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Inflammasome Genes Polymorphisms and Susceptibility to Gout. Is There a Link?

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ABSTRACT

Background: The inflammatory response in gout disease is induced by the activation of NLR family pyrin domain-containing 3 (NLPR3) signaling pathway mediated by IL-1β release. **Objective:** The objective of the study was to determine the association between single nucleotide polymorphisms (SNPs) within NLRP3 inflammasome genes and gout susceptibility. **Methods:** Mexican patients with gout from the National Rehabilitation Institute and General Hospital of Mexico were enrolled. A healthy control group was also included. We analyzed the frequency and allelic distribution of eight SNPs from seven different genes within the NLRP3 inflammasome signaling pathway: *TLR4* rs2149356, *CD14* rs2569190, *NLRP3* rs3806268, *NLRP3* rs10754558, *CARD8* rs2043211, *IL-1*β rs1143623, *P2RX7* rs3751142, and *PPARGC1B* rs45520937 SNPs. **Results:** We found that the SNP rs45520937 of *PPARGC1B* was associated with the risk of developing gout when it was analyzed using the dominant model (Odds ratio [OR] = 2.30; 95% confidence interval [CI]: 1.09-4.86; p = 0.030), and it is proposed that the adaptor molecule *CD14* rs2569190 polymorphism could be associated with a lower risk of gout under an additive model (OR= 0.41;95% CI: 0.16-1.05; p = 0.064). No significant associations were identified for the remaining SNPs. Conclusion: Our findings suggest that the *PPARGC1B* rs45520937 SNP is associated with gout susceptibility. (REV INVEST CLIN. 2022;74(3):147-55)

Keywords: Gout. SNPs. NLRP3. Inflammasome.

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INTRODUCTION

Gout is a chronic inflammatory disease induced by the deposition of monosodium urate crystals (MSUc) in joints and other soft tissues as a result of chronic hyperuricemia (values above 6.8 mg/dL)[1]. Its prevalence ranges between 1 and 10% worldwide, being 3.6-times more prevalent in men compared to women[2]. As a result of an active process of MSUc nucleation, gout presents a wide range of clinical manifestations, including acute attacks of arthritis, renal failure, hypertension, and cardiovascular disease. In fact, the inflammatory response in gout is triggered by MSUc deposition, which is recognized by Toll-like receptors types 2 and 4 (TLR2/TLR4), and CD14, as an adaptor molecule[3]. The NLR family pyrin domain-containing 3 (NLRP3) can be activated by the purinergic receptor P2X ligand-gated ion channel 7 (P2X7R) that senses the ATP concentration and induces downstream events that include the release of pro-inflammatory cytokines such as interleukins 1β (IL-1β) and 18 (IL-18)[4-6]. The ending of the inflammatory response is regulated by the proliferator–activated receptor gamma (PPARγ) that acts as an anti-inflammatory response[7].

Although the mechanism of gout pathogenesis is still an enigma, it is known that gout is a multifactorial syndrome where genetics is a key factor[8]. Recently, it has been proposed that single nucleotide polymorphisms (SNPs) may be related to gout susceptibility, suggesting that some SNPs may be involved in over activation and regulation of the NLRP3 inflammasome. To date, only a few reports have focused on SNPs as precursors of gout[9]. In addition, these studies have reported preliminary data exclusively from the European or Asian population, and no data regarding Latin American population are currently available on this topic. This latter population is of high interest because of its rich genetic background due to its diverse ancestries. Hence, studying this population could increase the knowledge on the role of SNPs in the pathogenesis of gout, allowing for the analysis of differences and analogies between heterogeneous populations. Therefore, we investigated the possible association between the SNPs within NLRP3 inflammasome genes and gout susceptibility in Mexican population.

METHODS

Study population

The study included a cohort of Mexican patients hospitalized or attending the outpatient Rheumatology clinic of the National Rehabilitation Institute Luis Guillermo Ibarra Ibarra (INRLGII) and General Hospital of Mexico Eduardo Liceaga. Inclusion criteria for gout patients were having a diagnosis of gout according to the American College of Rheumatology (ACR) and the European League Against Rheumatism classification criteria (EULAR)[10]. Exclusion criteria included the presence of concomitant chronic or autoimmune rheumatic disease, history of cancer, and ongoing immunosuppressive treatment. Healthy subjects, matched by sex, were included as a control group. These subjects were recruited from an internal database of our institution in which inclusion criteria were the absence of any type of chronic, autoimmune, or dysmetabolic disease, and with normal uric acid values (below 6.8 mg/dL). Body mass index (BMI) and serum biochemical parameters were obtained from all the participants. The Research Ethics Committee from the INRLGII approved the protocol (number INR16/16). The study was conducted according to the Declaration of Helsinki[11]. All the patients and participants signed the written informed consent which includes the approval for publication.

