Population diversity of three variants of the SLC47A2 gene (MATE2-K transporter) in Mexican Mestizos and Native Americans

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Received: 22 April 2021 / Accepted: 5 August 2021 / Published online: 12 August 2021
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Abstract

Background  MATE2-K is an efflux transporter protein of organic cation expressed mainly in the kidney and encoded by the SLC47A2 gene. Different variants of this gene have shown an impact on the pharmacokinetics of various drugs, including metformin, which represents one of the most widely used drugs in treating type 2 diabetes. The SLC47A2 gene variants have been scarcely studied in Mexican populations, especially in Native American groups. For this reason, we analyzed the distribution of the variants rs12943590, rs35263947, and rs9900497 within the SLC47A2 gene in 173 Native Americans (Tarahumara, Huichol, Maya, Puerépecha) and 182 Mestizos (admixed) individuals from Mexico.

Methods and results  Genotypes were determined through TaqMan probes (qPCR). The Hardy–Weinberg agreement was confirmed for all three SLC47A2 gene variants in all the Mexican populations analyzed. When worldwide populations were included for comparison purposes, for alleles and genotypes a relative interpopulation homogeneity was observed for rs35263947 (T allele; range 23.3–51.1%) and rs9900497 (T allele; range 18.6–40.9%). Conversely, heterogeneity was evident for rs12943590 (A allele, range 22.1–59.1%), where the most differentiated population was the Huichol, with high frequencies of the risk genotype associated with decreased response to metformin treatment (A/A = 40.9%).

Conclusions  Although the SLC47A2 gene variants allow predicting favorable response to the metformin treatment in Mexican populations, the probable high frequency of ineffectiveness should be discarded in Huichols.

Keywords  SLC47A2 · MATE2-K · Native Americans · Mestizos · Mexico · Metformin

Introduction

Multidrug and toxin extrusion proteins (MATE), also named MATE1 and MATE2, mediate organic cations' efflux through the luminal membrane on renal proximal tubule cells and canalicular membrane of hepatocytes. Near to 1000 substrates of MATE are investigated; some are endogenous, such as creatinine and thiamine, while antibiotics and antidiabetic drug metformin have been reported as exogenous substrates MATE [1–3]. Therefore, the function or expression of MATE receptors may be contributing to the interindividual variability of drug response. To date, the interest in studying MATE transporters has increased since apical efflux by the MATE family is considered one of the drug-drug interaction sites, in addition to OCT (Organic Cation Transporter) in the basolateral membrane [1, 3]. The MATE2 transporter is encoded by the SLC47A2 gene (Solute carrier family 47; multidrug and toxin extrusion, member 2), found within the short arm of
chromosome 17 position 11.2 (17p11.2). Two functional isoforms are known: hMATE2 (NP_690872.2, 602 amino acids) and a shorter variant with partial deletion of exon 7 called hMATE2-K (NP_001093116.1, 566 amino acids) [1, 4]. Approximately 1054 single nucleotide variants (SNVs) of SLC47A2 have been reported in the NCBI-SNP (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/SNP), but only a few SNVs are functional genetic variants. One of the most relevant clinical gene variants is rs12943590 (g.-130G > A), which is in the 5′-UTR promoter region. The presence of the A allele produces a "gain-of-function" and increase of metformin depuration; thus, patients with G/A and A/A genotypes present higher levels of glycated hemoglobin (HbA1c) and higher metformin dose requirements for these patients [5–10]. Interestingly, the allele A frequency varies along with worldwide human populations (13.1 to 49.5%) [11]. Conversely, the SLC47A2 intronic variants rs35263947 (c. IVS7-30C > T) and rs9900497 (IVS2-107G > T) do not produce amino acid substitution [7]. Although the clinical relevance for these two gene variants has not been demonstrated, rs9900497 has been associated with paliperidone response [12]. Because the intronic variants rs35263947 and rs9900497 are in linkage disequilibrium with rs12943590 (r2 > 0.8), these SNPs also could be associated to the increased glycemic response in patients treated with metformin [6, 7]. It is worth mention that metformin is used as a first-line treatment in patients with type 2 diabetes mellitus (T2DM), and its therapeutic response is apparently affected by the presence of triplotypes composed by the interaction of rs72552763, rs622342 for OCT1, and rs12943590 for SLC47A2 [2, 9, 13].

