Differential gene expression of ABCG2, SLC22A12, IL-1β and ALPK1 in peripheral blood leukocytes of primary gout patients, was associated to hiperuricemia and their comorbidities. A case control study

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Research

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Abstract

Background. Genes ABCG2, SLC22A12, and ALPK1 have been strongly associated with dysfunction of urate metabolism in patients with gout, but it is unknown how these transporters are expressed in patients with acute or chronic gout. Our objective was to analyze the gene expression of urate transporters and inflammation genes in peripherial blood from gout patients and controls, to determinate if the metabolic profile of gout patients can influence at the gene expression profile. Our objective was to analyze the expression of urate transporters ABCG2, SLC22A12 and inflammation molecules ALPK1 and IL-1β in peripheral blood leukocytes from gout patients and to compare them with their metabolic profile and with the gene expression of people without gout and without hyperuricemia.

Methods. A total of 36 chronic and acute patients and 52 controls were recruited, ABCG2, SLC22A12, IL-1β and ALPK1 gene expression was evaluated by quantitative real-time PCR. Correlations of gene expression with clinical and laboratory parameters of patients were also analyzed.

Results. IL-1β was significantly increased in PBMCs of patients when compared with their PMNLs ($p<0.05$). A significant increase in ABCG2 and IL-1β were found in PMNLs from patients compared to controls ($p<0.05$). Correlations of gene expression in patients were related to levels of serum uric acid (sUA), serum creatinine, CRP, triglycerides, BMI, kidney disease, hypertension, and metabolic syndrome.

Conclusions. Our data suggest that the leukocyte cells of the patients respond to the presence hyperuricemia and comorbidities expressing ABCG2 and IL-1β genes differentially compared to normouricemic and non disease state. Hyperuricemia, dyslipidemias and obesity should stimulate the differential gene expression of peripheral blood leukocytes (neutrophils and monocytes) even in an asymptomatic state.

Background

Gout is a multifactorial disease characterized mainly by acute joint pain, due to the immune response activated by the precipitation of uric acid crystals in the joints. This disease is currently associated with components of the metabolic syndrome such as obesity, hypertension and cardiovascular diseases (1, 2). Worldwide, gout is a frequent disease, reaching a prevalence of over 1% in developed countries and up to 3.9% in the United States (3). In Mexico, the reported prevalence is 0.35%, although it is suggested that it may reach 3% (4, 5). The inflammatory mechanisms of gout are known from in vitro studies and in animal models (6), but there are not much studies about inflammatory events outside the joint that serve as indicators of damage or the evolution of the disease. The intestine, liver, blood and saliva of patients can be exposed to high concentrations of sUA, even in an asymptomatic state and is not information about the perypheric molecular events involved in these conditions (7–9).

In recent years, have been studied genes related to the reabsorption and elimination of uric acid in the kidney and its association with gout (10–13). Currently, the function of each of these genes and their possible role in the gout etiology has begun to be studied in vitro or animal models (14–17). Human

genomic studies have linked various urate transporter genes such as ABCG2, SLC22A12 (Urate transporter 1 : URAT1 protein), SLC2A9 (GLUT9), OAT4 among others as possible risk factors (18, 19). Although low basal expression of urate transporters such as ABCG2 and SLC22A12 has been reported in peripheral blood of healthy people, we do not know if their leucocyte expression is increased in patients with gout and hyperuricemia and this may show some altered local cellular process; we also do not know whether these changes in expression are dependent on an inflammatory process or only depend on a hyperuricemic state or both

IL-1β cytokine is important in the response to MSU in gout attack has long been known, but little about its effect on ABCG2 and its interaction with other urate transporters. Studies in human intestinal knockdown cells of ABCG2 stimulated with MSU (Urate monosodium crystal) showed increases in IL-1β and IL8 respectively, therefore this and other studies suggest a relationship between transporters and inflammatory cytokines (14, 20). The ALPK1 kinase has been associated with the regulation of the expression of URAT1 and of testosterone in the kidney in murine models (21–24), therefore, there seems to be an interaction between the expression of inflammatory molecules and the activation of transporters at least in kidney and intestine. Monocytes and neutrophils are important cells in the inflammatory microenvironment of gout and are found in a large percentage in the peripheral blood. Both cell groups can induce their proliferation and activation depending on different pro-inflammatory stimuli, however monocytes have the main function of differentiating into macrophages or dendritic cells and amplifying the immune response, while neutrophils directly eliminate microorganisms (25–28). We hypothesized that there are differences in the gene expression of transporters and pro-inflammatory molecules between leukocytes (PBMCs or PMNLs) from gout patients and with controls and these differences are related to hyperuricemia.

