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ORIGINAL ARTICLE

PRL -1149T allele (rs1341239) is associated with decreased risk of rheumatoid arthritis in population from southern Mexico: analysis of mRNA expression and PRL serum levels

J. E. Navarro-Zarza[1] · J. F. Muñoz-Valle[2,3] · C. J. Baños-Hernández[4] · I. Parra-Rojas[4] · Z. Reyes-Castillo[2] · H. Rangel-Villalobos[5] · J. Hernández-Bello[2,3]

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Abstract

Introduction Prolactin (PRL) is a sex hormone with immunomodulatory properties, and it is associated with the clinical activity of rheumatoid arthritis (RA). The -1149G>T polymorphism at the prolactin (*PRL*) gene has been associated with autoimmune diseases, but its functional effect is unclear.

Objective To analyze the association of the *PRL* -1149G>T polymorphism with disease susceptibility, mRNA, and protein expression of PRL in RA patients from Southern Mexico.

Methods We included 300 RA patients and 300 control subjects (CS). Genotypes were identified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, the *PRL* mRNA expression was determined by real-time PCR, and PRL serum levels were measured by enzyme-linked immunosorbent assay.

Results Applying genetic models of inheritance (dominant, recessive, and additive), we found an association between the T allele and decreased RA susceptibility (OR = 0.55, 95% CI 0.35–0.87, $p = 0.009$; OR = 0.09, 95% CI 0.012–0.76, $p = 0.011$; OR = 0.49, 95% CI 0.32–0.76, $p = 0.001$, respectively). RA patients had higher mRNA expression and soluble levels of PRL than CS ($p < 0.05$). The PRL serum levels were similar in RA and CS according to genotypes. However, in CS, carriers of GT and TT genotypes showed lower *PRL* mRNA expression than GG genotype carriers (7.1-fold and 20-fold respectively, $p = 0.006$).

Conclusions This study demonstrated that the *PRL* -1149T allele is a genetic marker of decrease risk to RA in population from Southern Mexico, and it is associated with low *PRL* mRNA.

Key Points

- *PRL -1149T allele is a marker of decreased RA susceptibility in population from southern Mexico.*
- *PRL -1149TT genotype is associated with low PRL mRNA expression.*
- *RA patients have higher mRNA expression and soluble levels of PRL than healthy subjects.*
- *PRL serum levels are higher in those RA patients with < 2 years of disease evolution.*

Keywords -1149G>T polymorphism · mRNA expression · *PRL* gene · Prolactin · Rheumatoid arthritis

✉ J. Hernández-Bello
jorge89_5@hotmail.com

[1] Departamento de Medicina Interna-Reumatología, Hospital General de Chilpancingo "Dr. Raymundo Abarca Alarcón", Chilpancingo de los Bravo, Guerrero, Mexico

[2] Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada 950, Independencia Oriente, 44340 Guadalajara, Jalisco, Mexico

[3] Instituto Transdisciplinar de Investigación y Servicios, Universidad de Guadalajara, Zapopan, Jalisco, Mexico

[4] Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Chilpancingo de los Bravo, Guerrero, Mexico

[5] Instituto de Investigación en Genética Molecular, Centro Universitario de la Ciénega, Universidad de Guadalajara (CUCiénega-UdeG), Guadalajara, Mexico

Introduction

Rheumatoid arthritis (RA) is an autoimmune and chronic systemic disease of the connective tissue, characterized by progressive destruction of the joints [1]. Genome-wide association studies have identified more than 30 loci contributing to RA risk; of these, the strongest associations have been reported for the *HLA* genomic region, *PTPN22*, and *IL23R* genes [2]. In our research group, we have also confirmed the association of RA with some of these loci (*PTPN22*, *CTLA4*, *PADI4*, *MIF*, and *PRL*) in RA patients from the western Mexican population [3–7].

The increased prevalence of RA in women suggests that female hormones could be a key factor in the development of this disease [8]. A variety of physiological conditions associated with excess estrogen and progesterone production (e.g., during the postovulatory phase of the menstrual cycle and during pregnancy) seem to be protector factors for RA in women, as decreased joint symptoms are shown in those conditions [9]. Conversely, other female hormones, such as prolactin (PRL), appear to be detrimental to RA at high levels [10].

