Polymorphisms of the genes \textit{ABCG2}, \textit{SLC22A12} and \textit{XDH} and their relation with hyperuricemia and hypercholesterolemia in Mexican young adults [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Hyperuricemia is a pathological condition associated with risk factors of cardiovascular disease. In this study, three genetic polymorphisms were genotyped as predisposing factors of hyperuricemia.

Methods: A total of 860 Mexicans between 18 and 25 years of age were genotyped for the \textit{ABCG2} (rs2231142), \textit{SLC22A12} (rs476037), and \textit{XDH} (rs1042039) polymorphisms, as predisposing factors of hyperuricemia. Biochemical parameters were measured by spectrophotometry, while genetic polymorphisms were analyzed by real-time PCR. An analysis of the risk of hyperuricemia in relation to the variables studied was carried out using a logistic regression.

Results: Male sex, being overweight or obese, having hypercholesterolemia or having hypertriglyceridemia were factors associated with hyperuricemia ($p \leq 0.05$). The \textit{ABCG2} polymorphism was associated with hyperuricemia (OR = 2.43, 95% CI: 1.41-4.17, $p = 0.001$) and hypercholesterolemia (OR = 4.89, 95% CI: 1.54-15.48, $p = 0.003$), employing a dominant model, but only in male participants.

Conclusions: The \textit{ABCG2} (rs2231142) polymorphism increases the risk of hyperuricemia and hypercholesterolemia in young Mexican males.

Keywords

\textit{ABCG2}, \textit{SLC22A12}, \textit{XDH}, hyperuricemia, hypercholesterolemia
Introduction

Hyperuricemia is an abnormal metabolic trait defined as serum uric acid levels above 6 and 7 mg/dL for women and men, respectively (Bardin & Richette, 2014), and is associated with other cardiometabolic risk factors such as obesity, diabetes, hypertension, and dyslipidemia (Martínez-Quintana et al., 2016; Zuo et al., 2016). Similarly, hyperuricemia can be a predictor of impaired kidney functions as well as kidney disease progression (Galán et al., 2018; Hsu et al., 2009, and Pérez-Navarro et al., 2020). Serum uric acid levels depend on many factors including diet, sex, lifestyle, and alcohol consumption, as well as genetic heredity. In fact, some genes involved in the metabolism of purines or urates have versions that appear to predispose to a hyperuricemic condition. Some of these are the ABCG2 gene that encodes a membrane transporter, which exports urates to the kidney and intestine (Woodward et al., 2009); and the SLC2A9 gene that encodes a kidney protein called GLUcose Transporter 9 (GLUT9), an important flow regulator of urates in the proximal tubules (Caulfield et al., 2008). In the same way, the XDH gene gives rise to an enzyme denominated xanthine dehydrogenase, which breaks down purines from nucleic acids, specifically the hypoxanthine–xanthine–urate conversion pathway (Harrison, 2002).

Recent studies have shown that genetic polymorphisms in ABCG2 and SLC2A9 genes are prevalent in the Mexican population and may contribute to an abnormal condition of hyperuricemia, even at young ages (Macías-Kauffer et al., 2019; Rivera-Paredez et al., 2019). Interestingly, there appears to be no reports to date in the literature on XDH gene polymorphisms in the Mexican population. Therefore, the aim of this study was to genotype the polymorphisms of the genes ABCG2 (rs2231142), SLC22A12 (rs476037), and XDH (rs1042039) in 860 young Mexican volunteers aged between 18 and 25 years of age as predisposing factors of hyperuricemia associated with risk factors of cardiovascular disease.

Methods

Study design and ethical statement

A cross-sectional design and a covenance sample of 860 subjects was conducted in the City of San Luis Potosí, México. All participants were college applicants during the spring period of 2017. Data collection was carried out according to the recruitment protocols of the University of San Luis Potosí, since applicants receive a clinical evaluation as part of the admission process. This project was approved by two Bioethics Committees: the former at the National Institute of Medical Sciences and Nutrition Salvador Zubirán (Approval # FNU-669-13-15-2), and the latter at the Faculty of Chemistry of the University of San Luis Potosí (Approval # CEID2017105-S). All participants provided written informed consent.

