Population Diversity of Three Variants of the SLC47A2 Gene (MATE2-K transporter) in Mexican Mestizos and Native Americans

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

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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 (C allele; range: 48.9–76.7%) and rs9900497 (G allele; range: 59.1–81.4%). Conversely, heterogeneity was evident for rs12943590 (G allele, range 40.9–77.9%), 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, hhttp://www.ncbi.nlm.nihgov/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 rs35263947 and rs9900497 are in linkage disequilibrium concerning rs12943590, it has been observed that they may contribute to the increase in the glycemic response in patients treated with metformin [10]. 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 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 (Figure 1). Besides, four Native American populations from different geographic regions of Mexico were analyzed: Tarahumara (North), Huichol (West), Purépecha (Center), and Maya (Southeast) groups. 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.

FIGURE 1

DNA extraction, quantification, and genotyping

Genomic DNA extracted by the standard phenol-chloroform method was quantified into a Nanodrop 2000^{TM} (Thermo Scientific, USA). DNA samples 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^{TM} Real-Time PCR system (Applied Biosystems), under the following protocols: C___2593951_10, C___2593964_20, and C____446702_10 (Thermo Fisher Scientific), respectively.

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 and rs35263947, the modal alleles in Mexican populations were G (range: 40.91–77.91%) and C (range: 48.86–76.74%), respectively. On the other hand, the G allele of rs9900497 showed high frequencies in all Mexican Mestizo and Native American populations (59.09–81.39%). The genotype distribution agreed with Hardy-Weinberg's expectations for all three *SLC47A2* gene variants in the eight Mexican populations (Table 1).

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.

TABLE 2

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 (Figure 2).

ONLINE RESOURCE 1, FIGURE 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 *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). According to previous reports, 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]. Based on this background, Huichol would be the principal 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 *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; Figure 2). The genetic drift effects due to the higher level of geographic isolation where Huichols live, including Canyons and 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]. 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]. Interestingly, although higher genetic risk can be deduced in Huichols from the rs12943590 variant, a contrary more significant response to metformin and decreased transport could be inferred due to the allele's high frequencies T in rs35263947 and rs9900497 [10]. This peculiar distribution of the *SLC47A2* gene variants in Huichols could be helpful to evaluate their presumable clinical impact, analyzing the response to metformin in Huichol T2DM patients. Similarly, the relative high frequency of the G/A

genotype for the rs12943590 variant (41.30-43.18%) in Mayas and Mestizos from the Northwest (MestizoCHI), Southeast (MestizoYUC), and West (MestizoJAL) populations (Table 1). Presumably, this finding could increase the function of the SLC47A2 transporter and –consequently– will decrease both the cellular exposure and response to metformin, reducing the risk of adverse drug reactions [10].

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.

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.

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Author contributions

The concept and design of the experiments were performed by Favela-M, Rangel-V. Performance of the experiments: Cuevas-S, Favela-M. Data analysis: Aguilar-V, Fricke-G, Favela-M. Contributed reagents/materials/analysis tools: Favela-M, Martinez-C, Rangel-V. Wrote the paper: Favela-M, Rangel-V.

Declarations

Not applicable

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Conflicts 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.

References

1. Nies AT, Damme K, Kruck S, Schaeffeler E, Schwab M (2016) Structure and function of multidrug and toxin extrusion proteins (MATEs) and their relevance to drug therapy and personalized medicine. Arch Toxicol 90(7):1555-1584. doi:10.1007/s00204-016-1728-5

2. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE (2012) Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 22(11):820-827. doi:10.1097/FPC.0b013e3283559b22

3. Motohashi H, Inui K (2013) Multidrug and toxin extrusion family SLC47: physiological, pharmacokinetic and toxicokinetic importance of MATE1 and MATE2-K. Mol Aspects Med 34(2-3):661-668. doi:10.1016/j.mam.2012.11.004

4. Staud F, Cerveny L, Ahmadimoghaddam D, Ceckova M (2013) Multidrug and toxin extrusion proteins (MATE/SLC47); role in pharmacokinetics. Int J Biochem Cell Biol 45(9):2007-2011. doi:10.1016/j.biocel.2013.06.022

5. Nishimura K, Ide R, Hirota T et al (2014) Identification and functional characterization of novel nonsynonymous variants in the human multidrug and toxin extrusion 2-K. Drug Metab Dispos 42(9):1432-1437. doi:10.1124/dmd.114.056887

