Identification of genetic variants in pharmacogenetic genes associated with type 2 diabetes in a Mexican-Mestizo population

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Abstract. Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic pathologies in the world. In developing countries, such as Mexico, its prevalence represents an important public health and research issue. Determining factors triggering T2DM are environmental and genetic. While diet, exercise and proper weight control are the first measures recommended to improve the quality of life and life expectancy of patients, pharmacological treatment is usually the next step. Within every population there are variations in interindividual drug response, which may be due to genetic background. Some of the most frequent first line T2DM treatments in developing countries are sulfonylureas (SU), whose targets are ATP-sensitive potassium channels (K\text{ATP}). Single nucleotide polymorphisms (SNPs) of the K\text{ATP} coding genes, potassium voltage-gated channel subfamily J member 11 (KCNJ11) and ATP binding cassette subfamily C member 8 (ABCC8) have been associated with SU response variability. To date, there is little information regarding the mechanism by which these SNPs work within Mexican populations. The present study describes the distribution of three SNPs [KCNJ11 rs5219 (E23K), ABCC8 rs757110 (S1369A) and rs1799854 (-3C/T)] among Mestizo Mexican (MM) T2DM patients, and compares it with published data on various healthy subjects and T2DM populations. Through this comparison, no difference in the KCNJ11 rs5219 and ABCC8 rs757110 allelic and genotypic frequencies in MM were observed compared with the majority of the reported populations of healthy and diabetic individuals among other ethnic groups; except for African and Colombian individuals. By contrast, ABCC8 rs1799854 genomic and allelic frequencies among MM were observed to be significantly different from those reported by the 1000 Genomes Project, and from diabetic patients within other populations reported in the literature, such as the European, Asian and Latin-American individuals [T=0.704, G=0.296; CC=0.506, CT=0.397, TT=0.097; 95% confidence interval (CI); P≤0.05]; except for South Asian and Iberian populations, which may reflect the admixture origins of the present Mexican population. This genetic similarity has not been observed in the other Latin-American groups. To the best of our knowledge, this is the first study of ABCC8 rs757110 and rs1799854 SNP frequencies in any Mexican population and, specifically with diabetic Mexicans. Knowledge of the genetic structure of different populations is key to understanding the interindividual responses to drugs, such as SU and whether genotypic differences affect clinical outcome.

Introduction

Diabetes is a type of metabolic disease characterized by hyperglycemia resulting from either defective insulin secretion, insulin action or the two (1). The most prevalent type of diabetes is type 2 diabetes mellitus (T2DM), which is one of the leading causes of morbidity globally, as well as the third-highest risk factor for premature mortality (2).

In Mexico, T2DM has led mortality rates since 2005 and today it represents the leading cause of death in the country (3,4) with a prevalence of 11.8% (5). Costs associated with medical treatment of T2DM are ~450 million dollars annually (6), while ~75% of diagnosed patients do not observe adequate glycemic control even with medical assistance. These factors make T2DM a critical concern for the Mexican State’s public health and research systems.

While genetic factors causing T2DM have not yet been sufficiently defined, they are currently under extensive study. Numerous T2DM-associated genes present as single nucleotide polymorphisms (SNPs) whose frequencies vary among

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Abbreviations: MM, Mestizo Mexican; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; SU, sulfonylurea; K\text{ATP}, ATP-sensitive potassium channel; ME, metformin

Key words: diabetes, pharmacogenetics, potassium voltage-gated channel subfamily J member 11, ATP binding cassette subfamily C member 8, Mestizo Mexican
different populations. Genetic variations associated with either pharmacological targets or drug metabolism are of particular interest, as different responses to pharmacological treatments may be explained by the presence of a genetic mutation or the combination of various genotypes (7).