Methods

Patients and controls

This study enrolled 36 gout patients and 52 controls (normouricemic without gout) (43 male and 9 female), through the Laboratory of Musculoskeletal Articular Ultrasound, Neuromuscular Diseases Laboratory at the Rheumatology department of the National Institute of Rehabilitation (INR) and volunteers from the National Institutes of Health of the metropolitan area were recruited from Mexico City.

The patients inclusion criteria was: diagnosis of gout according to the criteria of ACR/EULAR 2015: (1) the presence of sodium urate crystals seen in the synovial fluid using a polarized microscope or (2) at least six of 12 clinical criteria being met (29, 30). Exclusion criteria was: a) autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, b) bone dysplasias, c) diabetes mellitus, d) kidney stones. Controls, did not have hyperuricemia, gout, joint diseases, heart disease and kidney stones. The protocol was approved by the Ethics Committee of the National Institute of Rehabilitation (51/14).

Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments. The study was approved by the Ethics and Research Committee of the National Institute of Rehabilitation “Luis Guillermo Ibarra Ibarra” (Ref. number 51/14).

Informed consent

Informed consent was obtained from all individual participants included in the study. All participants were formally informed about the blood sample study and consented in writing to participate.

The clinical information, anthropometric and sociodemographic data of all participants was collected including height and weight were measured by trained medical personnel, and the body mass index (BMI, in kg/cm2) was calculated subsequently. The medical history was recovered from each patient to confirm the following: comorbidities of interest, e.g. hypertension, diabetes mellitus, hyperlipidaemia, obesity, tophi, chronic kidney disease (CKD), frequency of alcohol consumption (times / week), smoking and frequency of cigarette consumption (times / week). All patients underwent a clinical evaluation to establish or rule out the diagnosis of MS at the time of the study, according to the criteria of the Adult Treatment Panel III report (ATP III) (31). A total of 15 patients had the mono-allopurinol prescription (300 mg/day), indomethacin (50 mg/day), losartan (50 mg/day), metaprolol (100 mg/day), prednisone (50 mg/day), bezafibrate (400 mg/day) colchicine (1 mg/day) and aspirin (>325 mg/day). Determination of clinical parameters/biochemical data were made with a UniCel DxC 600 device, Synchron Clinical System. A peripheral blood sample was taken from fasting participants metabolic parameters how: serum uric acid (sUA), glucose (GLC), total cholesterol (TC), triglycerides (TG), high lipoprotein density (HDL), low density lipoprotein (LDL), creatinine (Cr) and C-reactive protein (CRP).

Isolation of cells and RNA extraction

The PBMCs and PMNLs, were isolated using gradient centrifugation (1.077 g/ml) with Polymorphprep. Total RNA of human PBMCs and PMNLs, was extracted using NucleoSpin (Nagel) Kit. RNA obtained was preserved at -20 ° C until RTqPCR was performed.

Analysis of gene expression by RT PCR

The total RNA was amplified by RTqPCR using the GoTaq® 1-Step RT-qPCR System real kit (Promega) and the Qiagen Rotor Gene Q kit (Qiagen). The components of the reaction were GoTaq qPCR Master Mix (13.5 µl), forward and reverse primers 1: 100 (2 µl and 1.5 µl, respectively) and total RNA (150-200 ng / µl); only the samples of total RNA with 1.8-2.0 absorbance at 260 nm were used to ensure their quality.

The analysis of gene expression required the extraction of RNAt (200 ng / µl) from mononuclear and polymorphonuclear cells of peripheral blood. The amplification conditions were: 37 ° reverse transcription for 15 min, activation of Taq Polymerase at 95 ° C for 10 minutes, 45 cycles of denaturation 95 ° C for 10 s, alignment 62 ° C for 10 s and elongation of 72 ° C for 10 s. RTqPCR was performed for each sample in duplicate. The primers are presented in suplemental table 1.

The ΔCt method of Livak and Schmittge was used to compare mRNA expression of the target genes in patients versus controls. The ΔΔCt method was used to compare mRNA expression between target genes and PMNL versus PBMC. GAPDH gene is an internal control to normalize the expression of each gen.

Statistical analysis

The statistical analysis of the data was assessed by using Shapiro Wilk to ensure the normality of the dates and followed by the Student's t test, U Mann Whitney or by a one-way analysis of variance (ANOVA) and Bonferrini, Games Howell post hoc test. Results were considered significant when $p<0.05$, $p<0.01$ or $p<0.001$. All tests were performed with SPSS 22 software (SPSS, Inc., Chicago, IL, USA). The calculation of the statistical power P = (1-β) in the analysis of gene expression was carried out using the GPower 3.1.9.2 program.

The correlations between the clinical data, clinical history and expression levels of the evaluated population were made with Spearman's Rho test or Pearson test. The values are significant $p<0.05$ or $p<0.01$.