Biochemical parameters determination

Glucose, total cholesterol, and triglycerides concentrations were analyzed in blood plasma by an enzymatic–colorimetric method using the commercial Kit Uric Acid FS TBHBA, Kit Triglycerides FS, Kit Cholesterol FS, and Kit Glucose GOD FS (Diagnostic Systems, Germany). Quantification was made by spectrophotometry in a Beckman Coulter DTX 880 Multimode Detector.

SNPs selection and bioinformatic analysis

A detailed search to identify publications related to NLRP3 inflammasome polymorphism and gout susceptibility was performed in the electronic databases PubMed and ENBASE. Finally, eight SNPs from seven different genes were chosen (Table 1). The SNPs with

Table 1. Single-nucleotide polymorphisms within TLPR3 inflammasome genes and ancestry informative markers

Gene	dbSNP ID	Chromosome Location	SNP Type	Polymorphism
Inflammasome				
TLR4	rs2149356	9:120474199	Intron variant	G/T, Transversion Substitution
CD14	rs2569190	5:140012916	5 prime UTR variant	A/G, Transition Substitution
NLRP3	rs3806268	1:247587477	Synonymous variant	A/G, Transition Substitution
NLRP3	rs10754558	1:247612036	3 prime UTR variant	C/G, Transversion Substitution
CARD8	rs2043211	19:48737706	Stop gained	A/T, Transversion Substitution
IL-1β	rs1143623	2:113595829	Upstream gene variant	C/G, Transversion Substitution
P2RX7	rs3751142	12:121622419	Synonymous variant	T/G, Transversion Substitution
PPARGC1B	rs45520937	5:149212430	Missense variant	A/G, Transition Substitution
AIMs				
–	rs2695	9:82884577	Intergenic variant	C/T, Transition Substitution
POGZ	rs4528122	1:151441314	Intron variant	C/T, Transition Substitution
FMN2	rs986690	1:240288119	Intron Variant	A/G, Transition Substitution
AQR	rs2862	15:34853352	3 prime UTR variant	C/T, Transition Substitution
SAP30L	rs3340	5:154452307	Intron Variant	C/T, Transition Substitution
CKM	rs4884	19: 45306777	Synonymous variant	A/G, Transition Substitution
–	rs722098	21: 15313279	Intergenic variant	A/G, Transition Substitution
CA10	rs203096	17:51934409	Intron variant	G/T, Transversion Substitution
CCL17	rs223830	16: 57418059	3 prime UTR variant	C/T, Transition Substitution
DRD2	rs1800498	11: 113420866	Intron variant	A/G, Transition Substitution
PRKCE	rs281478	2: 46173272	Intron variant	C/G, Transversion Substitution

SNP: single nucleotide polymorphism; dbSNP: the NCBI database of genetic variation; AIMs: ancestry informative markers.

a minor allele frequency (MAF) above 1% for the Mexican population were selected; the identification was made using data from the 1000 Genomes Project that provides the description of common human genetic variation[12] (Table 1). Genetic variation data for all the SNPs were obtained from the HapMap project (http://www.hapmap.ncbi.nlm.nih.gov/) and the public National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp). The SNPs should not be in linkage disequilibrium. Since the Mexican–Mestizo population is admixed, the ancestry was analyzed using 11 ancestry informative markers (AIMs) differentiating mainly the Amerindian, African, and European ancestries (Table 1) as previously reported[13,14].

Samples

A peripheral blood sample was collected from participants by specialized health staff. Genomic DNA (gDNA) was obtained using the commercial kit QIAmp DNA® Blood Mini Kit (QIAGEN, Hilden, Germany); gDNA concentration and purity were analyzed using the NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific). A Biobank of gDNA (50 ng/mL) and blood sera were kept at −80°C for further analysis.