6. Stocker SL, Morrissey KM, Yee SW et al (2013) The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. *Clin Pharmacol Ther* 93(2):186-194. doi:10.1038/clpt.2012.210

7. Choi JH, Yee SW, Ramirez AH et al (2011) A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. Clin Pharmacol Ther 90(5):674-684. doi:10.1038/clpt.2011.165

8. Santoro AB, Botton MR, Struchiner CJ, Suarez-Kurtz G (2018) Influence of pharmacogenetic polymorphisms and demographic variables on metformin pharmacokinetics in an admixed Brazilian cohort. Br J Clin Pharmacol 84(5):987-996. doi:10.1111/bcp.13522

9. Marta M, Sánchez-Pozos K, Jaimes-Santoyo J et al (2020) Pharmacogenetic Evaluation of Metformin and Sulphonylurea Response in Mexican Mestizos with Type 2 Diabetes. Curr Drug Metab 21(4):291-300. doi:10.2174/1389200221666200514125443

10. Raj GM, Mathaiyan J, Wyawahare M, Priyadarshini R (2018) Lack of effect of the SLC47A1 and SLC47A2 gene polymorphisms on the glycemic response to metformin in type 2 diabetes mellitus patients. Drug Metab Pers Ther 33(4):175-185. doi:10.1515/dmpt-2018-0030.

11. Auton A, Abecasis G, Altshuler D et al (2015) A global reference for human genetic variation. Nature 526, 68–74. https://doi.org/10.1038/nature15393

12. Li Q, Wineinger NE, Fu DJ, et al (2017) Genome-wide association study of paliperidone efficacy. Pharmacogenet Genomics 27(1):7-18. doi:10.1097/FPC.0000000000000250

13. Chung H, Oh J, Yoon SH, Yu KS, Cho JY, Chung JY (2018) A non-linear pharmacokinetic-pharmacodynamic relationship of metformin in healthy volunteers: An open-label, parallel group, randomized clinical study. PLoS One. doi:10.1371/journal.pone.0191258

14. INEGI (2020) Censo de Población y Vivienda 2020. INEGI. https://www.inegi.org.mx/temas/lengua/. Accessed 07 April 2021.

15. Martínez-Cortés G, Salazar-Flores J, Gabriela Fernández-Rodríguez L et al (2012) Admixture and population structure in Mexican-Mestizos based on paternal lineages. J Hum Genet 57: 568–574. https://doi.org/10.1038/jhg.2012.67

16. García-Chapa EG, Leal-Ugarte E, Peralta-Leal V, Durán-González J, Meza-Espinoza JP (2017) Genetic Epidemiology of Type 2 Diabetes in Mexican Mestizos. Biomed Res Int. doi:10.1155/2017/3937893

17. Martorell R (2005) Diabetes and Mexicans: why the two are linked. *Prev Chronic Dis*. 2(1):A04.

18. Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28(19), 2537-9.

19. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1, 47-50.

20 Yoo J, Lee Y, Kim Y et al (2008) SNPAnalyzer 2.0: a web-based integrated workbench for linkage disequilibrium analysis and association analysis. BMC Bioinformatics 9, 290.

21. Rangel-Villalobos H, Martínez-Sevilla VM, Martínez-Cortés G, Aguilar-Velázquez JA, Sosa-Macías M, Rubi-Castellanos R, González-Martín A (2016) Importance of the geographic barriers to promote gene drift and avoid pre- and post-Columbian gene flow in Mexican native groups: Evidence from forensic STR Loci. Am J Phys Anthropol 160(2):298-316. doi: 10.1002/ajpa.22969.

22. García PA, Carrillo JM, Madero L, Gomez FE, Ortiz MG, Rosales AM, Rangel MR, Garcia AF, Gutierrez, RS, Bernés SR (2011) Prevalencia de factores de riesgo asociados a la diabetes mellitus tipo 2 en huicholes expuestos a un estilo de vida urbano. Revista Fuente 2(7): 77-84. http://dspace.uan.mx:8080/jspui/handle/123456789/752

23. Quiñonez-Tapia F, Vargas-Garduño ML, Soltero-Avelar R (2019) The wixárika with diabetes mellitus and its links to food. Rev Esp Nutr Comunitaria 25(1): 18-22. DOI:10.14642/RENC.2019.25.1.5270

Figure Leyends

Fig 1. Geographic location of the Mexican population samples studied herein for three *SLC47A2* gene variants.

Fig 2. MDS plot representing Fst genetic distances among worldwide populations based on the three *SLC47A2* variants (rs12943590, rs35263947, and rs9900497).