Results

Clinical characteristics and biochemical parameters of patients and controls.

A total of 36 patients and 52 controls participated in the study. Clinical characteristics and biochemical parameters determined at the time of sampling are presented in Table 2. The values between patients and normouricemic controls without gout were compared, in general the patients presented a higher percentage of obesity (35.3%), hyperuricemia (7.88 ± 0.34 mg/dl), high TG (244.45 ± 28.92 mg/dl), very low HDL (38.15 ± 1.69 mg/dl) and high creatinine above the reference values (1.21 ± 0.18). These values were significantly higher than in the controls as shown in Table 3.

An important part of this work was to determine the profile of the metabolic status of the volunteers at the time of the study and to record their clinical history. All the patients were Mexican and lived in Mexico City. Mexico has approximately 70% of its adult population between 30 and 60 years old who are overweight (32, 33). The clinical data analysis of the patients showed that the subjects did not have adequate treatment or follow-up of their comorbidities associated with gout, since regardless of the age or stage of the disease, acute gout was (15.55%) or asymptomatic (84.44 %) we found metabolic alterations in most of them (1, 12, 34). 67.56% of the patients presented hyperuricemia, 75% hypertriglyceridemia, 67.64% low HDLs, 19.44% high creatinine, a high percentage of obesity (35.3%) and overweight (41.2%) and 10.8% of them had kidney disease previously diagnosed. It is important to mention that only 18.91% had hyperglycemia and that none of them had a diagnosis of diabetes. Therefore, this cohort of patients with asymptomatic or acute gout had poor monitoring of their comorbidities, which puts them at risk of new episodes of gout and of developing cardiovascular and kidney diseases. The percentage of people with a family history of gout in our study was 45.7%.

Analysis of gene expression in PBMC and PMNL of patients

We first analized gene expression in PBMCs and in PMNLs of patients to know how is gene expression between cells groups, This is important to know if a group enriched in neutrophils could express more or less each gene compared to the other rich in monocytes. We compared their relative expression as shown in the figure 1. According results, IL-1β increased its expression significantly in PBMCs compared to PMNLs in patients, therefore the other genes were expressed in a similar way between different cell groups. Neutrophils are approximately 50% of the total leukocytes in peripheral blood and 10 times more abundant than monocytes, so that under inflammation conditions their number increases this proportion (35, 36). Our results showed that there were significant differences in IL-1β ($p<0.01$) expression between PBMCs and PMNLs from patients . These results could be explained if we consider that monocytes and macrophages are producers of IL-1β. They express IL-1β as a regulator and amplifier of the immune response to activate other cells such as neutrophils, B and T lymphocytes and natural killers (37, 38).

To date, it is not clear if there is a gene expression profile in the blood in the patient with gout that can help us to know if the organism responds or is altered by frequent episodes of gout, by the frequency of hyperuricemia or if this profile Gene expression can change and leave a mark as the disease progresses. We compare the expression of the 4 genes between both cell groups in the patient samples.This is to know which genes are expressed more than others in the same group of patient's leukocytes. In PBMCs ABCG2 were expressed more than the other genes, but were only significantly higher with respect to URAT1 gene ($p <0.05$), while IL-1β was significantly higher than SLC22A12 ($p <0.05$) as shown in figure 2. In PMNLs, it was observed that ABCG2 are expressed more than the other genes, but it was only significant with respect to ALPK1 ($p <0.05$) and IL-1β ($p <0.01$). These results show that ABCG2 is the gene that is most expressed in patients, with a recent acute attack and regardless of the time with the disease, however a larger study population would help us to have these groups better represented to analyze differences with respect to to the progression or control of the disease.

Finally gene expression was analyzed in order to know if transporter genes increase their expression in the blood due to frequent exposure to high concentrations of uric acid and due to the presence of dyslipidemia and metabolic syndrome. We analized gene expression in PMNLs and PBMCs of ABCG2, ALPK1, SLC22A12 and IL-1β to compare results between patients and controls as showed in figure 1. ABCG2 expression in PMNLs had a significantly higher expression ($p <0.05$) in patients ($n=18$) than in controls ($n=18$) with a $P= 0.8875$. In PBMCs ABCG2 had a significantly higher expression ($p <0.05$) in patients ($n=15$) than in controls ($n=13$) see figure 3. IL-1β expression in PMNLs had a significantly higher expression ($p <0.05$) in patients ($n=13$) than in controls ($n=14$).

The higher expression of IL-1β in PMNLs from patients, compared to controls ($p <0.05$), could indicate that neutrophils are activated and, therefore, are capable of inducing cytokine expression due to the effect of circulating sUA in patients with hyperuricemia or by MSU in an affected joint during an acute attack.