The initial pharmacological treatments for T2DM are oral hypoglycemics (OH), with sulfonylureas (SU) and metformin (ME) being the two most commonly administered in developing countries. SUs are a type of oral OHs, which inhibit ATP-sensitive potassium channels (K\textsubscript{ATP}), thus inducing glucose-independent insulin release by the β-pancreatic cells (8). However, not all T2DM patients respond to the anti-diabetic action of SU (primary failure) and those who initially respond adequately may experience a decrease in its efficacy following the years (as variable as 1-2 to 10 or more years) of treatment (secondary failure) (9,10). SUs are frequently combined with ME, a drug which reduces hepatic glucose production and insulin resistance (11). It has been observed that the short-term reduction of glycated hemoglobin A\textsubscript{lc} (HbA\textsubscript{lc}) is similar in SU and ME monotherapies (12), and that this drug combination reduces HbA\textsubscript{lc} more efficiently than SU alone (13). When compared, observations on the adjuvant effects of SU/ME-based treatments are inconclusive; while certain authors have associated hypoglycemic events of different severity and weight increase to SUs, either alone or in combination with ME (14), others have reported no effect on body weight when combining SUs with ME (15). However, due to their low cost and accessibility, SUs (alone or combined with ME) remain the most frequent first-line T2DM treatment in the world, particularly in developing countries, such as Mexico (16,17). The UK Prospective Diabetes Study demonstrated that only 25% of patients achieved glycemic control of <7% in HbA\textsubscript{lc} over a nine-year follow-up period of monotherapy on either SU or ME (10).

Of the genetic polymorphisms that have already been reviewed extensively and whose clinical implications have already been analyzed (18,19), there are a number, which appear to be well suited for pharmacogenetic studies. Various SNPs have been reported among K\textsubscript{ATP}-channel encoding genes (potassium voltage-gated channel subfamily J member 11 (KCNJ11) and ATP binding cassette subfamily C member 8 (ABCC8)) as the therapeutic target of SU (20). Many of these genes are associated with T2DM predisposition or progression, as well as with SU response variability. A specific SNP frequency may vary between different populations; therefore, it is important to evaluate and compare its distribution among different human populations, in order to better understand whether there is an association between drug response variability, patients' glycemic control and genetic architecture.

In the present study, the frequencies of three pharmacologically important SNPs are described in a Mestizo Mexican (MM) population and, in order to compare these with other reported populations, the distribution of KCNJ11 rs5219 (E23K), ABCC8 rs757110 (S1369A) and rs1799854 (-3C/T) is presented in MM T2DM patients. The aim of the present study is to increase the understanding of the genetic characteristics of specific populations. This may facilitate with elucidating the causes of therapeutic failure and the findings may also be extrapolated and/or compared to other populations in order to improve treatment options and patient management.

Materials and methods

Patient selection and study design. This study was observational and included 247 T2DM patients recruited from July 2014 to October 2016 from two health centers: 145 from Centro de Salud Portales and 102 T2DM patients from Centro de Salud Mixcoac, both located in Mexico City's Benito Juárez Health Jurisdiction (Mexico). Out of the total adult patient population, 165 were females while 82 were males. Patient eligibility criteria were as follows: Self-proclaimed MM ancestry of at least three generations; age between 18 and 90 years; individuals diagnosed with T2DM according to the American Association of Diabetes criteria (1); individuals taking OHs, the SU glibenclamide alone or combined with ME, for at least 3 months. All participants were enrolled in their health centers after providing written informed consent. Patient clinical history, anthropometrics and biochemical characteristics were obtained from their clinical records (a summary of this data is presented in Table 1).

Genotyping. Genomic DNA was obtained by taking 6 ml peripheral blood through arm phlebotomy in glass EDTA-tubes (Vacutainer\textsuperscript{®}; BD Biosciences, Franklin Lakes, NJ, USA) from each patient. Patients were fasted at the time of blood sampling. Genomic DNA was isolated from 200 µl total blood, using the UltraClean\textsuperscript{®} BloodSpin\textsuperscript{®} DNA Isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA), and the DNA-extraction protocol was performed according to the manufacturer's instructions. DNA quality was achieved by spectrophotometry (Jenway 7305; Cole-Parmer Ltd., Staffordshire, UK). The KCNJ11 rs5219 (E23K), ABCC8 rs757110 (S1369A) and rs1799854 (-3C/T) polymorphisms were determined using 20 ng total genomic DNA per reaction, by allelic discrimination via quantitative polymerase chain reaction (qPCR) using a ViiA\textsuperscript{™} 7 Real-Time PCR system and TaqMan\textsuperscript{®} SNP assays (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the standard cycling conditions as follows: An initial denaturation stage 95°C for 10 min, 40 cycles of denaturation 95°C for 15 sec, and annealing at 60°C for 1 min, and post read stage or final extension at 60°C for 30 sec.