Participants

A total of 860 applicants to the state University of San Luis Potosí, Mexico in 2017, were included in this study (448 men and 412 women). All participants were approached to take part in the study by phone and email by means of an open invitation explaining the goals of the study. Even though the invitation was open, only some accepted to participate. Participants were not paid to take part. All individuals who provided written informed consent and met the inclusion criteria were included. The inclusion criteria which were as follows: aged between 18–25 years; born in the Mexican state of San Luis Potosí; and provided written informed consent.

Clinical assessment and sample collection

Since all participants at the University of San Luis Potosí, Mexico receive a clinical evaluation as part of the admission process, we accessed the university medical records. The weight in kilograms and height in meters of each participant was obtained for anthropometry using a digital scale (UM-081 model; Tanita, Tokyo, Japan) and a stadiometer (Seca 213, 2009; Seca, Hanover, MD, USA), respectively. From this data, the Body Mass Index (BMI) was calculated (kg/m²). Systolic and diastolic blood pressure were taken as the mean of two readings at a 5-min interval after 5 min in a seated position, employing the Omron model HBP-1300 portable meter (Omron Healthcare, Inc., IL, USA). Also, 6 mL of blood was obtained by venipuncture after a 12-h fast; serum was obtained by centrifuging at 1,000 x g for 10 min using a laboratory centrifuge Z306 Benchmark Scientific (NY, USA), and processed immediately. An addition, 3 mL of blood was collected and stored in ethylenediaminetetraacetic acid (EDTA) tubes (Vacutainer®) for subsequent DNA purification. All samples were maintained at -20°C until their analysis.

Measurement of biochemical profiles

Serum was used to measure uric acid, glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides by spectrophotometry utilizing chemistry reagents (Paramedical S.r.l., Italy), specially developed to work with Mindray BS 300 Auto Chemistry Analyzer (Mindray, Shenzhen, China), with the following catalog numbers: uric acid (PDIBS200040), glucose (PDIBS200020), total cholesterol (PDIBS200030), LDL-C (PDIBS200170) HDL-C (PDIBS200160), and triglycerides (PDIBS200060).

Genotyping of polymorphisms

DNA was isolated from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The quality of DNA samples was verified by spectrophotometry using NanoDrop™ 2000. Polymorphisms of the genes ABCG2 (rs2231142), SLC22A12 (rs476037), and XDH (rs1042039) were measured by allelic discrimination real-time polymerase chain reaction (PCR) with the Tagman probe (Applied Biosystems®). Briefly, the sequences of the probes were the following:

ABCG2: GAACT(G/T)AACTTTCTCCTCAGGTAGTGTG;
SLC22A12: CCCTCCAGCCCTCAGCCACCTGCCC(A/G)CCA CATCCTCTGCTGCTGCTGCCC, and XDH: TTTATCTTTTC CAATGATTCAAGA(C/T)GGTAGAGGATTTAACCCT TCCA.
All PCR reactions were carried out using an ABI Prism 7900 HT detection system in 96-well plates (Applied Biosystems®). Reactions were adjusted to a final volume of 10 µL including Master mix probes and 50 ng/µL of genomic DNA, with the following amplification protocol: denaturation 94° for 9 min; followed by 50 cycles of denaturation at 95°C for 30 s, and annealing and extension at 68°C for 11 min. Negative controls and duplicated samples were included to check the accuracy of the genotyping.

Statistical analysis
After analyzing the data distribution, comparison of medians between groups (normal uricemia vs. hyperuricemia) was carried out with the Mann–Whitney U test for continuous variables. Allelic and genotypic frequencies as well as the Hardy–Weinberg equilibrium were calculated for each polymorphism utilizing the chi-square test. Likewise, a logistic regression analysis was used to calculate the risk of hyperuricemia in relation to the variables studied and the influence of the different genotypes on uric acid levels in blood through a dominant model. The level of significance was set at $p < 0.05$. Data were analyzed using SPSS version 19.0 statistical software.