The present-day Mexican population can be classified into two principal groups: (i) the Spanish-speaking Mestizos (admixed) constituting around 90% of the total population, and Native American populations representing approximately 6.2% [14]. Mestizos are characterized by a complex genetic structure originated by heterogeneous admixture after the European contact with the New World between Native Americans, Spaniards, and -to a lesser extent- Africans [15]. The high prevalence of diabetes in Mexican populations—related to both lifestyle and ancestral genetic susceptibility—justifies carrying out pharmacogenetic studies of MATE2-K transporter substrates, such as metformin, elimination in this country [9, 16, 17]. However, the SLC47A2 gene variants have been scarcely studied in Mexican populations [9, 11], especially in Native Americans where these genetic data are absent. For this reason, the aim of this study was to determine the distribution of the SLC47A2 gene variants rs12943590, rs35263947, and rs9900497 in Mestizos (admixed) and Native American populations from Mexico.

Subjects and methods

Population sample

A total of 355 unrelated volunteers were included in this study. Mexican population samples included Mestizos (admixed) from the Chihuahua (MestizoCHI), Nuevo León (MestizoNL), Jalisco (MestizoJAL), and Yucatán (MestizoYUC) states, which are in the north-center, north-east, west, and southeast regions of the Mexican territory, respectively (Fig. 1). Besides, four Native American populations from different geographic regions of Mexico were analyzed: Tarahumara (North), Huichol (West), Purépecha (Center), and Maya (Southeast) groups. We asked to these volunteers for the indigenous origin of their parents and grandparents, particularly to confirm that they spoke the corresponding Native American language. Because in these indigenous communities the Spanish language is extensively spoken, translator was not necessary. However, leader(s) of each indigenous community kindly helped us to invite and clarify the purposes of the study. All individuals signed a written informed consent according to the ethical guidelines of the Helsinki Declaration. Ethical approval was obtained from the Committee of Ethics and Research of the Centro Universitario de la Ciénega of the Universidad de Guadalajara (CUCI-UdeG, Mexico). The anonymity of the recruited individuals will be always preserved.

DNA extraction, quantification, and genotyping

Genomic DNA was extracted from 5 mL of peripheral blood samples by the standard phenol–chloroform method. DNA samples were quantified into a Nanodrop 2000™ (Thermo Scientific, USA), and they were diluted to 25–30 ng/μL. The genotypes for the SLC47A2 variants rs12943590 G > A, rs35263947 C > T and rs9900497 G > T were determined quantitative polymerase chain reaction (qPCR) using Taqman probes in the StepOne™ Real-Time PCR system (Applied Biosystems), under the following protocols: C___2593951_10, C___2593964_20, and C____446702_10 (Thermo Fisher Scientific), respectively. The Thermal cycling conditions included an initial denaturation for 95 °C for 10 min, and 48 cycles of denaturation at 95 °C for 15 s followed by the extension at 60 °C for 60 s.

Data analysis

We used the Excel complement GenAlex 6.5 to estimate allele and genotype frequencies through the gene counting method for all Mexican populations [18]. The Hardy–Weinberg expectations (HWE) were verified by
exact tests for each genetic variant and population. In addition, we assessed the pairwise population differentiation by Fst distances and Fst $P$-values, as well as the Analysis of Molecular Variance (AMOVA) using the Arlequin 3.0 software [19]. Genetic distances were graphically represented in a Multidimensional scaling (MDS) plot by the software SPSS 19.0 for Windows. The linkage disequilibrium (LD) between three $SLC47A2$ gene variants, and corresponding haplotype frequencies by population were estimated with the SNPanalyzer 2.0 software [20].

**Results**

**Allelic and genotype frequencies**

The allele frequencies of the three $SLC47A2$ gene variants estimated by population are shown in Table 1. For rs12943590, rs35263947, and rs9900497, the modal alleles in most of the Mexican populations were the wild type alleles, whereas the variants had minor frequencies as follows: A (range 22.1–59.1%) and T (range 23.3–51.1%), and T (range 18.6–40.9%), respectively. Interestingly, the Tarahumara and Huichol indigenous groups displayed the minor and largest variant allele frequencies, respectively (Table 1). The genotype distribution agreed with Hardy–Weinberg’s expectations for all three $SLC47A2$ gene variants in the eight Mexican populations (Table 1).