SLC22A12 is expressed mainly in the luminal membrane of the proximal tubule of the kidney, although it can also be expressed in other tissues such as the liver, brain or in adipocytes (21, 24). URAT1 the

product of SLC22A12 is important for the reabsorption of uric acid and mutations and polymorphisms of gain of function have been associated with hyperuricemia and gout (39, 40). We did not find differences in the expression between PBMCs and PMNLs, as seen in Figure 2; This is probably due to the fact that it was the gene with the lowest expression in both PBMCs and PMNLs.The main function of URAT1 is more important in the kidney to reabsorb urate and not only eliminate it as ABCG2 does, there could also be more restrictions for its expression in blood as the interaction with regulatory proteins such as PDZK (PSD-95, Drosophila discs- large protein, Zonula occludens protein 1) 1 or hepatocyte nuclear factor (HNF1α / β) as mentioned in some studies (41-43).

Correlation Analysis of gene expression of patients

A correlation analysis of gene expression was performed with the biochemical parameters and the clinical history of the patients and of the total population (patients more controls). The results are shown in Table 4. In PBMCs from patients we found a positive correlation of the expression of ABCG2 with the creatinine levels of patients, this correlation was maintained when analyzed with the total study population. The mean levels of serum creatinine were found to be high in the patients as seen in Table 3; from the demographic data of the patients, we know that the history of kidney disease ranged from 10 to 16% as shown in Table 2. These data in PBMC suggest that the increase in the expression of ABCG2 in blood could be related to the presence of renal dysregulation.

Monocytes induce the expression of IL-1β releasing it to amplify the response and allow the recruitment of neutrophils, which also express the cytokine when activated (44). The expression of IL-1β significantly correlated in PMNLs with the history of hypertension of the patients as shown in Table 4. Hypertension has been associated with chronic inflammatory diseases, aging, diabetes and gout (45, 46). IL-1β is important in the inflammatory process that leads to endothelial dysfunction of blood vessels to develop hypertension. Comorbidities associated with gout such as obesity and hyperuricemia also favor endothelial dysfunction and dysregulation of the renin angiotensin system. Therefore, the increase in the expression of this cytokine could be a biomarker of damage and inflammation in patients with gout and its increase together with ABCG2 in the blood could be associated with a pre-symptomatic state.

In PBMCs from patients, we found significant positive correlations between SLC22A12 expression with metabolic syndrome, sUA and triglyceride levels (these correlations were not found in controls, data not shown). These correlations may suggest that despite the low expression of the gene, the gene could be activated by metabolic alterations in the patient. An obesity / metabolic syndrome model in mice on a high-fat diet found increased SLC22A12, GLUT9, and ABCG2 expression in the kidney after 8 weeks but not in other transporters (47), While in another study the expression of SLC22A12 and GLUT9 was measured in mice after a diet rich in fructose, inducing metabolic syndrome and hyperuricemia in the animals, they reported an increase in the expression of these two genes (36). There are also different reports that link hyperuricemia with increased expression of URAT1 in the kidney, blood and salivary gland (48, 49). In PMNLs, as seen in Table 4, the expression of SLC22A12 correlated positively with hypertension only in patients. A possible relationship in humans of URAT1 with metabolic syndrome and

hypertriglyceridemia has been little studied. In humans, associations of metabolic syndrome and obesity with variants of SLC22A12 (rs11602903 and rs11231825) that predispose to gout have been reported (50, 51). Finally, in PMNLs we also found a correlation of the expression of SLC22A12 with the presence of kidney disease in patients and this was maintained in the total population, as seen in Table 4.

In PBMCs from patients we found correlations of ALPK1 expression with creatinine and uric acid levels. In human monocytes, induction of ALPK1 in response to MSU has been reported in monocytes and macrophages (52), its could indicate that leukocytes express ALPK1 in response to high uric acid concentrations or subsequent local activation of monocytes and macrophages during an acute attack. ALPK1 is known to be associated with kidney disease through association studies and its expression in animal models can regulate the release of the chemokines CCL2 and CCL5 in kidney cells (53). However, we did not find a correlation with a history of kidney disease, so the expression of ALPK1 in PBMCs may be increased by inflammatory signals in the blood, kidney or intestine due to frequent hyperuricemia in patients even in an asymptomatic state.