Table 1. Characteristics of the study participants classified by serum uric acid levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All participants median (p25-p75)</th>
<th>With hyperuricemia median (p25-p75)</th>
<th>With normal uricemia median (p25-p75)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>860 (100)</td>
<td>129 (15)</td>
<td>731 (85)</td>
<td>$&lt;0.001^a$</td>
</tr>
<tr>
<td>Men</td>
<td>448 (52)</td>
<td>88 (19.6)</td>
<td>360 (80.4)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>412 (48)</td>
<td>41 (10)</td>
<td>371 (90)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.0 (18.0-20.0)</td>
<td>19.0 (18.0-20.0)</td>
<td>19.0 (18.0-20.0)</td>
<td>0.138$^b$</td>
</tr>
<tr>
<td>BMI (Kg/m$^2$)</td>
<td>23.03 (20.56-26.58)</td>
<td>25.7 (23.0-29.7)</td>
<td>22.6 (20.2-26.1)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>110 (100-110)</td>
<td>110 (100-120)</td>
<td>110 (100-110)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>70 (60-70)</td>
<td>70 (70-80)</td>
<td>70 (60-70)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>79.0 (74.5-84.0)</td>
<td>81.0 (75.0-86.0)</td>
<td>79.0 (74.0-84.0)</td>
<td>0.087$^b$</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.3 (4.2-6.2)</td>
<td>7.6 (6.9-8.2)</td>
<td>4.9 (4.1-5.7)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>96.0 (71.0-133.0)</td>
<td>126.0 (96.0-178.0)</td>
<td>90.0 (68.0-126.5)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>150.0 (131.0-170.0)</td>
<td>164.0 (144.0-188.5)</td>
<td>146.0 (128.0-166.0)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>58.5 (40.8-70.05)</td>
<td>69.1 (49.7-94.3)</td>
<td>57.1 (40.2-73.0)</td>
<td>$&lt;0.001^b$</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>67.9 (58.8-76.9)</td>
<td>64.8 (56.1-74.9)</td>
<td>68.3 (59.3-76.9)</td>
<td>0.083$^b$</td>
</tr>
<tr>
<td>$ABCG2$ (rs2231142)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>G</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1298 (75)</td>
<td>183 (71)</td>
<td>1115 (76)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>422 (25)</td>
<td>75 (29)</td>
<td>347 (24)</td>
<td></td>
</tr>
<tr>
<td>Genotypes GG</td>
<td>490 (57)</td>
<td>64 (49.6)</td>
<td>426 (58)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>318 (37)</td>
<td>55 (42.5)</td>
<td>263 (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52 (6)</td>
<td>10 (7.9)</td>
<td>42 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Results
In total, 860 participants were included in this study: 52% men and 48% women (Alegría-Torres, 2021). Participant characteristics, as well as the allelic and genotypic frequencies of the $ABCG2$ (rs2231142), $SLC22A12$ (rs476037) and $XDH$ (rs1042039) gene polymorphisms, are presented in Table 1. Medians and genotypic distribution between hyperuricemic and normouricemic participants were analyzed using the Mann–Whitney U test, finding differences for sex, BMI, systolic and diastolic pressure, triglycerides, total cholesterol, and LDL-C ($p < 0.001$ for all), as well as a marginal distribution difference for the $ABCG2$ (rs2231142) polymorphism corresponding to G/T alleles ($p = 0.056$), therefore other genetic models were tested later. All the genotypes analyzed were in Hardy–Weinberg equilibrium: $ABCG2$ (rs2231142) $X^2 = 1.82 \times 10^{-6}$, $p = 0.9$; $SLC22A12$ (rs476037) $X^2 = 0.03$, $p = 0.84$; and $XDH$ (rs1042039) $X^2 = 0.08$, $p = 0.76$. Both allelic and genotypic distributions of the three polymorphisms studied did not differ significantly between men and women (Table 2).

Considering the statistically significant differences among the variables, a multivariate logistic regression analysis was performed, including sex, BMI, hypercholesterolemia, and
hypertriglyceridemia as predictors of hyperuricemia (Figure 1). A predisposition to abnormal serum uric acid levels was found in some conditions as follows: male sex (odds ratio (OR): 2.14, 95% confidence interval (CI): 1.41-3.24, \( p < 0.001 \)); overweight or obese (OR: 2.6, 95% CI: 1.71-3.88, \( p < 0.001 \)); having hypercholesterolemia (OR: 2.8, 95% CI: 1.47-5.46, \( p = 0.002 \)); or having hypertriglyceridemia (OR: 1.7, 95% CI: 1.04-2.69, \( p = 0.033 \)).