PRL is a 23-kD sex hormone secreted in the pituitary gland, through the hypothalamic–pituitary–adrenal axis. However, this hormone can also be produced in extra-pituitary sites, such as peripheral blood mononuclear cells (PBMCs), on which exerts different bioactivities, including the inhibition of negative selection of autoreactive B lymphocytes, which may promote autoimmunity [10].

Hyperprolactinemia (HPRL) has been observed in 6% of RA patients compared to 3% in the normal population, and this condition was associated with increased disease activity in RA patients [11, 12]. HPRL can affect B cell tolerance induction by impairment of BCR-mediated clonal deletion, deregulation of receptor editing, and reduction of the threshold for activation of anergic B cells, thereby promoting autoreactivity [13].

The *PRL* gene is located on the short arm of chromosome 6, near the *HLA-DRB1* region. This gene has two promoters, one of which controls the pituitary PRL production and the other controls extra-pituitary PRL production [14]. The extra-pituitary promoter has the -1149G>T polymorphism (rs1341239), which seems to be associated with serum prolactin levels [10, 15, 16] and mRNA expression [17]. Electrophoretic mobility shift assays have shown that the *PRL* -1149G>T polymorphism alters the binding of a member of GATA-transcription factor family, which regulates *PRL* gene expression. The peripheral blood lymphocytes from patients with the -1149GG genotype treated with phytohemagglutinin expressed higher *PRL* mRNA than from those with the -1149TT genotype [18].

There are few studies published to date evaluating the .-1149G>T polymorphism as a genetic marker in RA [17, 19], which agree in the association between the -1149T allele with decreased susceptibility to RA; however, studies in RA have demonstrated that some genetic variants are restricted to specific ethnic groups and its magnitude of risk may be influenced by interactions with other genes and environmental factors. Therefore, there is little evidence to generalize regarding the association between *PRL* gene and RA pathogenesis. In this study, we examined the relationship between the *PRL* -1149G>T polymorphism and RA risk in patients from a Southern Mexican population, and its effect on PRL serum levels and *PRL* mRNA expression.

Materials and methods

Study population

The study was performed in 300 patients with RA and 300 control subjects (CS) from Guerrero State (South of Mexico), sequentially recruited from the Hospital General de Chilpancingo "Dr. Raymundo Abarca Alarcón," Guerrero, Mexico. The patients were matched with CS, considering age, gender, geographic region, and ethnicity. A rheumatologist evaluated all patients, considering physical assessment, evaluation of the clinical activity according to the Disease Activity Score-28 (DAS-28), as well as the extent of disability according to the Spanish version of health assessment questionnaire disability index (Spanish HAQ-DI). Before enrollment, all patients and controls gave informed consent.

Laboratory assessment and autoantibodies titers

Peripheral blood for analysis was collected from RA patients and CS. The erythrocyte sedimentation rate (ESR) was measured using the Wintrobe method. Serum levels of high-sensibility C-reactive protein (hsCRP) and rheumatoid factor (RF) were quantified by a turbidimetric assay (COD31029 and COD31030, respectively; BioSystems, Spain). Serum anti-cyclic citrullinated peptide (anti-CCP) antibodies were measured with a second-generation enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (FCCP400; DIASTAT, Axis-Shield Diagnostics, UK). For autoantibodies, the cut-off value of seropositivity was defined by the manufacturer as follows: RF > 20 IU/mL and anti-CCP > 5 U/mL.

PRL concentration

Quantification of PRL serum levels was performed only in 100 female RA patients and 100 female CS matched by age, excluding those conditions such as pregnancies, endocrine-treatment, renal or any other disease, and medication that are known to affect serum PRL concentrations. Serum PRL concentrations were measured according to the manufacturer's instructions, using the EIA-1291ELISA assay from DRG

International. The sensitivity of the assay was 0.35 ng/mL. Hyperprolactinemia (HPRL) was defined as PRL serum levels > 20 ng/mL.