Genotyping assay

SNPs were genotyped using pre-designed Taqman probes (NLRP3, C_26646014_10 and C_26052028_10; TLR4, C_11270899_20; CD14, C_16043997_10; CARD8, C_11708080_1_; IL-1β, C_1839941_10; and PPARGC1B, C_86507005_10). To perform the genotyping assay, 2.5 ng/μL of gDNA were mixed with 2.5 μL of TaqMan® Universal PCR Master Mix (Applied BioSystems) on 96-well plates and amplified using the StepOnePlus Real-Time PCR Systems (Applied Biosystems) according to the manufacturer protocol.

Table 2. Clinical and biochemical characteristics of the study population (n = 463)

Variable	Control (%) 243 (52.48)	Gout (%) 220 (47.52)	p-value
Male (%)/Female (%)	199 (95)/11 (5)	208 (98)/4 (2)	
Age (years)	44.51 ± 8.38	52.37 ± 12.49	< 0.0001*
Weight (kg)	76.52 ± 10.36	79.47 ± 14.93	0.0097*
Height (m)	1.69 ± 0.07	1.67 ± 0.07	< 0.0001*
BMI (kg/m2)	26.63 ± 2.70	28.77 ± 4.28	< 0.0001*
Uric Acid (mg/dL)	5.43 ± 1.00	6.77 ± 2.01	< 0.0001*
Fasting Glucose (mg/dL)	100.0 ± 3392	98.19 ± 18.52	0.0525
Triglycerides (mg/dL)	137.0 ± 51.35	198.7 ± 120.5	< 0.0001*
Total Cholesterol (mg/dL)	159.8 ± 37.33	171.3 ± 38.19	< 0.0001*

Data are means ± SD; p values were assessed by Student's t test ($\alpha = 0.05$) and *p values were calculated by the Mann–Whitney *U* test ($\alpha = 0.05$). BMI: body mass index.

Conditions were as follows: an initial denaturation step at 94°C for 10 min, the amplification was performed by 40 cycles (95°C during 15 s), and the final elongation cycle at 60°C for 1 min. gDNA of gout patients and healthy subjects was genotyped by duplicate on the same plate. The allelic discrimination was analyzed by the Step One software V2.3. No discordant genotypes were observed in the duplicate samples.

Statistical analysis

The STATA version 14.0 (Texas USA) and the SPSS version 25.0 (Chicago, IL) were used for statistical analysis. Descriptive analysis was used for the demographic and clinical characteristics between groups (cases and controls). Significant differences were assessed by Student's t-test. Parametric data were reported as mean ± standard deviation (SD), ranges or percentages as appropriate. The Hardy-Weinberg (HW) equilibrium calculation was performed using Chi-square test with one degree of freedom. Allelic and genotype frequencies were estimated for all polymorphisms and groups were compared using Fisher's exact test. To analyze the AIMs genotypes, we inferred the ancestry percentage of the three main parental populations (European, Amerindian, and African) for each subject using the STRUCTURE v2.3.4 software (Pritchard Lab, Stanford University, USA) and the following parameters: a burning period of 150 thousand, 700 thousand Markov chain Monte Carlo repeats, an admixture model with an alpha initial value of one and using an individual alpha for each population, independent allele frequencies among populations, and a constant lambda.

Multiple conditional logistic regression model analyses were carried out to estimate the association of each SNP with gout; results were estimated by the odd's ratio (OR) and 95% confidence interval (CI). The analysis was adjusted by sex, age, BMI. and ancestry, taking into account dominant, codominants, recessive, and additive inheritance models. The association between SNPs and the metabolic values was determined by linear regression analyses. $p < 0.05$ was used for declaring significant association.

RESULTS

Characteristics of the study population

We included 504 subjects; 42 of them were excluded (17 did not give their consent to participate in the study; 12 had incomplete clinical information, and in 13 samples were insufficient or contaminated). Finally, a total of 463 subjects were included in the study, 220 gout patients and 243 healthy subjects. The study population came from 20 different states of Mexico, 71.1% from the central area, and 28.9% from the rest of the country. The study included individuals with generations born in the same state. Table 2

Table 3. HW Equilibrium and MAF of 8 SNPs of NLPR3 Inflammasome in controls and diverse populations

