) is a complex pathophysiological disease that various environmental and genetic factors are contributed to its development (1, 2). According to the International Diabetes Federation, 371 million people have been diagnosed with diabetes as of 2012, and this figure is expected to reach 552 million by 2030, which is equates to 3 new person is diagnosed with diabetes per second (3). The highest rate of increase in the incidence of the disease is observed in the developing countries (3). Moreover, diabetes mellitus is responsible for retinopathy and blindness, nephropathy, neuropathy and the increased risk of cardiac diseases and stroke (which is twice to four times the risk in non-diabetic individuals) in diabetics (4).

Several drugs are administered for the treatment of T2D including metformin, which is used as first-line therapy for T2D (5, 6). Owing to the variability in response to metformin, about 35%–40% of patients receiving this biguanide derivative fail to the expected glucose-lowering response (7, 8). The mechanism of action of metformin involves the reduction of hepatic glucose output and intestinal glucose absorption, and improving insulin sensitivity (6, 9). Furthermore, it can increase insulin release in the response to glucose (10). Metformin is not metabolized by the cytochrome-P450 system, but is expelled by the kidneys instead (11).

Metformin is an organic hydrophilic cation that acts as a substrate for organic cation transporters (OCTs) including OCT3 (2, 12). OCT3 gene belongs to the SLC22A gene family and is coded by the SLC22A3

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gene (13). The transporter has an extensive tissue distribution in the body mostly in the liver, brain and skeletal muscles (13, 14). One of the most important problems about metformin efficacy is that a sufficient response is not achieved in certain metformin users (15). Previous studies have estimated that 20% to 95% of variability in different responses to the same medication can be due to interindividual variations (15). The importance of metformin transporters in metabolizing this major anti-diabetic drug and allowing its effectiveness, show the need for determining the genetic variants of these transporters in different ethnic populations. The OCT3-1233G>A (rs2292334) is a synonymous variant in which the displacement G>A converts an alanine codon into another codon of alanine (A411A) (16). This variant affects the OCT3 mRNA expression levels (17). Furthermore, it may use as a cryptic splice acceptor site (14). Although OCT3 is a major metformin transporter, only a few studies have been conducted on the effects of variants in OCT3 on metformin effectiveness (18). The present study was therefore conducted to determine the allele and genotype frequencies of the variant OCT3-1233G>A in newly diagnosed T2D patients and its relationship with HbA1c levels following metformin therapy. Moreover, the study analyzed the role of this variant in the clinical response to metformin in Iranian patients with T2D.

Materials and Methods

Study subjects

One hundred and fifty newly diagnosed Iranian patients with type 2 diabetes (according to the WHO criteria) participated in the study. This study was conducted in northern province of Iran, Mazandaran. Participants had a mean age of 52.7 years. The patients were followed for three months and during this period; metformin (1000 mg/day) was orally prescribed alone (without other oral antidiabetes drugs and/or insulin). None of the patients were taking antidiabetic drugs prior to their diabetes diagnosis. Information about medical history, demographic parameters and medication use was obtained using a questionnaire. Patients were classified into two groups according to response to metformin (6): (1) responder group (decrease in HbA1c values by more than 1% compared to before receiving metformin) and (2) non-responder group (decrease in HbA1c values less than 1% compared to before receiving metformin). The study exclusion criteria consisted of having type 1 diabetes, being under insulin therapy, having previous history of renal failure, having chronic and autoimmune diseases. This study was approved by the Ethical Committee at Mazandaran University of Medical Sciences. All participants submitted their written consent prior to participating in the study.

Laboratory assays

Blood samples were collected from all participants after an overnight fast. Sera were isolated by low-speed centrifugation and were stored at -70 °C. Serum concentrations of total cholesterol (TC), triglycerides (TGs), high density lipoprotein-cholesterol (HDL-C), fasting blood glucose (FBG) and alanine aminotransferase (ALT) were determined using standard enzymatic automated methods (Pars Azmoon Inc., Iran). Levels of low density lipoprotein-cholesterol (LDL-C) were calculated using Friedewald formula (19). The HbA1c levels were determined by Axis-Shield kit (Oslo, Norway; accuracy, failure <5%), which is based on affinity technique. All of the tests were made before and after 3 months of metformin treatment.

Genotyping

The OCT3-1233G>A genotypes was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The designed primers were: F 5′-GTGGTGGAACTGCCAGGA-3′ and R 5′-CTACAGAACCAATCTCTTACTTCG-3′. The PCR cycling conditions were as follows: 35 cycles of 50 sec at 93 °C, 40 sec at 52 °C, and 40 sec at 72 °C, with a final extension at 72 °C for 5 min. The 307 bp products were digested with the restriction enzyme *AciI* (Thermoscientific, Lithuania) at 37 °C overnight and resulted in 70 and 237 bp fragments. The digested products were separated on 2% agarose gel. The restriction digest reveals 70 and 237 bp fragments in the presence of GG genotype, the fragments of 70, 237 and 307 bp in the presence of AG genotype, and a non-digested 307 bp fragment in the presence of AA genotype.