Genotyping

DNA was extracted from the peripheral blood leucocytes using a classical salting out method. Genotypes of the *PRL* -1149G>T polymorphism were obtained by PCR-RFLP method, using previously reported conditions [17]. Further genotyped validation was done by DNA sequencing of a subset of samples randomly selected, through a DNA Genetic Analyzer ABI Prism 310 (Applied Biosystems, Foster, California) (data not shown). Also, we carefully re-genotyped 30% of the individuals using internal controls for wild-type, heterozygous, and mutant genotypes for each amplification process.

RNA extraction and quantitative real-time PCR

The *PRL* mRNA expression was determined by quantitative real-time PCR (qPCR). Total RNA was extracted from 5 mL of peripheral blood of 25 RA patients and 25 CS matched by age, according to Chomcyznski and Sacchi technique [20]. One microgram of total RNA was converted to cDNA using oligo-dT and M-MLV reverse transcriptase (Promega Corp., Madison, WI, USA). *PRL* mRNA quantification was performed in a LightCycler® 96 (Roche Applied Science, Penzberg, Germany) equipment using FastStart Essential DNA Green master kit (Roche Applied Science). *GAPDH* was used as a reference housekeeping gene; all *PRL* and *GAPDH* qPCR reactions were run in triplicate. A melting curve analysis was performed immediately after PCR reactions for each gene to determine the reaction's specificity. Relative expression analysis was performed by the $2^{-\Delta Cq}$ and $2^{-\Delta\Delta Cq}$ methods.

Statistical analysis

Statistical analysis was performed using STATA Software v12.0 and GraphPad Prism v6.0, and a p value < 0.05 was accepted as the level of statistical significance. Chi-square (χ^2) test was used for comparison of the genotype and allele frequencies as to Hardy–Weinberg equilibrium (HWE) calculations, and the dominant, recessive, and additive genetic models for the polymorphism were also evaluated. A Cochran–Armitage test was also applied for gene/disease association as suggested when the single-nucleotide polymorphism largely deviates from HWE [21]. Risk of disease occurrence was determined by calculating odds ratios (OR) and 95% confidence intervals (95% CI). Comparisons between groups were performed using Kruskal–Wallis or Mann–Whitney *U* tests, as appropriate.

Results

Study subjects

Demographics and clinical characteristics of RA patients and CS are described in Table 1. The median age was 46 (25–70) years for patients and 47 (27–71) years for CS. Most individuals were females (93%) in both study groups. At the time of inclusion, RA patients had a median of 6 years of disease evolution with low disease activity (median DAS28 = 2.91) and some extent of functional disability to perform any daily activity (median HAQ-DI = 0.32). Most patients were treated mainly with methotrexate (77%) and non-steroidal anti-inflammatory drugs (NSAIDs) (68%). The serum levels of autoantibodies (RF and anti-CCP) and acute phase reactants (ESR and CRP) were higher in RA than in CS ($p < 0.001$).

Allelic and genotypic frequencies of the *PRL* -1149G>T polymorphism

The distribution of genotypic and allelic frequencies of the *PRL* -1149G>T polymorphism in RA patients and CS is shown in Table 2. The control group was not in HWE due to an excess of homozygosity ($\chi^2 = 9.635$, $p = 0.002$), which was measured by the intrapopulation inbreeding coefficient (Fis = 0.203).

We found significant differences in genotype and allele frequencies between RA patients and CS for the *PRL* -1149G>T polymorphism ($p < 0.05$). We also analyzed the distribution of the polymorphism by applying genetic models of inheritance (dominant, recessive and additive), and according to all models, there is an association between the T allele (GT and/or TT genotypes) and decreased RA susceptibility (OR = 0.55, 95% CI 0.35–0.87, $p = 0.009$; OR = 0.09, 95% CI 0.012–0.76, $p = 0.011$; and OR = 0.49, 95% CI 0.32–0.76, $p = 0.001$, respectively), Table 2. In addition, the clinical parameters of RA patients were compared according to the genetic models of the *PRL* -1149G>T polymorphism, but no significant differences were found ($p > 0.05$, data not shown).