Gene	SNP	HWE Control (n = 243)	HWE Gout (n = 220)	Minor Allele	MAF Control	MAF (1000 Genomes)		
						Mexican-Americans	Europeans	Africans
TLR4	rs2149356	0.534	0.006	T	0.29	0.32	0.31	0.82
CD14	rs2569190	0.480	0.246	G	0.49	0.45	0.51	0.70
NLRP3	rs3806268	0.683	0.683	A	0.42	0.46	0.55	0.01
NLRP3	rs10754558	0.110	0.652	G	0.16	0.20	0.46	0.25
CARD8	rs2043211	0.615	0.405	T	0.25	0.27	0.33	0.18
IL-1β	rs1143623	0.108	0.223	C	0.45	0.54	0.71	0.92
P2RX7	rs3751142	0.179	0.622	T	0.41	0.31	0.08	0.16
PPARGC1B	rs45520937	0.087	0.226	A	0.28	0.28	0.04	0.00

HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency.

describes the demographic and clinical data of gout patients and healthy subjects. Most of the participants were male (98% and 95% in the gout group and healthy controls, respectively). The gout group was older than the control group ($p < 0.0001$). Patients with gout had higher BMI compared with healthy individuals ($p < 0.0001$). Similarly, uric acid values ($p < 0.0001$), total cholesterol ($p < 0.0001$), and triglycerides ($p < 0.0001$) were higher in gout patients with respect to healthy controls. Serum fasting glucose levels were not different between groups ($p > 0.0525$).

Regarding the ancestry of the overall population, after an accurate analysis, we found a median of 35% for both, European and Amerindian ancestry, and 30% for African. No statistically significant differences in terms of percentage of genomic content in European, Amerindian, and African were found between gout patients and healthy controls ($p = 0.44$, $p = 0.58$, and $p = 0.48$, respectively) (Supplementary table).

Allele and genotype distributions of NLRP3 inflammasome SNPs

The genotype distributions were consistent with those expected from the HW equilibrium analysis between the study groups in almost all the SNPs ($p > 0.05$), except for the rs2149365 (TLR4) in the gout group ($p = 0.006$) (Table 3). The MAF of the 8 SNPs in the control group was similar as the reported in 1000 Genomes Project (Table 3).

Association analysis of NLRP3 inflammasome genes

The association analysis was performed adjusting for age, sex, BMI, and ancestry under a logistic regression model. When analyzing the dominant model, only the missense SNP rs45520937 of *PPARGC1B* was associated with risk of developing gout (OR = 2.30; 95% CI: 1.09-4.86; $p = 0.030$), while neither codominant, recessive, nor additive models showed a significant association (Tables 4 and 5).

A lower risk for gout was found in the adaptor molecule *CD14* rs2569190 polymorphism when it was analyzed under an additive model, adjusted for age, BMI, and ancestry; nevertheless, the p-value showed only a tendency to the significance (OR = 0.41; 95% CI: 0.16-1.05; $p = 0.064$) (Table 4). The remaining SNPs did not present differences in the genotype and allele frequencies between gout patients and healthy controls in any of the inheritance models (Table 4).

Association of SNPs with biochemical values in the control group

To analyze whether SNPs were associated with metabolic values in the control group, a linear regression analysis was performed. Interestingly, under a dominant model adjusted for age, sex, BMI, and ancestry, the *IL-1β* rs1143623 SNP was associated with lower glucose levels (B [SE] = −7.32 [2.47]; $p = 0.004$),

Table 4. Association of 8 SNPs of NLPR3 inflammasome genes with gout disease

z	SNP	MAF		OR (95% CI)	p-value
		Controls (n = 243)	Gout (n = 220)		
TLR4	rs2149356	28.7	31.8	0.68 (0.33-1.40)	0.295
CD14	rs2569190	48.7	45.9	0.53 (0.25-1.13)	0.101
				0.41 (0.16-1.05)	0.064[&]
NLRP3	rs3806268	41.9	39.0	0.70 (0.33-1.47)	0.346
NLRP3	rs10754558	16.3	15.6	0.56 (0.26-1.22)	0.147
CARD8	rs2043211	24.7	24.1	1.35 (0.67-2.71)	0.407
IL-1β	rs1143623	44.7	37.0	1.20 (0.57-2.48)	0.632
P2RX7	rs3751142	41.0	39.3	1.12 (0.48-2.61)	0.802
PPARGC1B	rs45520937	28.3	34.9	2.30 (1.09-4.86)	0.030

Associations with gout were adjusted for age, sex, BMI and ancestry. p-values were calculated By logistic regression, using dominant model and [&]additive model. MAF: minor allele frequency; OR: odds ratio; CI: confidence interval.