Statistical analyses

The distribution normality of the variables was analyzed using the Kolmogorov–Smirnov test. The parametric variables were analyzed by independent t-test or paired t-test, and Mann–Whitney U test were applied to analyze the nonparametric variables. Deviation from the Hardy-Weinberg equilibrium was analyzed using the chi-square test. Two-tailed *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS (version 16.0) software.

Results

The changes in study parameters following 3 months of metformin therapy are shown in Table 1. Most of the parameters including HbA1c (P<0.001), fasting glucose (P<0.001), BMI (P<0.001), systolic blood pressure and diastolic blood pressure (P<0.001) were significantly reduced after 3 months of treatment compared with before treatment. Compared with baseline levels, LDL-C (P<0.001), TG

Table 1. Change in the study variables from before treatment to three months of metformin therapy (n=150)

Variable	Before treatment	After 3 months	*P* value
Diastolic blood pressure (mmHg)	80.3±9.7	76.4±9.59	<0.001
Systolic blood pressure (mmHg)	130.37±15.52	125.38±16.66	<0.001
BMI (kg/m²)	31.18±5.2	30.6±5.23	<0.001
HbA1c (%)	7.65±0.81	7±1.15	<0.001
Fasting glucose (mmol/l)	7.87±1.5	7.16±1.83	<0.001
LDL-C (mmol/l)	2.7±0.89	2.34±0.7	<0.001
HDL-C (mmol/l)	1.21±0.39	1.26±0.37	0.101
TC (mmol/l)	4.9±1.05	4.54±0.85	0.001
TG (mmol/l)	2.11±0.9	1.85±0.69	<0.001
LDL-C/HDL-C	2.46±1.09	2±0.77	<0.001

Data are means±SD

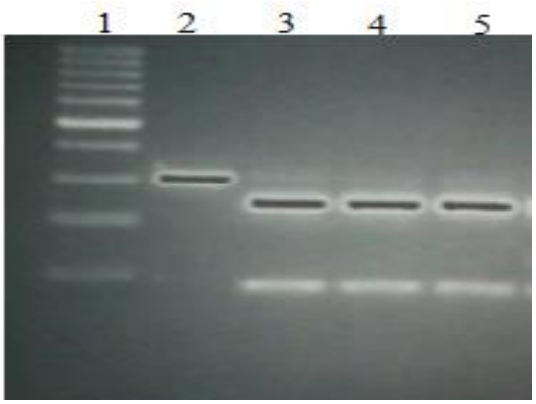

Figure 1. Restriction fragment length polymorphism (RFLP) of PCR-amplified products. The amplicons (307 bp) were subjected to restriction analysis with *AciI* resulting in 237 and 70 bp fragments (Lanes 3, 4 and 5). A non-digested 307 bp fragment is shown in lane 2. 100 bp DNA ladder is indicated in lane 1

(*P*<0.001), total cholesterol (*P*=0.001) and HDL-C (*P*=0.101) were improved after 3 months of treatment.

Amplification products (307 bp) from each sample were digested with the restriction enzyme *AciI*, which were subjected to electrophoresis on a 2% agarose gel (Figure 1).

There was no deviation from Hardy-Weinberg equilibrium for the study variant (*P*=0.544). The frequency of the GG, GA and AA genotypes was 41.4%, 48%, and 10.6%, respectively, in the diabetic patients. The G allele, thus, had a frequency of 0.65 and the A allele a frequency of 0.35. It should be noted that the designed PCR-RFLP as a simple, low-cost and applicable method could be useful to assess the genotypes of OCT3-1233G>A variant in other studies. The results of assessments following 3 months metformin therapy were compared to their baseline values and presented in GG and GA+AA groups (Table 2). As indicated in Table 2, a significant reduction was observed in the majority of the clinical parameters after the metformin therapy, including in fasting glucose, BMI, systolic and diastolic blood pressures, TG, total cholesterol and LDL-C in both the GG genotype group and the group containing A allele. There was an increase in HDL-C concentrations (*P*=0.097) in patients with GA + AA genotype after three months of treatment with metformin. According to our findings, the HbA1c levels in both GG genotypes and GA + AA group decreased significantly (*P*<0.001) after 3 months of treatment with metformin (Figure 2). It should be mentioned that reduction in average HbA1c values after 3 months was higher in patients with the GA + AA genotype (0.77% reduction from baseline) than in those with the GG genotype (0.54% reduction from baseline).