In PBMCs of the total population we found the correlation of the expression of ALPK1 with the BMI. In our patients the expression of the gene was not different from the controls that have a lower percentage of obesity and overweight, however the controls do have a positive correlation of BMI with the expression of ALPK1 ($p < 0.05$ $n = 22$). This could be due to the larger differences in BMI between controls than between patients, since 56.6% were in normal weight as opposed to 23.5% of patients. There are few studies on the relationship of ALPK1 with obesity. A study in transgenic murines reported that testosterone could negatively regulate ALPK1 by decreasing the production of IL1-β and TNF-α. Kuo et al., Proposed ALPK1 as a negative regulator of SLC22A12 in kidney, they measured in ALPK1-deficient transgenic mice the expression of SLC22A12 in kidney, which was higher than in wild types, while testosterone levels decreased in blood and testicles (16). In our work we did not find a negative or positive correlation between ALPK1 and SLC22A12 in the blood, however it does not mean that it is not found in the kidney or intestine.

In PMNLs of patients we found a correlation of ALPK1 with CRP and although there are no significant differences in CRP with controls, the average CRP in patients was high (5.98 ± 4.6 mg / L), however, this increase could indicate a pro-inflammatory state in some patients which together with the presence of hyperuricemia favors the recruitment of neutrophils and a new attack of gout. Correlation of ALPK1 gene expression with sUA could support this idea, while the correlation with serum creatinine levels could support the presence of pro-inflammatory factors (54). The increased expression of ALPK1 and SLC22A12 in the blood of patients with gout could be associated with their altered metabolic status, which favors a long-term chronic condition with recurrent acute episodes if hyperuricemia and comorbidities are maintained (55). A genomic study analyzed the epistasis of ALPK1 with the loci of GLUT9, ALPK1, SLC22A12 and ABCG2, finding a positive prediction value (PPV≥81%) to measure the risk of gout (OR≥12.30) using variants of these genes in different populations. with gout, so this gene seems to continue to be linked to gout, although its importance in its progression is not evident (56).

Discussion

Urate transporters are important in the homeostasis of the balance of uric acid elimination and absorption in the kidney and intestine; however, many questions remain about its role in the development and progression of gout. Mutations and SNIPs of SLC22A12 and ABCG2 transporters have been found in different populations that suggest that dysfunction of their proteins favors hyperuricemia and gout, while ALPK1 polymorphisms in populations with predisposition to gout suggest that this protein could be active during acute attack (10, 52, 57). Most patients with gout have a different profile of gene polymorphisms associated with the disease, so genetics do not seem to be sufficient to explain why some people develop gout and others do not.

In this work we analyze the expression of 3 specific genes associated with gout in peripheral blood leukocytes of patients with acute, chronic and asymptomatic gout. ABCG2 was the gene with the most important findings because it showed greater expression than the others in PMNLs and PBMCs and also with respect to the controls in both cell groups. We interpret this as a response of different cell types to the presence of uric acid in high concentrations in serum.

The profile of the patients in this study showed that despite having differences in time with the disease, type of treatment and symptomatology, ABCG2 gene expression was higher that people with normouricemia and without gout. Despite this, it is not clear whether this increase in expression reflects what can happen in other organs specialized in eliminating uric acid, such as the intestine or the kidney. It is known that salivary and brain cells respond by overexpressing ABCG2 by increasing uric acid levels in their environment, so that different cells could induce the expression of the gene under similar conditions (41, 48). Chen et al., Reported that exposure to sUA increased the expression of ABCG2 in intestinal cells, which activated the inflammasome and the PI3K / Akt pathway, so there is a possibility that soluble uric acid can activate the expression of abcg2 in peripheral blood leukocytes (14). Unlike controls, in patients there is a high probability of the presence of inflammation, caused by hyperuricemia or its comorbidities, which may favor the expression of ABCG2. Pro-inflammatory factors such as TNF-α, IL-1β and IL-6 have been reported to increase ABCG2 expression in various in vitro models (8-10). It is possible that ABCG2 increases its expression in blood and tissues in a state of hyperuricemia, trying to eliminate sUA in different cell types. In people with mutations that decrease the elimination of urates, hyperuricemia could be latent. Ultimately, high concentrations of uric acid would activate the innate immune response by over-expressing IL-1β in neutrophils and monocytes, which will favor an acute episode of gout if urate homeostasis is not regulated. One posibility of exist another cause to explain the differences in ABCG2 expression between patients and controls; such as the ingestion of drugs in the patients prior to taking the sample, this could alter the expression of ABCG2, however, most of the patients in treatment did not show high levels of expression of ABCG2 compared to the average of all patients and either we found correlation of gene expression with medicated patients.