In Table 3, an analysis by genotype is shown for the ABCG2 (rs2231142), SLC22A12 (rs476037), and XDH (rs1042039) polymorphisms, employing a dominant model for all three. With respect to the ABCG2 (rs2231142) polymorphism, statistically significant differences were found between the GT+TT genotypes vs. GG for serum uric acid (\( p = 0.003 \)) total cholesterol (\( p = 0.005 \)) and HDL-C levels (\( p = 0.003 \)). GA+AA genotypes vs. GG of the SLC22A12 (rs476037) polymorphism only showed to influence LDL-C levels (\( p = 0.043 \)). Finally, no statistically significant differences were found by genotype for the XDH (rs1042039) polymorphism (\( p > 0.05 \)).

When the OR and the association between genotypes for the ABCG2 (rs2231142) and SLC22A12 (rs476037) polymorphisms and cardiovascular risks were calculated by sex, some significant results were found utilizing a dominant model. Table 4 reports that GT+TT genotypes for the ABCG2 (rs2231142) polymorphism significantly statistically increases the risk of hyperuricemia (OR = 2.43, 95% CI: 1.41-4.17, \( p = 0.001 \)) and hypercholesterolemia (OR = 4.89, 95% CI: 1.54-15.48, \( p = 0.003 \)), but only in male participants. On the other hand, no significant results were found for the genotypes of the SLC22A12 (rs476037) and XDH (rs1042039) polymorphisms.
Table 3. Analysis of variables by genotype for the *ABCG2* (rs2231142), *SLC22A12* (rs476037), and *XDH* (rs1042039) genetic polymorphisms.

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>ABCG2</em> (rs2231142)</th>
<th><em>SLC22A12</em> (rs476037)</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GT+TT median (25th-75th percentile range)</td>
<td>GG median (25th-75th percentile range)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.1 (20.4 – 27.0)</td>
<td>22.7 (20.2 – 26.3)</td>
<td>0.393</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>110 (100.0 – 110.0)</td>
<td>110 (100.0 – 110.0)</td>
<td>0.632</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>70.0 (60.0 – 70.0)</td>
<td>70.0 (60.0 – 70.0)</td>
<td>0.220</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>80.0 (76.0 – 85.0)</td>
<td>80.0 (75.0 – 84.0)</td>
<td>0.129</td>
</tr>
<tr>
<td>Uric Acid (mg/dL)</td>
<td>5.00 (4.2 – 6.2)</td>
<td>4.7 (3.9 – 5.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>94.0 (69.5 – 134.0)</td>
<td>92.0 (68.0 – 127.0)</td>
<td>0.634</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>151 (132.0 – 173.0)</td>
<td>145 (125.0 – 164.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>59.2 (42.5 – 74.8)</td>
<td>53.8 (38.8 – 72.7)</td>
<td>0.070</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>70.9 (62.3 – 78.5)</td>
<td>67.3 (57.8 – 76.8)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Figure 1*. Association of sex, body mass index and dyslipidemias as predictive factors of hiperuricemia using a multivariate logistic regression analysis. The cutoff criteria were based on the World Health Organization and the National Cholesterol Education Program Adult (ATP III). Overweight: body mass index ≥ 25, Obesity: body mass index >30; hypercholesterolemia: fasting serum cholesterol levels > 200 mg/dL; hypertriglyceridemia: fasting serum triglycerides levels > 150 mg/dL.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Dominant model for the ABCG2 (rs2231142) polymorphism GT+TT vs. GG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All participants</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Overweight and obesity</td>
<td>1.10 (0.78-1.56)</td>
</tr>
<tr>
<td>SP &gt;130 mmHg</td>
<td>1.15 (0.38-3.47)</td>
</tr>
<tr>
<td>DP &gt;85 mmHg</td>
<td>0.33 (0.03-2.99)</td>
</tr>
<tr>
<td>Fasting glucose &gt;100 mg/dL</td>
<td>4.1 (0.82-20.49)</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td><strong>1.49 (1.00-2.21)</strong></td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>0.84 (0.54-1.31)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td><strong>3.27 (1.62-6.59)</strong></td>
</tr>
<tr>
<td>LDL-C ≥130 mg/dL</td>
<td>3.1 (0.81-12.47)</td>
</tr>
<tr>
<td>Low HDL-C</td>
<td>0.37 (0.07-1.83)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; ABCG2, gene that encodes an ABC transporter subfamily G member 2; SLC22A12, gene that encodes an urate transporter 1 gene; XDH, gene that encodes a xanthine dehydrogenase.

*Mann–Whitney U test. The level of significance was set at p <0.05.

