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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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.\textsuperscript{[9,10]} Metformin reduces triglyceride (TG) levels, free fatty acids, and low-density lipoprotein (LDL) in plasma and increases high-density lipoprotein (HDL).\textsuperscript{[11]} Due to slight hydrophobic property, metformin needs a transporter to cross plasma membrane into the cell.\textsuperscript{[12]} A family of transporters called organic cation transporters (OCTs) is among transporters with an important role in bioavailability, clearance, and pharmacodynamics of metformin.\textsuperscript{[12]} OCTs have three members: OCT1-3. OCT3 is coded by SLC22A3 gene located on chromosome 6q26-q27.\textsuperscript{[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.\textsuperscript{[14,15]} This transporter contributes to the transfer of endogenous physiological amino compounds such as catecholamines.\textsuperscript{[14]} Like two other members of OCT family, OCT3 is an excellent transporter for metformin.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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), triglycerides (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.\textsuperscript{[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’-AGATTGCATGGAGGATGAC-3’ and R 5’-TGTTACAGGAGGTGCCAG-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 Acil (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.

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)         | 130.3±15.52  | 125.3±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)        | 4.91±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

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 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)  | P   |
|--------------------|---------------|-----------------|-----|
| SBP (mmHg)         | 130.3±14.9    | 124.7±12.3      | <0.001 |
| DBP (mmHg)         | 80.5±9.07     | 76.4±9.9        | <0.001 |
| BMI (kg/m²)        | 30.5±5.7      | 29.9±5.7        | <0.001 |
| FBG (mmol/L)       | 7.85±1.6      | 7.37±2.09       | 0.005 |
| HbA1c (%)          | 7.7±0.8       | 7.09±1.23       | <0.001 |
| ALT (µkat/L)       | 0.43±0.16     | 0.43±0.2        | 0.296 |
| TG (mmol/L)        | 2.11±0.91     | 1.8±0.66        | 0.001 |
| TC (mmol/L)        | 4.91±1.18     | 4.36±0.77       | 0.001 |
| HDL-C (mmol/L)     | 1.19±0.39     | 1.23±0.41       | 0.238 |
| LDL-C (mmol/L)     | 2.73±0.96     | 2.26±0.58       | 0.002 |
| LDL-C/HDL-C        | 2.6±1.2       | 1.9±0.69        | 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 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)         | 128.8±11.83 | 125.42±13.69 | 0.229 |
| DBP (mmHg)         | 77.0±11.27  | 77.95±8.66  | 0.762 |
| BMI (kg/m²)        | 30.1±5.47   | 30.3±4.65   | 0.352 |
| 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)         | 119.3±10.91 | 121.8±26.8 | 0.328 |
| DBP (mmHg)         | 75.47±7.76  | 75.4±10.73 | 0.922 |
| BMI (kg/m²)        | 29.64±6.01  | 31.32±4.7  | 0.194 |
| 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

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. Moreover, in the study of Aoyama et al., 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 Japanese population is lower than that of healthy Caucasian and Japanese populations. According to an investigation recently published by Li et al., 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.

Interindividual variations are involved in drug disposition and response. For example, a sufficient response is not achieved in diabetic patients with T2D. It is possible that one reason for this poor response is a variant in the 3’-UTR region of OCT3, OCT1, and OCT2. OCT3 is an important transporter for the antidiabetic drug, metformin. The expression of OCT3 is affected by some variants of OCT3 such as rs3123634 and rs2292334 in vitro. Moreover, Tsvekotov et al. 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. 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. Moreover, Nies et al. 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 is consistent with results obtained by other authors\(^\text{29-30}\) 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.

**REFERENCES**

1. Zinman B, Harris SB, Neuman J, Gerstein HC, Retnakaran RR, Raboud J, et al. Low-dose combination therapy with rosiglitazone and metformin to prevent type 2 diabetes mellitus (CANOE trial): A double-blind randomised controlled study. Lancet 2010;376:103-11.

2. Gao Y, Yoon KH, Chuang LM, Mohan V, Ning G, Shah S, et al. Efficacy and safety of exenatide in patients of Asian descent with type 2 diabetes inadequately controlled with metformin or metformin and a sulphonylurea. Diabetes Res Clin Pract 2009;83:69-76.

3. Burcelin R, Serino M, Chabo C, Blasco-Baque V, Amar J. Gut microbiota and diabetes: From pathogenesis to therapeutic perspective. Acta Diabetol 2011;48:257-73.

4. Weber MB, Narayan KM. Preventing type 2 diabetes: Genes or lifestyle? Prim Care Diabetes 2008;2:65-6.

5. Nolan CJ, Damm P, Pretinki M. Type 2 diabetes across generations: From pathophysiology to prevention and management. Lancet 2011;378:169-81.