Table 2. Change in the study variables from before treatment to 3 months of metformin therapy according to the genotypes of OCT3-1233G>A

	GG (n=62)			GA + AA (n=88)		
Variable	Before treatment	After 3 months	*P* value	Before treatment	After 3 months	*P* value
Diastolic blood pressure (mmHg)	80.75±8.58	77±9.88	0.004	80.24±10.52	76.49±9.84	<0.001
Systolic blood pressure (mmHg)	129±14.31	124.75±11.8	0.005	132.03±16.39	124.22±19.8	<0.001
BMI (kg/m²)	29.81±4.65	29.32±4.71	<0.001	31.75±5.38	31.09±5.44	<0.001
Fasting glucose (mmol/l)	8.08±1.53	7.54±2.25	0.004	7.76±1.51	6.98±1.47	<0.001
LDL-C (mmol/l)	2.71±0.97	2.26±0.59	0.004	2.71±0.85	2.41±0.79	0.014
HDL-C (mmol/l)	1.21±0.41	1.24±0.43	0.381	1.22±0.38	1.28±0.34	0.097
TC (mmol/l)	4.92±1.22	4.33±0.78	0.002	4.9±0.92	4.67±0.88	0.033
TG (mmol/l)	2.1±0.96	1.74±0.62	0.002	2.15±0.89	1.91±0.73	0.005
LDL-C/HDL-C	2.53±1.18	1.98±0.72	0.002	2.44±1.06	2.03±0.86	0.001

Data are means±SD

Table 3. Comparison of the study variables between responders and non-responders before treatment

Variable	Responder (n=69)	Non-responder (n=81)	*P* value
Diastolic blood pressure (mmHg)	79.75±9.97	81.37±10.19	0.069
Systolic blood pressure (mmHg)	128.75±15.93	133.85±15.13	0.095
BMI (kg/m²)	31.4±5.07	30.91±5.33	0.46
HbA1c (%)	7.86±0.82	7.45±0.74	0.003
Fasting glucose (mmol/l)	8.07±1.68	7.69±1.35	0.215
LDL-C (mmol/l)	2.83±0.9	2.6±0.86	0.072
HDL-C (mmol/l)	1.17±0.38	1.24±0.39	0.297
TC (mmol/l)	5.02±0.93	4.79±1.12	0.132
TG (mmol/l)	2.13±0.81	2.1±0.97	0.499
LDL-C/HDL-C	2.7±1.31	2.26±0.85	0.102

Data are means ± SD

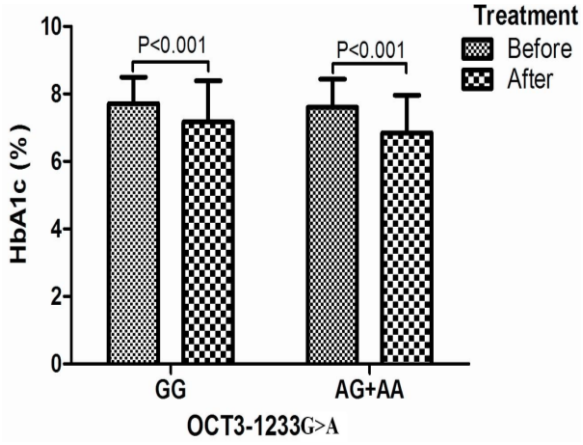

Figure 2. Change in HbA1c values from baseline to 3 months of metformin therapy according to the genotypes of OCT3-1233G>A

Based on our findings, there were no significant differences between responders and non-responders before treatment according to the study parameters, except HbA1c levels (Table 3). As shown in Table 4, when we examined the study parameters on the basis of clinical response to metformin according to OCT3-1233G>A genotypes, our results showed no significant differences between responders and non-responders with respect to the majority of the study parameters. In both responders and non-responders, the parameters such as LDL-C and TG levels and systolic and diastolic blood pressures had no significant differences according to OCT3-1233G>A genotypes. However, our results showed that in patients with GA + AA genotype compared with GG genotype, the mean reduction in HbA1c values was 0.34% for responders and 0.14% for non-responders compared with baseline.

Table 4. Change in the study variables after three months of metformin therapy in metformin responders and non-responders according to the genotypes of OCT3-1233G>A