Statistical analysis. The SNP frequencies of KCNJ11 rs5219, ABCC8 rs757110 and rs1799854 were determined through direct counting, the remaining analyses were performed using SPSS 23.0 for Windows (IBM Corp., Armonk, NY, USA). The SNP frequencies of the patients from both health centers were compared by performing $\chi^2$ test of independence. In addition, biochemical and anthropometric data were compared using two-way analysis of variance (ANOVA). The total frequencies for each SNP were compared with those of other populations using the $\chi^2$ test of independence. P<0.05 with 95% CI was considered to indicate a statistically significant difference.

Results

Mexican-Mestizo sample characteristics. No statistically significant differences were identified after comparing
biochemical and anthropometric data from the individuals from the two health centers (data not shown). Within the whole sample, there were approximately twice as many females as there were males (162 females and 83 males); 61.5% of the patients were overweight (55.7% of females and 73.8% of males); ~25% of the subjects presented with grade I obesity (27.9% in females and 20.0% in males). The mean period since the first T2DM diagnosis was 10 years, with a mean of 7.55±0.15% for HbA1c and 141.86±4.06 mg/dl for fasting plasma glucose (FPG). A summary of anthropometrics and biochemical characteristics of our subjects is presented in Table I.

No significant differences were observed in genotypic and allelic frequency distribution between the two health centers (data not shown). The genotyping frequencies of the three SNPs in the whole sample were in Hardy-Weinberg equilibrium (Table II). The obtained allelic and genotypic frequencies were compared with those reported for other non-diabetic populations using data from the 1000 Genomes Project (21) and from literature on T2DM patients; the comparative of each SNP is presented in Tables III-V (22-38). For the present study, studies were included that involved populations of T2DM subjects that were comparable with the current sample.

SNP comparison with other populations. KCNJ11 rs5219 (E23K) allelic and genotypic frequencies in MM individuals were not identified to be different between all of the T2DM populations that were compared [predominantly European (EUR) and Asian]. To the best of our knowledge, there were only two other studies from Mexican rs5219 allelic frequencies, which demonstrated no difference compared with the MM population: T2DM Mestizo from Yucatan and T2DM Mayans. In the 1000 Genomes Project populations, MM allelic and genotypic frequencies were significantly different from African individuals, East Asians, the majority of EUR individuals, and other admixed Americans from Colombia, Peru, Puerto Rico and individuals of Mexican ancestry from Los Angeles. Only in Iberic EUR and South Asian individuals did the 1000 Genomes Project report non-significant differences in allelic and genotypic frequencies compared with MM (Table V). Currently, to the best of our knowledge, there are no other studies regarding allelic or genotypic frequencies of this SNP in a Mexican population (Table IV).

ABCC8 rs1799854 (-3C/T) SNP allelic and genotypic frequencies from MM were significantly different from all of the compared T2DM populations (primarily European and Asian). In comparison to non-diabetic populations from the 1000 Genomes Project, MM allelic and genotypic frequencies were significantly different from African individuals (P=1.45E-13 and P=1.72E-23 for allelic and genotypic frequencies, respectively) and American-Colombians from Medellin (P=6.13E-04 and P=2.52E-06 for allelic and genotypic frequencies, respectively). Currently, to the best of our knowledge, there are no other studies on the frequencies of these SNPs among Mexican populations of non-diabetic or diabetic individuals.

Discussion
In the present study, in the MM from Mexico City, rs5219 allelic and genotypic frequencies were not different from the majority of the reported populations of healthy and diabetic individuals among other ethnic groups, except for the African and Colombian subjects; the same observation applies to

| Parameter                  | Total (n=247) | Female (n=165) | Male (n=82) |
|----------------------------|--------------|----------------|-------------|
| Age (years)                | 60.43±0.75   | 60.05±0.91     | 61.24±1.34  |
| Weight (kg)                | 70.87±1.06   | 69.25±1.23     | 74.40±1.98  |
| Body mass index (kg/m²)    | 29.51±0.37   | 30.26±0.45     | 27.89±0.62  |
| Diabetes diagnosis (years) | 10.27±0.61   | 9.57±0.64      | 11.92±1.34  |
| Triglycerides (mg/dl)      | 199.06±8.02  | 199.59±9.44    | 197.90±15.24|
| Glycated hemoglobin A1c (%)| 7.55±0.15    | 7.53±0.19      | 7.60±0.26   |
| Fasting plasma glucose (mg/dl) | 141.86±4.06 | 144.55±5.22   | 135.76±5.99 |
| Cholesterol (mg/dl)        | 194.82±2.71  | 197.24±3.45    | 189.39±4.12 |