6. Rizos CV, Elisaf MS. Metformin and cancer. Eur J Pharmacol 2013;705:96-108.

7. Kashi Z, Masoumi P, Mahrooz A, Hashemi-Soteh MB, Bahar A, Alizadeh A. The variant organic cation transporter 2 (OCT2)-T201M contribute to changes in insulin resistance in patients with type 2 diabetes treated with metformin. Diabetes Res Clin Pract 2015;108:78-83.

8. Zhou K, Pearson ER. OCT3 is a major determinant of metformin effect in skeletal muscle. Pharmacogenomics J 2011;12:708-9.

9. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: Pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820-7.

10. Mahrooz A, Parsanasab H, Hashemi-Soteh MB, Kashi Z, Bahar A, Alizadeh A, et al. The role of clinical response to metformin in patients newly diagnosed with type 2 diabetes: A monotherapy study. Clin Exp Med 2015;15:159-65.

11. Viollet B, Foretz M. Revisiting the mechanisms of metformin action in the liver. Ann Endocrinol (Paris) 2013;74:123-9.

12. Chen EC, Liang X, Yee SW, Geier EG, Stocker SL, Chen L, et al.
Targeted disruption of organic cation transporter 3 attenuates the pharmacologic response to metformin. Mol Pharmacol 2015;88:75-83.

13. Verhaagh S, Schweifer N, Barlow DP, Zwart R. Cloning of the mouse and human solute carrier 22a3 (Slc22a3/SLC22A3) identifies a conserved cluster of three organic cation transporters on mouse chromosome 17 and human 6q26-q27. Genomics 1999;55:209-18.

14. DeGorter MK, Kim RB. Hepatic drug transporters, old and new: Pharmacogenomics, drug response, and clinical relevance. Hepatology 2009;50:1014-6.

15. Bleasby K, Castle JC, Roberts CJ, Cheng C, Bailey WJ, Sina JF, et al. Expression profiles of 50+enobiotic transporter genes in humans and pre-clinical species: A resource for investigations into drug disposition. Xenobiotica 2006;36:963-88.

16. Wieland A, Hayer-Zillgen M, Bönisch H, Brüss M. Analysis of the gene structure of the human (SLC22A3) and murine (Slc22a3) extraneuronal monoamine transporter. J Neural Transm (Vienna) 2000;107:1149-57.

17. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett 2006;234:4-33.

18. Nies AT, Koepsell H, Winter S, Burk O, Klein K, Kerb R, et al. Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. Hepatology 2009;50:1227-40.

19. Li L, He M, Zhou L, Miao X, Wu F, Huang S, et al. A solute carrier family 22 member 3 variant rs3088442 G>A associated with coronary heart disease inhibits lipopolysaccharide-induced inflammatory response. J Biol Chem 2015;290:5328-40.

20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

21. Hengen N, Lizer MH, Kidd RS. Evaluation of genetic variations in organic cationic transporter 3 in depressed and nondepressed subjects. ISRN Pharmacol 2011;2011:161740.

22. Aoyama N, Takahashi N, Kitaichi K, Ishihara R, Saito S, Maeno N, et al. Association between gene polymorphisms of SLC22A3 and metformin action. Aliment Pharmacol Ther 2009;30:1644-9.

23. Bell DS. Drugs for cardiovascular risk reduction in the diabetic patient. Curr Diab Rep 2001;1:133-9.

24. Pacanowski MA, Hopley CW, Aquilante CL. Interindividual variability in oral antidiabetic drug disposition and response: The role of drug transporter polymorphisms. Expert Opin Drug Metab Toxicol 2008;4:529-44.

25. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. Pharmacogenomics 2008;9:415-22.

26. Kashi Z, Mahrooz A, Kianmehr A, Alizadeh A. The role of metformin response in lipid metabolism in patients with recent-onset type 2 diabetes: HbA1c level as a criterion for designating patients as responders or nonresponders to metformin. PLoS One 2016;11:e0151543.

27. Chen L, Pawlikowski B, Schlessinger A, More SS, Stryke D, Johns SJ, et al. Role of organic cation transporter 3 (SLC22A3) and its missense variants in the pharmacologic action of metformin. Pharmacogenet Genomics 2010;20:687-99.

28. Tzvetkov MV, Vormfeldte 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;86:299-306.

29. Bosi E. Metformin – The gold standard in type 2 diabetes: What does the evidence tell us? Diabetes Obes Metab 2009;11 Suppl 2:S3-8.

30. Abdul-Ghani MA, Puckett C, Triplitt C, Maggs D, Adams J, Cersosimo E, et al. Initial combination therapy with metformin, pioglitazone and exenatide is more effective than sequential add-on therapy in subjects with new-onset diabetes. Results from the efficacy and durability of initial combination therapy for type 2 diabetes (EDICT): A randomized trial. Diabetes Obes Metab 2015;17:268-75.