Table 5. Association of PPARGC1B rs45520937 SNP with gout disease

Model	Genotype	Control	Gout	OR (95% CI)	p-value
Codominant	G/G	39 (53.4%)	38 (40.9%)	1	0.074
	A/G	27 (37%)	43 (46.2%)	2.14 (0.97-4.71)	
	A/A	7 (9.6%)	12 (12.9%)	2.99 (0.88-10.12)	
Dominant	G/G	39 (53.4%)	38 (40.9%)	1	0.030
	A/G-A/A	34 (46.6%)	55 (59.1%)	2.30 (1.09-4.86)	
Recessive	G/G-A/G	66 (90.4%)	81 (87.1%)	1	0.210
	A/A	7 (9.6%)	12 (12.9%)	2.03 (0.65-6.37)	

Associations with gout were adjusted for age, sex, BMI, and ancestry. p-values were calculated by logistic regression. MAF: minor allele frequency; OR: odds ratio; CI: confidence interval.

while the rs3751142 SNP of *P2RX7* was associated with higher triglycerides levels (B [SE] = 25.03 [11.31], p = 0.041) (Table 6). Furthermore, under an additive model adjusted for age, sex, BMI, and ancestry, CD14 rs2569190 polymorphism was associated with lower glucose levels, although the p-value only showed a tendency to the significance (B [SE] = −2.99 [1.59], p = 0.064; Table 6). No significant associations were found in the rest of the SNPs analyzed.

DISCUSSION

To date, there is still a lack of evidence regarding the genetic variations in the pathogenesis of chronic inflammatory diseases, including gout[15], especially in Latin America. To the best of our knowledge, this is the first study demonstrating a potential association of the SNPs within the NLRP3 inflammasome genes with the risk for developing gout in a Mexican cohort. As previously described, NLRP3 inflammasome

Table 6. Association of 8 SNPs with biochemical values in the control group

Gene	SNP	Uric acid		Glucose		Triglycerides		Cholesterol	
		B (SE)	p-value	B (SE)	p-value	B (SE)	p-value	B (SE)	p-value
TLR4	rs2149356	0.08 (0.27)	0.541	−0.71 (2.54)	0.781	−10.95 (10.44)	0.456	−2.12 (7.20)	0.631
CD14	rs2569190	−0.21 (0.31)	0.549	−2.35 (2.79)	0.367	10.06 (12.01)	0.459	8.65 (7.95)	0.300
		−0.22 (0.18)	0.295[&]	−2.99 (1.59)	0.064[&]	8.98 (6.97)	0.337[&]	1.55 (4.68)	0.751[&]
NLRP3	rs3806268	−0.16 (0.28)	0.887	−4.78 (2.52)	0.087	−10.10 (10.83)	0.398	−1.21 (7.30)	0.846
NLRP3	rs10754558	−0.06 (0.29)	0.485	−0.11 (2.71)	0.950	12.83 (11.37)	0.344	−2.13 (7.64)	0.760
CARD8	rs2043211	−0.02 (0.27)	0.737	2.86 (2.52)	0.253	−4.58 (10.70)	0.638	6.07 (7.14)	0.360
IL-1β	rs1143623	−0.27 (0.29)	0.333	−7.32 (2.47)	0.004	2.05 (11.32)	0.883	−9.53 (7.40)	0.149
P2RX7	rs3751142	0.53 (0.29)	0.173	−0.40 (2.78)	0.884	25.03 (11.31)	0.041	1.36 (7.78)	0.789
PPARGC1B	rs45520937	0.31 (0.26)	0.117	−3.03 (2.47)	0.252	0.51 (10.50)	0.746	8.60 (6.94)	0.153

Models were adjusted for sex, age, BMI, and ancestry (n = 243), and [&]p-values for recessive models were adjusted for age, BMI, and ancestry (n = 243).

activation plays a key role in gout since it triggers swelling and inflammation mediated by MSUc recognition, mainly in dendritic and phagocytic cells.

In our study population, we analyzed 8 SNPs of seven different genes of the NLRP3 inflammasome signaling pathway, which were previously associated with gout disease in other populations[16-25]. Since those studies were centered exclusively on the European and Asian populations, we focused our study on a Mexican cohort taking into account their biological admixture population.