Values are presented as means ± standard error of the mean.

| SNP          | Frequency | %  | P-value |
|--------------|-----------|----|---------|
| E23K         | GG        | 93 | 37.7    |
|              | GA        | 112| 45.3    |
|              | AA        | 42 | 17.0    |
| Total        |           | 247| 100.0   |
| -3/C/T       | CC        | 125| 50.6    |
|              | CT        | 98 | 39.7    |
|              | TT        | 24 | 9.7     |
| Total        |           | 247| 100.0   |
| S1369A       | AA        | 75 | 30.4    |
|              | AC        | 131| 53.0    |
|              | CC        | 41 | 16.6    |
| Total        |           | 247| 100.0   |

P<3.84 using the χ² test for Hardy-Weinberg equilibrium.
It appears that the distribution of these polymorphisms prevails among the majority of populations, while the ancestral allele is most frequent among African individuals. The results from the Colombian population may reflect the ancestral admixture history of the country, where the African component is widely spread across the Pacific and Caribbean regions (39-41).

In Mexican populations, rs5219 is the only SNP reported for KATP-coding genes, exhibiting no differences between the alleles of healthy and T2DM subjects, even when the geographical and ethnic profile of the three Mexican samples were markedly different: The healthy volunteers were Mestizo from the South East (22), the T2DM group had Mayan Amerindian ancestry (23) and our group (MM) was formed by Mestizo individuals, primarily from the central area of Mexico.

Table III. Potassium voltage-gated channel subfamily J member 11 rs5219 (E23K) allelic and genotypic frequency comparison.

| Author (year) | Population | T | n | G | N | p¥ | TT | n | TG | n | GG | n | p¥ | Refs. |
|--------------|------------|---|---|---|---|----|----|---|----|---|----|---|----|------|
| 1000 Genomes Project | | | | | | | | | | | | | | |
| Auton et al (2015) | MM | 0.603 | 298 | 0.397 | 196 | - | 0.377 | 93 | 0.453 | 112 | 0.17 | 42 | (21) |
| | AFR | 0.977 | 1291 | 0.023 | 31 | 2.17E-12 | 0.956 | 632 | 0.047 | 27 | 0.003 | 2 | 2.31E-19 |
| | EAS | 0.667 | 667 | 0.338 | 341 | 0.387 | 0.429 | 216 | 0.466 | 235 | 0.105 | 53 | 0.386 |
| | SAS | 0.604 | 591 | 0.386 | 387 | 0.988 | 0.38 | 186 | 0.448 | 219 | 0.172 | 84 | 0.997 |
| | EUR | 0.647 | 651 | 0.393 | 355 | 0.52 | 0.4 | 201 | 0.495 | 249 | 0.105 | 53 | 0.406 |
| | EUR IBS | 0.617 | 132 | 0.383 | 82 | 0.839 | 0.374 | 40 | 0.486 | 52 | 0.14 | 15 | 0.815 |
| | AMR | 0.707 | 491 | 0.293 | 203 | 0.121 | 0.496 | 172 | 0.424 | 147 | 0.081 | 28 | 0.084 |
| | AMR CLM | 0.803 | 151 | 0.197 | 37 | 0.002 | 0.638 | 60 | 0.33 | 31 | 0.032 | 3 | 7.20E-05 |
| | AMR MXL | 0.594 | 76 | 0.406 | 52 | 0.897 | 0.328 | 21 | 0.531 | 34 | 0.141 | 9 | 0.54 |
| | AMR PEL | 0.682 | 116 | 0.318 | 54 | 0.243 | 0.435 | 37 | 0.494 | 42 | 0.071 | 6 | 0.092 |
| | AMR PUR | 0.712 | 148 | 0.288 | 60 | 0.104 | 0.519 | 54 | 0.385 | 40 | 0.096 | 10 | 0.086 |

rs757110 (Tables III and IV). It appears that the distribution of these polymorphisms prevails among the majority of populations, while the ancestral allele is most frequent among African individuals. The results from the Colombian population may reflect the ancestral admixture history of the country, where the African component is widely spread across the Pacific and Caribbean regions (39-41).