Table 4. Analyses for the ABCG2 (rs2231142), SLC22A12 (rs476037), and XDH (rs1042039) polymorphisms and risk associated by sex.
Discussion
A total of 860 college applicants between the ages of 18 and 25 years of both sexes were included in this study, an important age range to identify early disorders. Indeed, we found a 15% prevalence of hyperuricemia, being higher among males (19.6%) than females (10%), as previously observed in another study with comparable age groups in Mexico (Alegría-Díaz et al., 2018). Regarding uric acid levels, similar blood concentrations have also been reported in Mexican young persons (Pérez-Navarro et al., 2020). The subgroup with hyperuricemia was prone to higher levels of BMI, blood pressure, triglycerides, total cholesterol, and LDL cholesterol (Table 1). In fact, the association
between serum uric acid levels and the traits of metabolic syndrome has been previously analyzed (Lin et al., 2006; Zhang et al., 2020a), although some authors found no causal evidence of uric acid levels being associated with metabolic syndrome and its components (Wang et al., 2020). According to our results, being a man, being overweight or obese, or having dyslipidemia are related to high uric acid levels (Figure 1). Men have a lower capacity to eliminate urate via the kidney compared with females due to a deficiency of estrogen and progesterone (Hak et al., 2010). Likewise, the involvement of uric acid in lipid metabolism can lead to hyperuricemia, a condition considered as predictor factor of dyslipidemia (Kuwabara et al., 2020; Lima et al., 2015; Son et al., 2016) and can subsequently alter blood pressure (Teng et al., 2011; Zhang et al., 2020a). In this way, our results summarized in Figure 1 show that being male, being obese and having dyslipidemia increases the risk of hyperuricemia (Liu et al., 2020a).

The study of the genetic influence on blood uric acid levels has included the search for single nucleotide polymorphisms (SNP) in genes involved in purine metabolism and urate removal. Here, we analyzed three polymorphisms in three different genes, including ABCG2 (rs2231142), SLC22A12 (rs476037), and XDH (rs1042039). The first corresponds to the exchange of a glutamine for a lysine at position 141 of an adenosine triphosphate (ATP)-binding cassette transporter subfamily G member 2 (ABCG2). This exchange predisposes individuals to hyperuricemia (Nakashima et al., 2020; Wrigley et al., 2020). A frequency of 0.25 for the risk for T allele was found, a slightly lower prevalence than that reported in Asian and New Zealand populations (Kim et al., 2015; Liu et al., 2020b; Toyoda et al., 2019). The distribution of the T allele was marginal between hyperuricemic and normouricemic groups, being more frequent in the hyperuricemic group (p = 0.056). When a dominant model was carried out, the risk for the T allele was associated with hyperuricemia and hypercholesterolemia (total and HDL cholesterol). This association was confirmed in men, showing that the ABCG2 (rs2231142) polymorphism increases the risk for hyperuricemia 2.43 times (95% CI: 1.41-4.17, p = 0.001) and hypercholesterolemia 4.89 times (95% CI: 1.54-15.48, p = 0.003) in a dominant model. Other studies have also found a greater influence of this polymorphism in men (Narang et al., 2019) although, under some conditions, the ABCG2 (rs2231142) polymorphism could contribute to increased uric acid in women (Guo et al., 2020; Roman et al., 2020). Although initially our work focused on hyperuricemia in young people, the increase in serum cholesterol in men associated with the ABCG2 (rs2231142) polymorphism was evident. There are still few studies linking the ABCG2 gene and cholesterolemia. A relevant fact is that mRNA expression levels of ABCG2 appear to be higher in individuals with hypercholesterolemia (Rodrigues et al., 2009); likewise, the activity of ABCG2 has been associated with cholesterol levels both in vitro and in vivo (To et al., 2014).

The SLC22A12 (rs476037) polymorphism comprises the transition from guanine to adenine in the 3′-UTR region in the urate transporter 1 gene; this transition appears to modify uric acid levels (Flynn et al., 2013; Köttgen et al., 2013). In this study, the frequency of the minor allele was 0.32; however, there was no statistically different distribution of this between hyperuricemic and normouricemic groups. Although no differences in uric acid levels were associated with this polymorphism, lower LDL cholesterol levels were observed in the group of carriers of the A allele when data were analyzed in a dominant model (p = 0.043), suggesting a protective effect of this allele (Simon et al., 2014). Since the SLC22A12 (rs476037) polymorphism is located at a potential miRNA binding site (Flynn et al., 2013), the epigenetic mechanism involved in the regulation of uric acid levels needs to be studied.