Online Resource 1

1a. Fst genetic distances (above diagonal) and Fst p-values (below diagonal) among 14 worldwide populations for the rs12943590 variant.

1b. Fst genetic distances (above diagonal) and Fst p-values (below diagonal) among 13 worldwide populations for the rs35263947 variant.

1c. Fst genetic distances (above diagonal) and Fst p-values (below diagonal) among 13 worldwide populations for the rs9900497 variant.

Figures

Figure 1

Geographic location of the Mexican population samples studied herein for three SLC47A2 gene variants. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

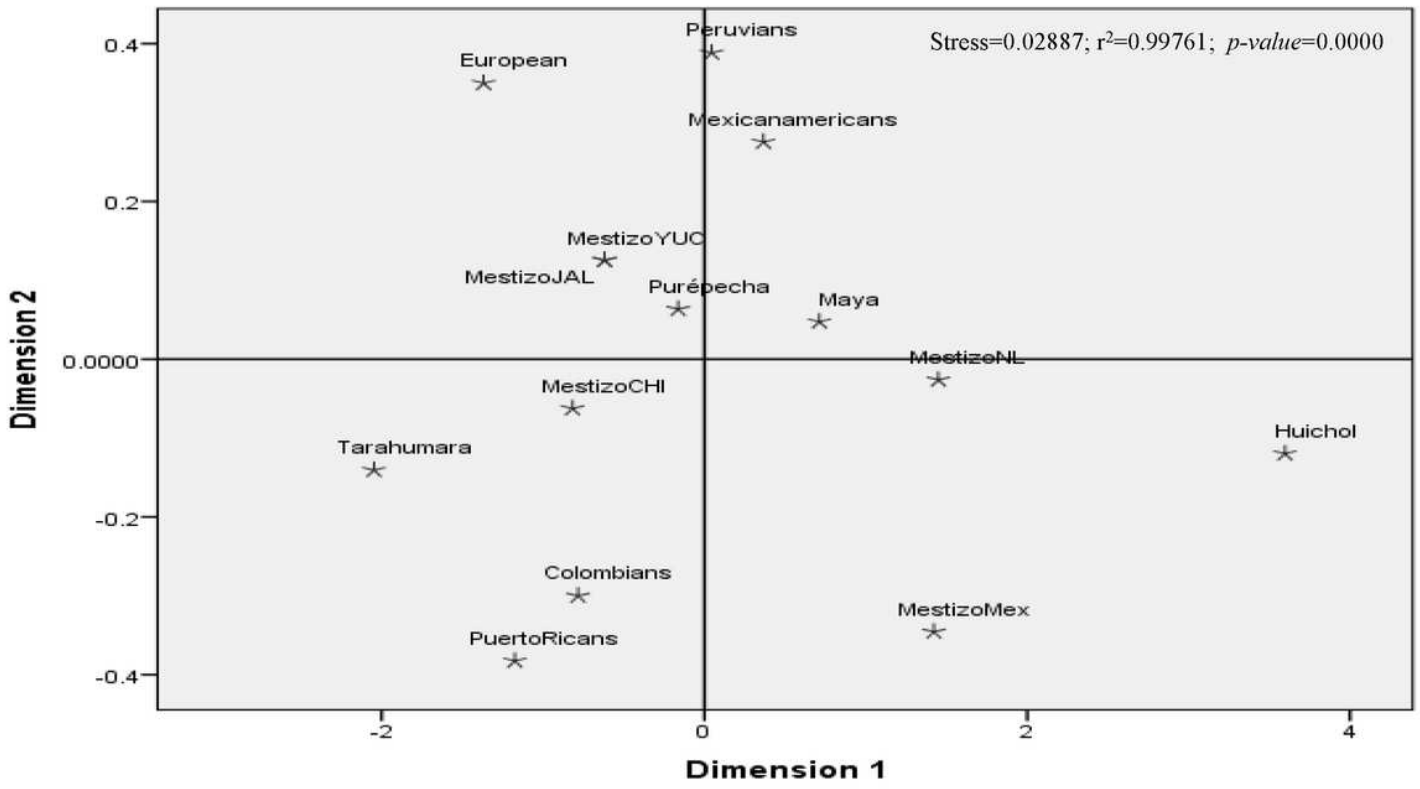

Figure 2

MDS plot representing Fst genetic distances among worldwide populations based on the three SLC47A2 variants (rs12943590, rs35263947, and rs9900497).

Supplementary Files

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- OnlineResource1.xlsx