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To the best of our knowledge, this is the first report of ABC28 rs757110 and rs1799854 SNPs in Mexican populations, and more specifically, in diabetic Mexican patients.
Table IV. ATP binding cassette subfamily C member 8 S1369A allelic and genotypic frequency comparison.

| Author (year)          | Population | T  | n   | G   | n   | p¥   | TT  | n   | TG  | n   | GG  | n   | p¥   | Refs. |
|-----------------------|------------|----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|------|-------|
| 1000 Genomes Project  |            |    |     |     |     |      |     |     |     |     |     |     |      |       |
| Auton et al (2015)    | MM         | 0.569 | 281 | 0.431 | 213 | 0.304 | 75 | 0.53 | 131 | 0.166 | 41 |  (21) |
|                       | AFR        | 0.975 | 1289 | 0.025 | 33 | 1.45E-13 | 0.953 | 630 | 0.044 | 29 | 0.003 | 2 |  1.72E-23 |
|                       | EAS        | 0.639 | 644 | 0.361 | 364 | 0.195 | 0.395 | 199 | 0.488 | 246 | 0.117 | 59 | 0.331 |       |
|                       | SAS        | 0.585 | 572 | 0.415 | 406 | 0.819 | 0.354 | 173 | 0.462 | 226 | 0.184 | 90 | 0.652 |       |
|                       | EUR        | 0.648 | 652 | 0.352 | 354 | 0.252 | 0.404 | 203 | 0.489 | 246 | 0.107 | 54 | 0.238 |       |
|                       | IBS        | 0.617 | 132 | 0.383 | 82 | 0.49 | 0.374 | 40 | 0.486 | 52 | 0.14 | 15 | 0.567 |       |
|                       | AMR        | 0.693 | 481 | 0.307 | 213 | 0.069 | 0.47 | 163 | 0.447 | 155 | 0.084 | 29 | 0.030* |       |
|                       | CLM        | 0.793 | 149 | 0.207 | 39 | 6.13E-04 | 0.606 | 57 | 0.372 | 35 | 0.021 | 2 |  2.52E-06* |       |
|                       | MXL        | 0.586 | 75 | 0.414 | 53 | 0.808 | 0.328 | 21 | 0.516 | 33 | 0.156 | 10 | 0.932 |       |
|                       | PEL        | 0.682 | 116 | 0.318 | 54 | 0.098 | 0.435 | 37 | 0.494 | 42 | 0.071 | 6 |  0.041 |       |
|                       | PUR        | 0.678 | 141 | 0.322 | 67 | 0.111 | 0.462 | 48 | 0.433 | 45 | 0.106 | 11 | 0.061 |       |
| Type 2 diabetics      |            |    |     |     |     |      |     |     |     |     |     |     |      |       |
| Zhang et al (2007)    | EAS (China)| 0.565 | 130 | 0.435 | 100 | 0.954 | 0.33 | 38 | 0.47 | 54 | 0.2 | 23 | 0.676 | (38) |
| Yokoi et al (2006)    | EAS (Japan)| 0.592 | 1884 | 0.408 | 1296 | 0.742 | 0.358 | 570 | 0.468 | 744 | 0.174 | 276 | 0.655 | (25) |
| Klen et al (2014)     | EUR (Slovenia)| 0.619 | 193 | 0.381 | 119 | 0.471 | 0.378 | 59 | 0.481 | 75 | 0.141 | 22 | 0.536 | (32) |
| Nicolac et al (2009)  | EUR (Croatia)| 0.607 | 277 | 0.393 | 179 | 0.585 | 0.395 | 90 | 0.425 | 97 | 0.18 | 41 | 0.301 | (33) |
| Sokolova et al (2015) | East Russia (West Asia)| 0.623 | 1763 | 0.377 | 1065 | 0.436 | 0.393 | 556 | 0.46 | 651 | 0.146 | 207 | 0.414 | (35) |