Our cohort study shows that uric acid, cholesterol, and triglycerides values were statistically significantly higher in the gout group compared to the control group. However, these parameters are in the normal range values for the overall population. Our analysis of SNP rs2149356 of *TLR4* gene located at intron four did not show an association with the risk of gout. This result is in line with Torres et al.[26], who studied this polymorphism in a Spanish cohort, reporting no association with a risk for developing gout. Contrary, Qing et al.[27] reported a solid association between the TT genotype of the SNP rs2149356 with the risk of gout in a Chinese population (OR = 1.96; p = 7.9 x 10-5). Moreover, they demonstrated an over-expression of *TLR4* and IL-b induced probably by this SNP. Finally, Rashed et al.[22] showed that T allele is associated with gout risk in European population (OR = 1.12, p = 0.012), but not in a Polynesian cohort, in which they contrarily reported a decreased risk of gout (OR = 0.80, p = 0.011).

In our analysis, SNP rs2569190 of the *CD14* gene, under a dominant model adjusted for age, sex, BMI, and ancestry, did not show significant differences in the genotype and allele frequencies, but when the analysis was adjusted for age, BMI, and ancestry under an additive model, there was a tendency to a significantly lower risk for gout (p = 0.064, Table 4). Interestingly, it may suggest that SNP rs2569190 plays a protective role for gout in the Mexican population, contrary to what has been reported for the European and New Zealand Polynesian populations[16]. Further, we found no association between the NLRP3 rs3806268 and rs10754558 SNPs and the risk of developing gout, in line with previous results of Meng et al.[17] for the rs3806268 SNP. However, Deng et al.[19] contrast these results since they

reported a positive association of the rs3806268 SNP with the risk of gout when compared with the AA allele (OR = 1.83, p > 0.03) in a Chinese population. With respect to the *NLRP3* rs10754558 SNP, the previous studies conducted by Mckinney et al., Deng et al., and Wang et al.[16,19,23], in line with our results, did not find a significant association with risk of gout in the European, Polynesian, and Chinese populations, respectively. In contrast, Zhang et al.[24] found that the allelic frequency G of this SNP was significantly increased in gout patients compared with the group control in Chinese population (OR = 1.30, p = 0.003).

To date, only one SNP has been analyzed within the *CARD8* gene (rs2043211 SNP), which previously showed an association with gout risk in Chinese population[18]. Contrary, in Korean population this association was not found[20], similar to our findings. About IL-1β polymorphism, our data showed no difference in the allelic and genotype frequencies, in contrast to Mckinney et al.[16] who found a nominal allelic association for *IL-1*β rs1143623 in the combined European and Polynesian populations for the G allele (OR = 1.10, p = 0.020).

In our population, rs3751142 SNP of *P2RX7* was not associated with gout risk, as was also reported by Lee et al.[20] in Korean population; nevertheless, they described that the C/A genotype had a trend toward a higher risk of gout when it was combined with the T/T *CARD8* rs2043211 genotype. The *P2RX7* gene is particularly polymorphic, and therefore, it is necessary to study other SNPs in our population[21].

The NLRP3 inflammasome is regulated by PPAR-γ, which is a key regulator controlling both metabolism and inflammatory response[28]. Our analysis demonstrated that in the Mexican population, the presence of the rs45520937 SNP *(PPARGC1B)* was associated with the risk of developing gout (A/G-A/A genotypes). This result is in agreement with Chang et al.[25] who reported that the rs45520937 SNP causes Arg-265Gln (p.R265Q) substitution in the exon 5 of *PPARGC1B* and demonstrated that rs45520937 augments the NLRP3 inflammasome response, and the upregulation of IL-1 production. We are aware that it is necessary to assess the functional impact of this SNP in our population study and taking into consideration the results of Chang et al.[25], we hypothesize that overexpression of *NLPR3* and the inflammatory cytokine IL-1β is involved in the pathogenesis of gout in our population. The differences between the associations of SNPs in other populations and our results could be explained by the different ancestry of the populations, and even more, by the variables taken for adjusting in the statistical analyses, which are different in all the reports.

This study also showed an association between the inflammasome SNPs with metabolic values in the control group. Under the dominant model, IL-1β rs1143623 SNP was associated with lower glucose levels and *P2RX7* rs3751142 SNP was associated with higher triglycerides levels; however, there is not enough evidence for this relationship. Delgado-Lista et al.[29] described that *IL-1*β SNP rs1143623 affects triglycerides levels, but there are no data on the influence over cholesterol levels. Our data suggest that the inflammasome SNPs could have a potential genetic influence on these metabolic parameters.