**Haplotypes frequencies**

Eight haplotypes based on the $SLC47A2$ gene variants rs12943590–rs35263947–rs9900497 and their corresponding frequencies were estimated in two Mexican population clusters: Mestizos and Native Americans. In both clusters, the most frequent haplotypes were G-C-G (range 54.82–56.37%) and A-T-T (range 27.41–22.82%) (Table 2). The linkage disequilibrium (LD) between gene variants was significant in all Mexican populations ($p = 0.000$). Because this result is expected by their chromosomal proximity in the same gene, and it agrees with previous studies [7, 10], this finding was not further discussed.

**Population pairwise comparisons**

For each $SLC47A2$ gene variant, pairwise comparisons were performed between the studied Mexican populations plus worldwide populations available in the literature (Online Resource 1a–c). The AMOVA test showed a worldwide homogeneous distribution for rs35263947 and rs9900497 (Fst $p$-value > 0.0038, considering Bonferroni correction). Conversely, rs12943590 showed significant differences (AMOVA Fst $p$-value = 0.0000). The most differentiated Mexican population was the Huichol Native American group. For instance, rs12943590 in Huichols showed differences with all populations ($p < 0.0035$, after Bonferroni correction), excepting with Mexican Mestizos (MestMex) and Peruvians, whereas for rs35263947 and rs9900497 Huichols
showed differences only with Colombian, Puerto Rico, and European populations (Online Resource 1a–c) which can be seen in the MDS plot (Fig. 2).

Discussion

In this work, we characterized geographically dispersed Mexican Mestizo and Native American populations distinguished by the elevated prevalence of diabetes and subsequent clinical importance of the \( \text{SLC47A2} \) gene variants for metformin’s metabolism first-line drug used for diabetes treatment in Mexico [9, 16, 17]. Although a relative worldwide homogeneity was observed among Mexican populations for rs35263947 and rs9900497, heterogeneity was observed for rs12943590 (Table 1; Online Resource 1a–c). For rs12943590, the most frequent genotype in most of the Mexican populations was the G/G genotype (Table 1), which is related with better glycemic response to metformin [10]. Conversely, the presence of the A allele and the A/A genotype for the variant rs12943590 is related to increased renal clearance of metformin and changes of the HbA1c in T2DM patients, as well as the need to increase the metformin dose [6, 7, 9, 10]. Consequently, whereas \( \text{SLC47A2} \) gene variants suggest a good response to metformin in most of the Mexican population, the Huichol group highlight as a population at risk of having a lack of glycemic control by metformin therapeutic failure (genotype A/A = 40.91%).

Huichol was the most differentiated Native American group for all the three \( \text{SLC47A2} \) gene variants rs12943590, rs35263947, and rs9900497, both between the studied Mexican Natives and Mestizos, as well as regarding the reference worldwide populations (Online Resource 1a–c; Fig. 2). The genetic drift effects due to the higher level of geographic isolation where Huichols live, including Canyons and

| Table 1 | Allele and genotype frequencies of three \( \text{SLC47A2} \) gene variants in Native American and Mestizo populations from Mexico

| Gene variant | Native Americans | Mestizos |
|--------------|-----------------|---------|
| rs12943590   |                 |         |
| G allele     | 0.6279          | 0.5000  |
| A allele     | 0.3721          | 0.5000  |
| C allele     | 0.0000          | 0.0000  |
| T allele     | 0.5698          | 0.4996  |
| G/G genotype| 0.3409          | 0.3023  |
| A/G genotype| 0.6591          | 0.3977  |
| C/G genotype| 0.0000          | 0.0000  |
| T/G genotype| 0.1028          | 0.3977  |

| Table 2 | Haplotype frequencies of three \( \text{SLC47A2} \) gene variants in Native Americans and Mestizo populations from Mexico

| Haplotypes | Frequencies |
|------------|-------------|
| rs12943590 |             |
| rs35263947 |             |
| rs9900497  |             |
| Native Americans | Mestizo |
| G          | C           | G         |
| A          | T           | T         |
| T          | G           | G         |
| G          | C           | T         |
| A          | C           | G         |
| T          | T           | T         |