Serum levels and mRNA expression of PRL in RA and CS

The concentration of PRL in serum was higher in RA patients than in CS (11.2 vs 9.9 ng/mL, respectively, $p = 0.037$, Fig. 1a). We observed HPRL status (values > 20 ng/mL) in 9% of the RA patients vs. 2% in CS. Moreover, we compared PRL serum levels in RA patients according to the evolution time of the disease, and we found that the patients with < 2 years of evolution (defined classically as early RA) had higher PRL serum levels compared to patients with > 2 years of evolution (defined classically as established RA) ($p = 0.013$; Fig. 1b). Also, we evaluated the relationship between

Table 1 Demographic and clinical characteristics of RA patients and CS

Variables	RA ($n = 300$)	CS ($n = 300$)	p value
Demographics			
Age, years[a]	46 (25–70)	47 (27–71)	0.319
Gender % (n)			
Male	7 (22)	7 (22)	1.00
Female	93 (278)	93 (278)	
Smokers % (n)	16 (48)	19 (57)	0.466
Clinical assessment			
Disease evolution, years[a]	6 (1–22)	–	
DAS28 score, 0–10 scale [a]	2.91 (1.82–7.05)	–	
DAS28% (n)			
Remission (< 2.6)	34 (102)	–	
Low activity (2.6 < 3.2)	21 (63)	–	
Moderate activity (≥ 3.2 < 5.1)	30 (90)	–	
High activity (≥ 5.1)	15 (45)	–	
Spanish HAQ-DI, 0–3 scale[a]	0.32 (0–1.61)	–	
ESR, mm/h[a]	31 (9–55)	27 (6.5–46.5)	< 0.001
CRP, mg/L[a]	15.8 (3.45–101.95)	10.7 (2.5–46.5)	< 0.001
RF (IU/mL)[a]	173.87 (3.5–300)	0 (0–14.9)	< 0.001
RF % (n)			< 0.001
Negative (< 20 IU/mL)	13 (26)	96 (192)	
Positive (≥ 20 IU/mL)	87 (174)	4 (8)	
Anti-CCP (U/mL)[a]	107 (0–900)	0 (0–3)	< 0.001
Anti-CCP % (n)			< 0.001
Negative (≤5 U/mL)	11.6 (23)	95.3 (142)	
Low positive (5.1–14.9 U/mL)	8 (16)	2 (3)	
High positive (≥ 15 U/mL)	80.4 (160)	2.7 (4)	
Drug treatment % (n)			
NSAIDs	68 (204)	–	
Steroids (Prednisone)	53 (159)	–	
DMARDs			
Azulfidine (Sulfasalazine)	15 (45)	–	
Chloroquine	39 (117)	–	
Methotrexate	77 (195)	–	

[a] Data provided in median (p5–p95). The p values were calculated by χ^2 test or Wilcoxon–Mann–Whitney test, as appropriate. *RA*, rheumatoid arthritis; *CS*, control subjects; *DAS28*, disease activity score 28; *Spanish HAQ-DI*, Spanish version of health assessment questionnaire disability index; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *RF*, rheumatoid factor; *anti-CCP*, anti-cyclic citrullinated peptide antibody; *NSAIDs*, non-steroidal anti-inflammatory drugs; *DMARDs*, disease modifying anti-rheumatic drugs

the PRL levels and the clinical characteristics of RA patients, but statistically significant correlations were not found (data not shown). On the other hand, the relative mRNA expression of *PRL* in RA patients was 2.4-fold higher than in the CS ($p < 0.001$; Fig. 1c).

Serum levels and mRNA expression of PRL according to the *PRL* −1149G>T polymorphism

There were no significant differences in the PRL serum levels according to the *PRL* −1149G>T genotypes in RA patients and CS. However, RA patients carrying the GT genotype tended to have lower levels of PRL than carriers of the GG genotype (Fig. 2a, $p = 0.872$). Similarly, we observed that CS carrying the TT genotype had lower PRL serum levels compared with the GG genotype carriers ($p > 0.05$; Fig. 2b).