We are aware that our study has some limitations. First, the sample size is limited to definitely support strong conclusions. However, we consider our results as a first step to open new research pathways on this topic, which has not been previously explored in our country. The expansion of the study is in progress, involving more patients and centers. This will allow us to elucidate better the role of SNPs in the genesis of gout, including its functional impact. Second, the cohort did not include all the regions of Mexico; however, we believe that the geographic cohort included may provide a global outlook to reach more solid results in a near future. Third, a hyperuricemia control group was not included. Fourth, the controls were only matched for sex, since age matching was difficult due to technical reasons. However, we will consider these aspects for the ongoing study, even the aim to increase the number of patients with at least four controls each. Finally, additional functional studies are necessary to further validate if *PPARGC1B* rs45520937 SNP has implications on the protein structure and function. Additional studies including more extensive geographic cohorts are needed to elucidate the solid role of NLRP3 inflammasome genes in gout. Despite these limitations, we believe that our study is relevant because we explored 8 of the most common SNPs reported so far in the literature, which allowed us to contrast our results with those in

other populations. This may open new research frontiers to determine the potential role of these SNPs, even in the newly established states of gout (i.e., tophaceous gout, erosive gout, first gout flare, and recurrent gout flares)[30], and also in asymptomatic hyperuricemia status, which is being frequently reported in recent years31. In conclusion, our findings suggest that rs45520937 SNP of *PPARGC1B* was associated with gout susceptibility.

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SUPPLEMENTARY DATA

Supplementary data is available at DOI: 10.24875/RIC.21000603. These data is provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