*P<0.05 (95% confidence interval); p¥, c² test of independence; CDMX, type 2 diabetics from Mestizo Mexico City (all of the following are 1000 Genomes Project third release): AFR, African; EAS, East Asian; SAS, South Asian; EUR, European; IBS, Iberian population in Spain; AMR, Admixed American; CLM, Colombians from Medellin, Colombia; MXL, Mexican ancestry from Los Angeles, USA; PER, Peruvians from Lima, Peru; PUR, Puerto Ricans from Puerto Rico.

rs757110 allelic and genotypic frequencies behaved similarly to rs5219 SNP, which was to be expected as it is well known that these SNPs form a haplotype. Yet, rs1799854 SNP frequencies seem extremely different to those reported by the 1000 Genomes Project and the literature on various diabetic populations (Table V). A comparison between allelic and genotypic frequencies in the investigated group and those populations reported by the 1000 Genomes Project, demonstrated differences between all but three samples: South Asian, European and Iberic. The Iberic population may have affected the comparison result of the EUR sample, as it was included in the EUR group in the 1000 Genome data. This was consistent with the fact that other EUR diabetic populations from different ethnic origins were statistically different when compared with the MM group (UK, Croatia and Poland). These results may reflect the Hispanic admixture dating from Mexico's colonial past; however, it is interesting and unexpected that this was not observed in the other Latin-American populations reported. It was demonstrated by the results of the previous Mexican health and nutrition national survey (ENSANUT 2012) that diabetes diagnosis and first level medical attention have improved considerably in Mexico (5), yet the percentage of treated patients actually maintaining adequate glycemic control remains poor. If this lack of control is genetic, at least partially, the differences observed in Mexican genetic SNP frequencies may significantly contribute to explaining treatment failure. This first screening may facilitate with understanding where focus and investigations are required to establish whether genetic structure and pharmacological failure are associated.

It would be interesting to observe whether a healthy control group of MM individuals from central Mexico behaves the same as diabetic subjects, and to analyze groups of different ethnicity within the country to establish whether those results are consistent with or different from the present study.

The SNPs, rs5219 (E23K) and rs757110 (S1369A) form a haplotype, where K23/A1369 has been identified as a risk genotype (35,42,43). While electrophysiological studies have demonstrated that channels containing the K allele and K23/A1369 are less sensitive to ATP inhibition (44,45), the SU response of these polymorphic channels appears to be different depending on the drug. For example, it has been shown that K23/A1369 channels are more sensitive to SU gliclazide, yet these same channels are less sensitive to inhibition by SUs, such as tolbutamide and glimepiride, while glibenclamide demonstrated no significant inhibition difference in any haplotype (45,46). In another study, K allele carriers exhibited significantly higher secondary treatment failure than E allele homozygous (29) treated with SU and ME; however, Dawed et al (19) demonstrated that secondary failure on patients treated with a combination of SU and ME, carrying the K allele polymorphism of rs5219 may be more involved with diabetes progression than with SU response (19).

In Mexico, the most common first level treatment for T2DM combines ME with glibenclamide administration (5).
In this first analysis, whose objective was mainly SNP frequency description, the authors included patients receiving glibenclamide or ME either as a mono- or combined therapy, as the SNP distribution is not affected by treatment. In future studies, the aim will be to investigate clinical implications of using ME-only treated patients as a control group to distinguish the ME effect.

The present results may contribute to future studies to clarify whether there is a real association.

The aim of the present study was to describe the genetic architecture of three pharmacogenetically important SNPs of ABCC8 and KCNJ11 within an MM population. To the best of our knowledge, this study is the first to report allelic and genotypic frequencies of ABCC8 rs757110 and rs1799854 SNPs in an MM population. Diabetes is a major concern in Mexico, and current pharmacological treatment is considered insufficient, as shown by the latest national health survey. Therefore, understanding the characteristics of our population is a priority for elucidating a viable hypothesis to improve our knowledge of this complex pathology. It is known that individual responses to SU are affected by clinical factors, such as baseline glucose levels, disease duration, β-cell function and insulin resistance levels (51). However, multiple gene interaction may explain the marginal impact of each individual SNP, indicating the necessity to construct an interaction model.