With respect to the XDH (rs1042039) polymorphism, we studied the xanthine dehydrogenase polymorphism located in the 3′-UnTranslated (UTR) region of the XDH gene. The replacement of T by C is considered a risk factor related to hypertension (Wu et al., 2015). We found that the minor allele frequency was 0.37 for the C allele, contrary to what was reported in a Chinese population, where the C allele had a higher frequency, in addition to its being associated with hypertension (Wu et al., 2015). However, the link between the XDH (rs1042039) polymorphism and hypertension was not demonstrated in Taiwanese women (Lee et al., 2019). In the present study, we did not find a statistically significant different distribution of the C allele between the hyperuricemic and normouricemic groups, and the risk allele was also not associated with any component of the metabolic syndrome.

Finally, hyperuricemia is an undesirable condition that has been seen as a minor trait of metabolic syndrome, cardiovascular risk, as well as other types of disorders such as psoriasis and alopecia, which have been associated with high levels of blood uric acid (Guo et al., 2020; Ma et al., 2020; Talebi et al., 2020; Zhang et al., 2020a). Even though our criterion for hyperuricemia were defined as blood uric acid >6 mg/dL for women and 7 mg/dL for men, cut-off points could be reconsidered according to the considerations made by Alegría-Díaz et al. (2018). In this regard, a recent study considers uric acid levels below 5 mg/dL for men and below 2-4 mg/dL for women to be optimal for a lesser risk of cardiometabolic diseases in a Japanese population (Kuwabara et al., 2020). Although there are modifiable factors related to lifestyle, genetic inheritance plays a decisive role in the control of uricemia.

This study has two main limitations: i) only one polymorphism was genotyped for every gene, analysis of multiple SNPs by haplotypes could be more appropriate; and ii) new cut-off values for hyperuricemia have been suggested (Alegría-Díaz et al., 2018); however the conservative criteria of >6 mg/dL for women and 7 mg/dL for men were considered in this study.

Conclusions

In this study, we found that the ABCG2 (rs2231142) polymorphism increases the risk of hyperuricemia as well as of hypercholesterolemia in young Mexican males. Since the ABCG2 (rs2231142) polymorphism can modify the efficacy of statins in reducing cholesterol (Zhang et al., 2020b), carriers of the risk allele represent a vulnerable group of interest for future
pharmacogenetic research. Some considerations for future studies are including lifestyle and diet factors, the monitoring of the study population, and exploring more polymorphisms in the ABCG2, SLC22A12, and XDH genes, as well as studying haplotypes.

Data availability
Underlying data
Data mendeley: Polymorphisms of the genes ABCG2, SLC22A12 and XDH and their relation with hyperuricemia and hypercholesterolemia in Mexican young adults.

This project contains the following underlying data:

- DATABASE ART ARCHIVES OF PHYSIOLOGICAL AND BIOCHEMISTRY.sav (spreadsheet of participant data)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

References


Open Peer Review

Current Peer Review Status: 

Version 1

Reviewer Report 24 August 2021

https://doi.org/10.5256/f1000research.49473.r91797

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Jing He
Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Guangzhou Women and Children’s Medical Center, Guangzhou Medical University, Guangzhou, China

In the current study, the authors investigate the association between three polymorphisms in the \textit{ABCG2}, \textit{SLC22A12}, and \textit{XDH} genes and hyperuricemia risk by including 860 subjects. They found the \textit{ABCG2} rs2231142 polymorphism increases the risk of hyperuricemia and hypercholesterolemia in young Mexican males.

I have some comments that should be addressed by the authors:

1. In the Abstract, it's better provide the number of cases and controls, as well as the nucleotide alterations for the polymorphisms they chose.

2. The authors only chose one polymorphism for each gene, that was not rationalised. It's better chose at least two potentially functional SNPs for each gene.

3. In the Materials and Methods, there was no need to provide the sequences of the probes. The authors should provide the function of the selected polymorphisms.

4. False-positive report probability and statistical power should be calculated as the positive findings for the samples included were so small (see the following articles, in which I have been involved with: He \textit{et al.} Mol Carcinog. 2013; 52 Suppl 1: E70-9\textsuperscript{1} and He \textit{et al.} Mol Ther Nucleic Acids. 2018; 11: 1-8\textsuperscript{2}).