All \( p \)-values of the LD test between these three variants were significant \((p < 0.0001)\)
Mountains of the Sierra Madre Occidental, could explain the genetic differentiation observed in this Native American group, as previously was described from analysis of autosomal STRs used in Human Identification [21]. In addition, the low European admixture level detected in Huichols (average 8%) [21], probably has been important to keep genetic differences, as principally observed in the rs12943590 gene variant. However, the Huichol group has undergone significant cultural changes during the last decade, especially in their lifestyle, including eating habits that predispose to generate diseases, such as type 2 diabetes mellitus (T2DM) commonly found in urban locations [22, 23].

The increased function of the SLC47A2 transporter is related with metformin therapeutic failure in A/A individuals for rs12943590. However, the resulting lower cellular exposure also allow predicting diminished adverse reactions in response to metformin, which could be certain for additional SLC47A2 substrates, such as cephalixin, cephradine, cisplatine, etc. [1–3]. Although adverse reactions in response to metformin have been estimated at 15.8% in India, differences across rs12943590 genotypes have not been demonstrated [10]. Interestingly, the peculiar distribution of the SLC47A2 gene variants found in this study in Huichols could be helpful to evaluate their presumable clinical impact with different substrates, and specifically analyzing the response to metformin in Huichol T2DM patients. Moreover, the influence of other genes such as OCT1, OCT2, and MATE1 influencing the metformin response must be considered [6, 9]. Differences in the metformin response have been recently claimed among Mexican Mestizos carrying the triplotype formed by rs72552763-Del and rs622342-C (OCT1 gene), and rs12943590-G (SLC47A2 gene) concerning individuals with the triplotype rs7255276-3Del, rs12943590-A, and rs622342-C (p < 0.05), respectively [9]. Unfortunately, because the OCT1 gene variants were not analyzed herein, the previous conclusions cannot be extrapolated in our study. The inclusion of different genes involved in the metformin transport, such as SLC47A2 [6], will provide a comprehensive overview of the pharmacogenetic implications in response to this drug.

Among the limitations of our study, we avoided the inclusion of related individuals through personal questionnaires to detect first-line relatives. Nevertheless, in some small Native American communities undetected endogamy probably exists, given the high frequency of some surnames. Moreover, although indigenous groups from different geographic regions were studied herein (North, West, Center, and Southeast), many Native American groups exist in Mexico [21]. However, a deep analysis of the Native American genetic diversity in Mexico is beyond the scope of the current study.

Conclusions

This study provides valuable information for prospective pharmacogenetic studies focused on the clinical implication of SLC47A2 gene variants on metformin response in Mexican populations. Based on the genotype distribution of rs12943590, although most individuals would respond favorably to the metformin treatment in this country, the high presence of the genotype A/A could promote accelerated elimination of metformin—and potential ineffectiveness—in the Huichol population.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06628-y.

Acknowledgements The authors wish to thank the volunteers who participate in this study and the PhD Elba Margarita Romero Tejeda for the technical support for the writing of the paper. We also thank...
the Universidad de Guadalajara (UdeG) for the economic support to this project to AFFM and HRV in the PROSNI 2019–2020 and infrastructure 2019 programs.

Author contributions The concept and design of the experiments were performed by F-MAF, R-VH. Performance of the experiments: C-SW, F-MAF. Data analysis: A-VJA, F-GI, F-MAF. Contributed reagents/materials/analysis tools: F-MAF, M-CG, R-VH. Wrote the paper: F-MAF, R-VH.

Funding The grant from Infrastructure 2019 program of the University of Guadalajara (UdeG) to HR-V and AFF-M.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval All Mexican volunteers signed a written informed consent according to the ethical guidelines of the Helsinki Declaration. The Committee of Ethics and Research of the Centro Universitario de la Ciénega of the Universidad de Guadalajara (CUCI-UdeG, Mexico) approved this project. The anonymity of the recruited individuals was always preserved.

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