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Table V. ATP binding cassette subfamily C member 8 -3C/T Allelic and genotypic frequency comparison.

| Authors (year) Population | C n | T n | p¥ | CC n | CT n | TT n | p¥ | Refs. |
|---------------------------|-----|-----|----|------|------|------|----|------|
| 1000 Genomes Project      |     |     |    |      |      |      |    |      |
| Auton et al (2015) MM     | 0.704 | 348 | 0.296 | 146 |      |      | 0.506 | 125 | 0.397 | 98 | 0.097 | 24 | - | (21) |
| AFR                       | 0.862 | 1139 | 0.138 | 183 |      |      | 0.006 | 0.741 | 490 | 0.241 | 159 | 0.018 | 12 | 7.96E-04* |
| EAS                       | 0.449 | 453 | 0.551 | 555 | 2.41E-04* | 0.198 | 100 | 0.502 | 253 | 0.3 | 151 | 2.20E-06* |
| SAS                       | 0.681 | 666 | 0.319 | 312 | 0.724 | 0.481 | 235 | 0.401 | 196 | 0.1  | 58 | 0.865 |
| EUR                       | 0.58  | 583 | 0.42  | 423 | 0.067 | 0.33  | 166 | 0.499 | 251 | 0.171 | 86 | 0.031* |
| EUR IBS                   | 0.612 | 131 | 0.388 | 83  | 0.17  | 0.393 | 42  | 0.439 | 47  | 0.168 | 18 | 0.169 |
| AMR                       | 0.464 | 322 | 0.536 | 372 | 5.35E-04* | 0.225 | 78  | 0.478 | 166 | 0.297 | 103 | 1.30E-05* |
| AMR CLM                   | 0.516 | 97  | 0.484 | 91  | 0.006* | 0.266 | 25  | 0.5   | 47  | 0.234 | 22 | 0.001* |
| AMR MXL                   | 0.422 | 54  | 0.578 | 74  | 5.10E-05* | 0.172 | 11  | 0.5   | 32  | 0.328 | 21 | 1.35E-07* |
| AMR PEL                   | 0.312 | 53  | 0.688 | 117 | 1.91E-08* | 0.071 | 6   | 0.482 | 41  | 0.447 | 38 | 3.15E-14* |
| AMR PUR                   | 0.567 | 118 | 0.433 | 90  | 0.044* | 0.346 | 36  | 0.442 | 46  | 0.212 | 22 | 0.022* |
| Type 2 diabetics          |     |     |    |      |      |      |    |      |
| He et al (2008) EAS (China)| 0.41 | 82  | 0.59  | 118 | 2.50E-05* | 0.14  | 14  | 0.54  | 54  | 0.32  | 32 | 1.03E-08* |
| Yokoi et al (2006) EAS (Japan) | 0.474 | 1507 | 0.526 | 1673 | 8.91E-04* | 0.233 | 371 | 0.481 | 765 | 0.286 | 454 | 2.90E-05* |
| Matharoo et al (2013) SAS (India) | 0.568 | 227 | 0.433 | 173 | 0.044* | 0.405 | 81  | 0.325 | 65  | 0.27  | 54 | 0.006* |
| Glyn et al (2001) EUR (UK) | 0.464 | 412 | 0.536 | 476 | 5.35E-04* | 0.191 | 85  | 0.545 | 242 | 0.264 | 117 | 3.50E-06* |
| Nicolac et al (2009) EUR (Croatia) | 0.489 | 223 | 0.511 | 233 | 0.002* | 0.197 | 45  | 0.583 | 133 | 0.219 | 50 | 1.30E-05* |
| Dvoracka et al (2007) EUR (Poland) | 0.45 | 36  | 0.55  | 44  | 2.54E-04* | 0.25  | 10  | 0.4  | 16  | 0.35  | 14 | 6.00E-06* |

*P<0.05 (95% confidence interval); p¥, c² test of independence; CDMX, type 2 diabetics from Mestizo Mexico City (all of the following are 1000 Genomes Project third release): AFR, African; EAS, East Asian; SAS, South Asian; EUR, European; IBS, Iberian population in Spain; AMR, Admixed American; CLM, Colombians from Medellin, Colombia; MXL, Mexican ancestry from Los Angeles, USA; PER, Peruvians from Lima, Peru; PUR, Puerto Ricans from Puerto Rico.
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