5. The authors found the \textit{ABCG2} (rs2231142) polymorphism increases the risk of hyperuricemia and hypercholesterolemia in young Mexican males. It is lacking functional validation for the function of \textit{ABCG2} (rs2231142) polymorphism. It's better to perform genotype-based mRNA expression analysis to further explore the potential role of the significant polymorphism (He \textit{et al.} Mol Ther Oncolytics, 2021; 20: 199-208\textsuperscript{3}; Zhuo \textit{et al.} Mol Ther Nucleic Acids, 2020; 22:17-26\textsuperscript{4}). From these two references, which I have been involved
with, the authors can perform genotype based mRNA expression analysis to further explore
the potential role of the ABCG2 (rs2231142) polymorphism.

References

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular Epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of
expertise to confirm that it is of an acceptable scientific standard, however I have
significant reservations, as outlined above.
The study assessed the relationship of ABCG2, SLC22A12, and XDH genes polymorphism on their relationship with hyperuricemia and risk factors of cardiovascular disease. It seems that the authors have provided a significantly huge amount of effort and cost in conducting this study. This polymorphism study could provide potential implications in the future on the prevention, diagnosis, and treatment of hyperuricemia associated with cardiovascular risk factors. The authors display the knowledge on good writing skills including grammar and word choice, sentence structure, and paragraph development. However, several points need to be addressed:

1. The study has provided a significant relationship on ABCG2 polymorphism gene in the risk of hyperuricemia on specific age of 18-25 years old. Detailed explanation has been provided for the reasons of relationship between ABCG2 polymorphism on hyperuricemia. However, are there any specific relations on the ABCG2 on specific young adults of age? Why perform the study in specific young adult age groups? Is it better to examine the relationship in a more general group?

2. Is there any consideration of excluding preexisting disease such as cardiovascular, liver, kidney, and secondary hyperuricemia (leukemia, myeloma, drugs induced, hypothyroidism, etc) in this study?

3. Is there any specific reason to choose the rs2231142 SNPs from all polymorphisms? The study should also describe the reasons to choose this rs2231142 SNP.

4. It has been revealed in the study that gender, obesity/overweight, hypercholesterolemia, and hypertriglyceridemia are associated with the risk of developing hyperuricemia. Therefore, in the analysis of the relationship of ABCG2 gene polymorphism and hyperuricemia, it is better to perform the analysis of other subgroups other than gender (such as BMI, cholesterol levels, and triglyceride).

5. The author should earlier define the diagnostic criteria used for hyperuricemia and its cut off value in the methods section.

Is the work clearly and accurately presented and does it cite the current literature?  
Yes

Is the study design appropriate and is the work technically sound?  
Yes

Are sufficient details of methods and analysis provided to allow replication by others?  
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Metabolic syndrome, hyperuricemia, insulin resistance, endocrinology, internal medicine, urology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Author Response 02 Apr 2021

**Jorge Alegría**, Universidad de Guanajuato, Guanajuato, Mexico

Regarding the five points discussed, we respond to each of them:

1. Although examining the relationship in a more general group could provide more information, this study included college applicants due to the facilities to be recruited. In addition, we directed the study to recognize risk factors in young population, an age group less likely to have metabolic diseases.

2. In fact, participants with pre-existing diseases were not excluded. As mentioned in the methods section: “All participants were approached to take part in the study by phone and email by means of an open invitation explaining the goals of the study”.

3. The ABCG2 (rs2231142) polymorphism is one of the most studied and it seems to have the greatest influence on uric acid levels in both men and women (Guo *et al*., 2020; Narang *et al*., 2019; Roman *et al*., 2020). Likewise, this polymorphism could be influencing both the mRNA expression levels and activity of ABCG2 modulated by cholesterol levels (Rodrigues *et al*., 2009; To *et al*., 2014), as this was mentioned in the discussion. In relation to the first point, most studies of polymorphisms focus on older patients or hemodialysis patients; however, we studied young people.

4. In order to summarize and present the most relevant results, the data were shown taking into account the genotype as a grouping criterion (Table 3). When the data was grouped and analyzed by another criterion (data not shown), there were no relevant findings.

5. Serum uric acid ≥6 and 7 mg/dL was considered as hyperuricemia for women and men, respectively (Table 1). We considered it more appropriate to include this information where the study group data are shown.
**Competing Interests:** No competing interests were disclosed.

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