REFERENCES

1. Neogi T, Jansen T, Dalbeth N, Fransen J, Schumacher HR, Berendsen D, et al. Gout classification criteria: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2015;74:1789-98.
2. Peláez-Ballestas I, Sanin LH, Moreno-Montoya J, Alvarez-Nemegyei J, Burgos-Vargas R, Garza-Elizondo M, et al. Epidemiology of the rheumatic diseases in Mexico. A study of 5 regions based on the COPCORD methodology. J Rheumatol Suppl. 2011;86:3-8.
3. Xiao J, Zhang XL, Fu C, Han R, Chen W, Lu Y, et al. Soluble uric acid increases NALP3 inflammasome and interleukin-1β expression in human primary renal proximal tubule epithelial cells through the toll-like receptor 4-mediated pathway. Int J Mol Med. 2015;35:1347-54.
4. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440:237-41.
5. Pouliot M, James MJ, McColl SR, Naccache PH, Cleland LG. Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes. Blood. 1998;91:1769-76.
6. Di Virgilio F, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 receptor in infection and inflammation. Immunity. 2017;47:15-31.
7. Meng QQ, Feng ZC, Zhang XL, Hu LQ, Wang M, Zhang HF, et al. PPAR-γ activation exerts an anti-inflammatory effect by suppressing the NLRP3 inflammasome in spinal cord-derived neurons. Mediators Inflamm. 2019;2019:6386729.
8. Smith EU, Díaz-Torné C, Pérez-Ruiz F, March LM. Epidemiology of gout: an update. Best Pract Res Clin Rheumatol. 2010;24:811-27.
9. Clavijo-Cornejo D, Hernández-González O, Gutiérrez M. The current role of NLRP3 inflammasome polymorphism in gout susceptibility. Int J Rheum Dis. 2021;24:1257-65.
10. Neogi T, Jansen T, Dalbeth N, Fransen J, Schumacher HR, Berendsen D, et al. 2015 Gout Classification Criteria: an American college of rheumatology/European league against rheumatism collaborative initiative. Arthritis Rheumatol. 2015;67:2557-68.
11. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. JAMA. 2013;310:2191-4.
12. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. Nature. 2015;526:68-74.
13. Choudhry S, Coyle NE, Tang H, Salari K, Lind D, Clark SL, et al. Population stratification confounds genetic association studies among Latinos. Hum Genet. 2006;118:652-64.
14. Fernández-Torres J, Zamudio-Cuevas Y, López-Reyes A, Garrido-Rodríguez D, Martínez-Flores K, Lozada CA, et al. Gene-gene interactions of the Wnt/β-catenin signaling pathway in knee osteoarthritis. Mol Biol Rep. 2018;45:1089-98.
15. Niño-Moreno P, Turrubiartes-Martínez E, Oceguera-Maldonado B, Baltazar-Benítez N, Negrete-González C, Oliva-Ramírez B, et al. The role of NRAMP1/SLC11A1 gene variant D543N (1730G/A) in the genetic susceptibility to develop rheumatoid arthritis in the Mexican Mestizo population. Rev Invest Clin. 2017;69:5-10.
16. McKinney C, Stamp LK, Dalbeth N, Topless RK, Day RO, Kannangara DR, et al. Multiplicative interaction of functional inflammasome genetic variants in determining the risk of gout. Arthritis Res Ther. 2015;17:288.
17. Meng DM, Zhou YJ, Wang L, Ren W, Cui LL, Han L, et al. Polymorphisms in the NLRP3 gene and risk of primary gouty arthritis. Mol Med Rep. 2013;7:1761-6.
18. Chen Y, Ren X, Li C, Xing S, Fu Z, Yuan Y, et al. CARD8 rs2043211 polymorphism is associated with gout in a Chinese male population. Cel Physiol Bioche. 2015;35:1394-400.
19. Deng J, Lin W, Chen Y, Wang X, Yin Z, Yao C, et al. rs3806268 of NLRP3 gene polymorphism is associated with the development of primary gout. Int J Clin Exp Pathol. 2015;8:13747-52.
20. Lee SW, Lee SS, Oh DH, Park DJ, Kim HS, Choi JR, et al. Genetic association for P2X7R rs3751142 and CARD8 rs2043211 polymorphisms for susceptibility of gout in Korean men: multi-center study. J Korean Med Sci. 2016;31:1566-70.
21. Ying Y, Chen Y, Li Z, Huang H, Gong Q. Investigation into the association between P2RX7 gene polymorphisms and susceptibility to primary gout and hyperuricemia in a Chinese Han male population. Rheumatol Int. 2017;37:571-8.
22. Rasheed H, McKinney C, Stamp LK, Dalbeth N, Topless RK, Day R, et al. The Toll-Like Receptor 4 (TLR4) variant rs2149356 and risk of gout in European and Polynesian sample sets. PLoS One. 2016;11:e0147939.
23. Wang LF, Ding YJ, Zhao Q, Zhang XL. Investigation on the association between NLRP3 gene polymorphisms and susceptibility to primary gout. Gen Mol Res. 2015;14:16410-4.
24. Zhang QB, Qing YF, He YL, Xie WG, Zhou JG. Association of NLRP3 polymorphisms with susceptibility to primary gouty arthritis in a Chinese Han population. Clin Rheumatol. 2018; 37:235-44.
25. Chang WC, Jan Wu YJ, Chung WH, Lee YS, Chin SW, Chen TJ, et al. Genetic variants of PPAR-gamma coactivator 1B augment NLRP3-mediated inflammation in gouty arthritis. Rheumatology (Oxford). 2017;56:457-66.
26. Torres RJ. Toll-Like receptor 4 (TLR4) polymorphism rs2149356 and risk of gout in a Spanish cohort. Nucleosides Nucleotides Nucleic Acids. 2020;39:1424-31.
27. Qing YF, Zhou JG, Zhang QB, Wang DS, Li M, Yang QB, et al. Association of TLR4 gene rs2149356 polymorphism with primary gouty arthritis in a case-control study. PLoS One. 2013; 8:e64845.
28. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature. 2000;405:421-4.
29. Delgado-Lista J, García-Ríos A, Pérez-Martínez P, Solivera J, Yubero-Serrano LM, Fuentes F, et al. Interleukin 1B variant -1473G/C (rs1143623) influences triglyceride and Interleukin 6 metabolism. J Clin Endocrinol Metab. 2011;96:E816-20.
30. Bursill D, Taylor WJ, Terkeltaub R, Abhishek A, So AK, Vargas-Santos AB, et al. Gout, hyperuricaemia and crystal-associated disease network (G-CAN) consensus statement regarding labels and definitions of disease states of gout. Ann Rheum Dis. 2019;78:1592-600.
31. Estévez-García IO, Gallegos-Nava S, Vera-Pérez E, Silveira LH, Ventura-Ríos L, Vancini G, et al. Levels of cytokines and microRNAs in individuals with asymptomatic hyperuricemia and ultrasonographic findings of gout: a bench-to-bedside approach. Arthritis Care Res. 2018;70:1814-21.