Variable	Responder (n=69)			Non-responder (n=81)		
	GG	GA + AA	*P* value	GG	GA + AA	*P* value
Diastolic blood pressure (mmHg)	76.04±7.22	75.13±10.41	0.598	77.64±11.37	77.32±8.98	0.723
Systolic blood pressure (mmHg)	119.37±10.35	121.32±24.56	0.464	128.33±11.46	126.27±13.83	0.405
BMI (kg/m²)	28.6±4.59	31.59±5.61	0.052	29.8±4.8	30.54±5.33	0.567
HbA1c (%)	6.45±0.81	6.11±0.7	0.12	7.64±1.23	7.5±1.03	0.738
Fasting glucose (mmol/l)	6.8±1.01	6.36±1.21	0.124	8.05±2.7	7.53±1.61	0.505
LDL-C (mmol/l)	2.34±0.71	2.27±0.69	0.6	2.2±0.5	2.53±0.87	0.145
HDL-C (mmol/l)	1.3±0.49	1.3±0.38	0.793	1.2±0.39	1.26±0.31	0.064
TC (mmol/l)	4.41±0.85	4.48±0.74	0.692	4.28±0.74	4.82±0.95	0.016
TG (mmol/l)	1.61±0.62	1.83±0.64	0.19	1.82±0.61	1.98±0.82	0.67
LDL-C/HDL-C	2.01±0.85	1.9±0.82	0.6	1.97±0.63	2.12±0.88	0.871

Data are means±SD

Discussion

The present study found the frequency of major G alleles and minor A alleles in patients with type 2 diabetes as 0.65 and 0.35, which is consistent with the frequencies reported in studies conducted by Chen *et al* (14) and Lazar *et al* (16) in other human populations. Chen *et al* (14) reported the frequency of minor A allele as 0.35 in a Mexican-American population and as 0.36 in a European-American population, which is closely similar to the results reported in the present study. Lazar *et al* reported the frequency of A allele in a healthy Caucasian population as 0.36 (16). Hengen *et al* (20) reported the frequency of G allele as 0.55 in their controls and as 0.6 in their group of patients with depression, and reported the frequency of A allele as 0.45 and 0.4 in the two groups, respectively. Chen *et al* also reported the frequency of A allele as 0.45 in an Asian-American population and as 0.13 in an African-American population (14). Overall, our findings confirm the results reported by Chen *et al* (14) that OCT3-1233G>A (rs2292334) can be considered as a polymorphic variant in different ethnic groups with an allele frequency even reaching 0.45 in some populations.

Studies have demonstrated that a sufficient response is not achieved in certain metformin users that is a main problem in efficacy of this important drug (21, 22). This poor response may result from variability in metformin transporters, which are metformin metabolizers (2, 23). Despite OCT3 is one of the major metformin transporters, there are limited studies on the effects of OCT3 variants on the effectiveness of metformin. According to the findings of Chen and colleagues (14) some OCT3 variants such as rs8187717 and rs68187715 influence metformin uptake *in vitro*. In a study, Tzvetkov *et al* did not find significant relationship between some variants of OCT3 such as rs2292334 (OCT3-1233G>A) and rs3123634 and clearance of metformin (24). It should be noted that OCT2 has a main role in the metformin clearance so that the transporter is account for 80% of the total metformin clearance (25).

According to studies, OCT3-1233G>A involves in the gene expression of OCT3 and influences the final production of mRNA (17, 23). This study assessed the role of the OCT3-1233G>A variant in the effectiveness of metformin after three months of metformin therapy according to the OCT3-1233G>A genotypes.

The present study showed an expected significantly reduction in HbA1c levels following three months of metformin therapy. When we analyzed the glycemic factor according to OCT3-1233G>A genotypes, it was significantly decreased in both GG genotypes and GA+ AA genotypes with the course of metformin therapy. A closer analysis revealed that the mean reduction in HbA1c values following 3 months metformin therapy was higher in patients with the GA + AA genotype than in those with the GG genotype. Additionally, when we analyzed HbA1c levels with respect to metformin response, our results showed that in patients with GA + AA genotype compared with GG genotype, reduction in average HbA1c values from baseline was higher for responders than for non-responders. In other words, it seems that OCT3-1233G>A may has a mild effect on response to metformin. It should be noted that metformin can also be transmitted through other transporters including OCT1 and OCT2 (6, 26). Therefore, to carry out a more comprehensive study and a better understanding of the role of OCT3 variants including OCT3-1233G>A in the response to metformin, the variants should be evaluated alongside OCT1 and OCT2 variants as well to study their synergistic effects.

The results obtained showed a significant improvement in lipid profile following the metformin therapy, which is consistent with previous studies (27, 28). The analysis of results according to the OCT3-1233G>A genotypes indicated that lipid profile is improved following three months of metformin therapy in both GG group and GA+AA group.

The relatively small sample size is a limitation of the current study, and thus a larger sample size would have allowed to clarify the role of the OCT3-1233G>A variant in the glycemic response following metformin therapy.

Conclusion

Considering the roles of genetic variations in the function of metformin transporters, the effect of variations such as 1233G>A in the OCT3, which is a high-capacity transporter widely expressed in various tissues cannot be ignored. Comparing the allele frequencies of OCT3-1233G>A variant in our findings and other populations confirm that this variant is a highly polymorphic variant. Moreover, the designed PCR-based RFLP as a simple, low-cost and applicable method could be applied to determine the genotypes of OCT3-1233G>A variant in other studies.

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