The expression of ABCG2 in the blood should be evaluated in the different stages of the progression of gout, the attention of the metabolic syndrome and control of uric acid levels in people with hyperuricemia

and patients with gout could help to better prevent kidney dysfunction and cardiovascular diseases (11, 12). Understanding the role of ABCG2 in different organs of the patient with gout will allow us to know the key points of its dysregulation and suggest more effective pharmacological therapies. A more complete study could show us if there are groups of patients that strongly increase the expression of ABCG2 in acute attack conditions, or with specific comorbidities that maintain subclinical inflammation, with hyperuricemia without treatment for a long time, etc. This would be important to know if the gene responds significantly in the early stages of the disease, in the chronic state, or is indistinct while there are acute periods, as suggested by our results. The implications of this knowledge would be reflected in more effective therapies for gout and its comorbidities

It is known about the risk of chronic kidney disease due to the presence of hyperuricemia and about the role of URAT1 in kidney function, and the importance of this transporter with different kidney diseases is recently being understood (58, 59). A study in murines has reported that animals previously treated with reverastrol reduced renal SLC22A12 expression and consequently hyperuricemia caused by uric acid reabsorption by URAT1 in the kidney (60, 61). Despite the low gene expression of the SLC22A12, they could be related to hyperuricemia and the consequent kidney damage in people with a longer time with the disease and poor control of hyperuricemia. In the case of patients with kidney disease, they could have overexpression of the transporter in the kidney if there are no mutations present, however a longitudinal study is needed that considers uric acid levels, renal dysfunction and the treatment of each patient to compare in these cases the expression of SLC22A12 (60).

Gene expression of ALPK1 may be increased by inflammatory signals in the blood, kidney or intestine due to frequent hyperuricemia in patients even in an asymptomatic state. The increased expression of the alpk1 protein and the urate transporters SLC22A12 and ABCG2 could be part of an altered molecular profile in response to continuous episodes of gout and comorbidities that favor inflammation. A follow-up study to analyze the variation of the gene and protein expression of these molecules will help us to know if they can be candidates for markers of damage by the disease.

Conclusions

Differential gene expression of ABCG2, SLC22A12, IL-1β and ALPK1 in peripheral blood leukocytes of primary gout patients (chornic or acute) correlated with hiperuricemia and their commorbidities such as dyslipidemias, obesity, hypertension, metabolic syndrome, kidney disease and high levels of creatinine in blood.

Our data suggest that the leukocyte cells of the gout patients respond differentially to hyperuricemia and comorbidities, increase gene expression of ABCG2 and IL-1β compared to normouricemic and non gout controls.

Declarations

Ethics approval and consent to participate

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments. The study was approved by the Ethics and Research Committee of the National Institute of Rehabilitation “Luis Guillermo Ibarra Ibarra” (Ref. number 51/14). Informed consent was obtained from all individual participants included in the study. All participants were formally informed about the blood sample study and consented in writing to participate.

Consent for publication

All authors agree to the writing sent to publish

Availability of data and materials

All the information in this manuscript and the full results are available for free review.

Competing Interest

The authors declare no conflict of interest.

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Author Contributions

Paniagua-Díaz Natsuko: support in carrying out the experiments, analysis of results and revision of the manuscript, Sanchez-Chapul Laura: support in the design of experiments, analysis of results and revision of the manuscript, Clavijo-Cornejo Denise, support in carrying out the experiments, analysis of results and revision of the manuscript, Ventura-Ríos Lucio support in the design of experiments, analysis of results and revision of the manuscript, Aguilar-Salinas Carlos support in the design of experiments, analysis of results, wrinting and revision of the manuscript, Sanchez-Muñoz Fausto, support in analysis of results, wrinting and revision of the manuscript, López-Macay Ambar support in carrying out and design of experiments, analysis of results wrinting and revision of the manuscript.

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Tables

Tables 1 through 4 can be found in the Supplemental Files section.

Figures

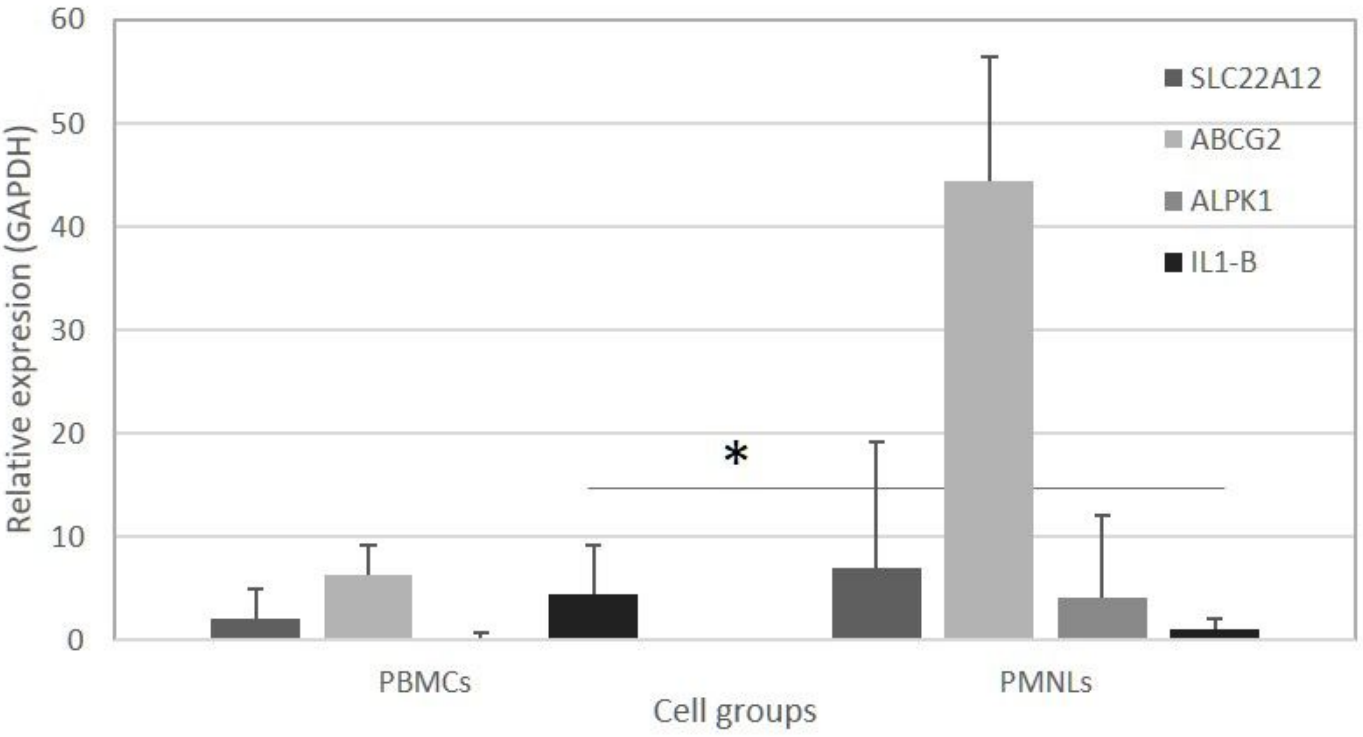

Figure 1

Comparison of the gene expression of ABCG2, SLC22A12, ALPK1 and IL-1β between PBMCs and PMNLs from patients. The graph shows the average and standard error SD. * represents significant differences ($p<0.01$).

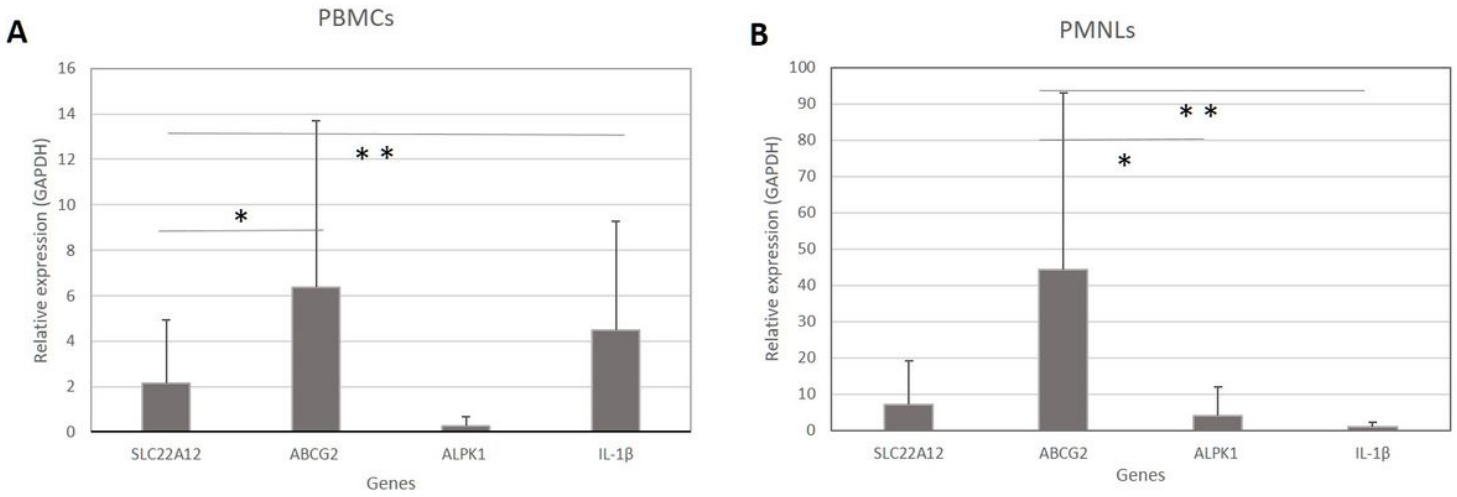

Figure 2

Comparison of ALPK1, SLC22A12, IL-1β and ABCG2 gene expression between PBMCs and PMNLs in gout patients. The relative expression of all genes evaluated in PBMCs A) and B) PMNLs is shown. * represents significant differences, ** ($p<0.01$), *($p<0.05$). The graph shows the average and standard error SD.

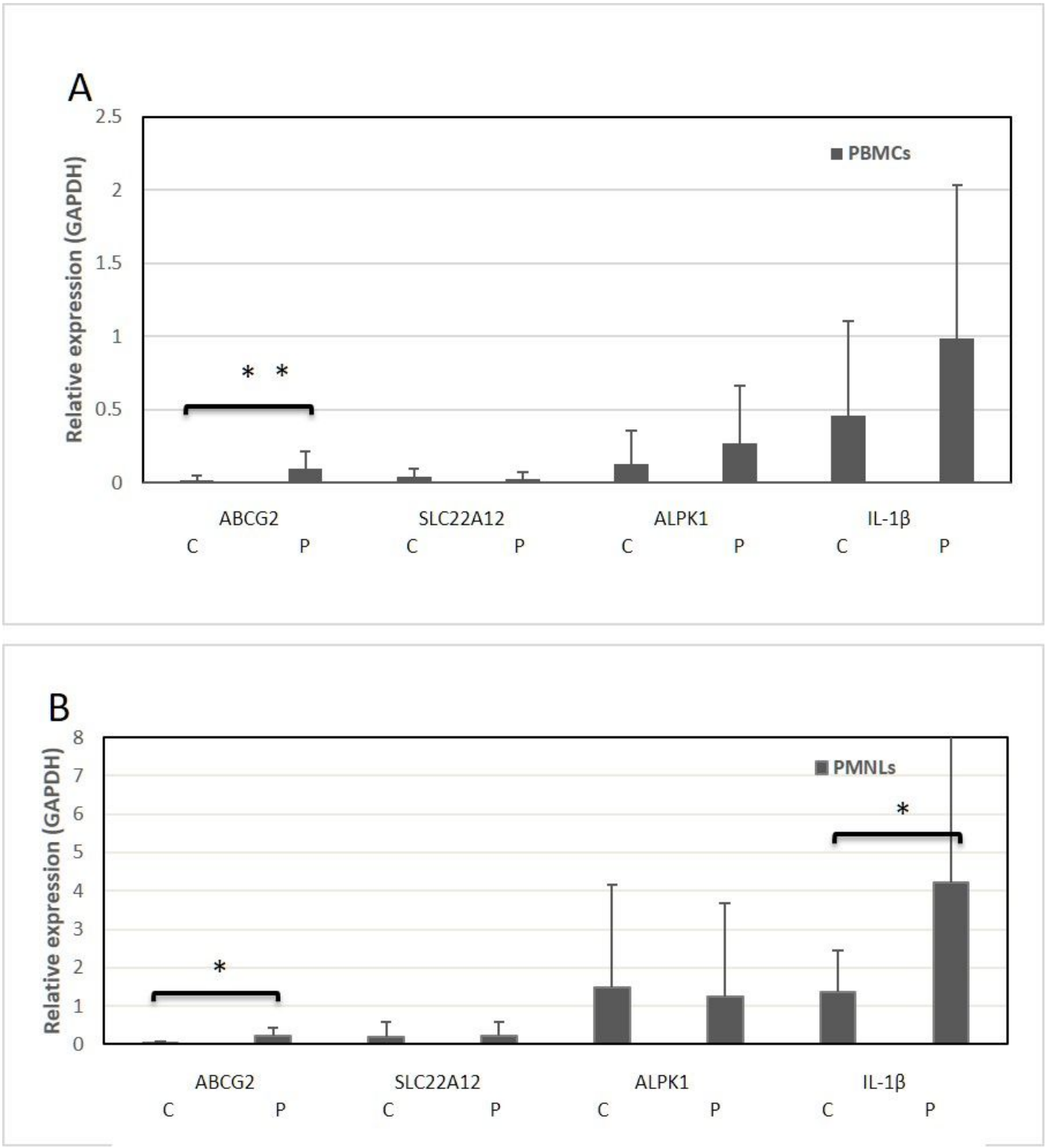

Figure 3

Comparison of the gene expression of patients vs controls in PBMCs and PMNLs. C = controls, P= patients. Analysis de la relative expression in PBMCs A) and B) PMNLs of patients and controls. *represents significant differences ($p<0.05$) or ** ($p<0.01$). The graph shows the average and standard error SD.

Supplementary Files

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- TABLE1.jpg
- TABLE2.jpg
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