Regarding *PRL* mRNA expression (Fig. 2c–d), we found that RA patients carriers of the GT genotype had lower relative expression in comparison to the GG genotype carriers, although the difference did not reach statistical significance (GG = 1 relative unit vs. GT = 0.72 relative unit, $p = 0.640$, Fig. 1c). On the other hand, in CS, GT genotype carriers and

Table 2 Genotypic and allelic frequencies of the *PRL* -1149G>T polymorphism among RA patients and CS

SNP -1149G>T	RA $n = 300$ n (%)	CS $n = 300$ n (%)	OR (95% CI)	p value
Genotype				
GG[a]	265 (88.4)	242 (81.7)	1.0	
GT	34 (11.3)	48 (16.0)	0.65 (0.40–1.03)	0.069
TT	1 (0.3)	10 (3.3)	0.09 (0.01–0.72)	0.004
Allele				
G[a]	564 (94)	532 (89)	1.0	
T	36 (6)	68 (11)	0.52 (0.33–0.76)	0.002
Assessed association model				
Do				
GG[a]	265 (88.3)	242 (80.7)	1.0	
GT+TT	35 (11.7)	58 (19.3)	0.55 (0.35–0.87)	0.009
Re				
GG+GT[a]	299 (99.7)	290 (96.7)	1.0	
TT	1 (0.3)	10 (3.3)	0.09 (0.012–0.76)	0.011
Ad				
GG+(GG+GT)[a]	564 (94)	532 (88.7)	1.0	
(GT+TT)+TT	36 (6)	68 (11.3)	0.49 (0.32–0.76)	0.001

RA, rheumatoid arthritis; *CS*, control subjects; *OR*, odds ratio; *95% CI*, 95% confidence interval. Percentages were obtained by count direct. *Do*, dominant genetic model; *Re*, recessive genetic model; *Ad*, additive genetic model

[a] Reference category. p values were calculated by logistic regression comparisons with the reference category and by Armitage's trend test

TT genotype carriers had 7.14 and 20-fold lower *PRL* expression than GG genotype carriers, respectively (1/0.14 = 7.14-fold; 1/0.05 = 20-fold; $p < 0.006$, Fig. 1d).

Discussion

The etiopathogenesis of RA is partially understood; however, it is well known that genetic, environmental and hormonal factors have important roles in disease development. The dimorphism between gender in autoimmune diseases is believed to rely on sex hormones [10]. There are many controversies regarding the role of hormonal factors in the development of RA. For example, high and very low estrogen exposure has been associated with increased risk of RA [8]. and this discrepancy could be explained by the dual effects of estrogens (having both pro- and anti-inflammatory activities) [22], similar to the reports regarding PRL in RA patients [23, 24].

PRL is a hormone with bioactive function as a cytokine, with the capacity to inhibit the negative selection of autoreactive B lymphocytes, therefore, promoting autoimmunity. Hyperprolactinemia has been described in relation to the pathogenesis and activity of several autoimmune disorders [10]. In this study, PRL serum concentrations were significantly higher in the female RA patients when these were compared to healthy-matched controls. This finding broadly supports the work of other previous studies [11, 12, 25], suggesting an increased PRL production, either systemic or locally secreted by immune cells, in putative relation with disease activity [10]; however, this study has been unable to demonstrate that PRL serum levels are associated with disease activity, inflammatory markers, or autoantibodies as reported by others [12, 17, 26], probably by the fact that most patients in our study were under DMARD and steroidal therapy (both of which modify these clinical parameters), which is supported with the overall low disease activity of the patients (median DAS28 = 2.9).

One interesting finding was that early RA patients (less than 2 years of disease) had higher PRL serum levels than patients with established RA (more than 2 years of disease). These results could suggest that PRL may be altered significantly early in the development of the disease manifestations, similar to observations previously reported in human and murine SLE [16, 27]. This could be one of the reasons why bromocriptine (a suppressor of PRL secretion) is not effective as adjuvant therapy of anti-rheumatic drugs in established RA [28, 29].

In contrast to earlier findings [17], we observed a higher relative expression of *PRL* in RA patients than CS (2.4-fold higher, $p < 0.001$); in this respect, it is the first study reporting differences at the level of mRNA expression, so we need more studies that support this finding. However, this result is consistent with reports of high PRL serum levels in RA, which may indicate that *PRL* gene is upregulated in RA at both the transcriptional and translational level.

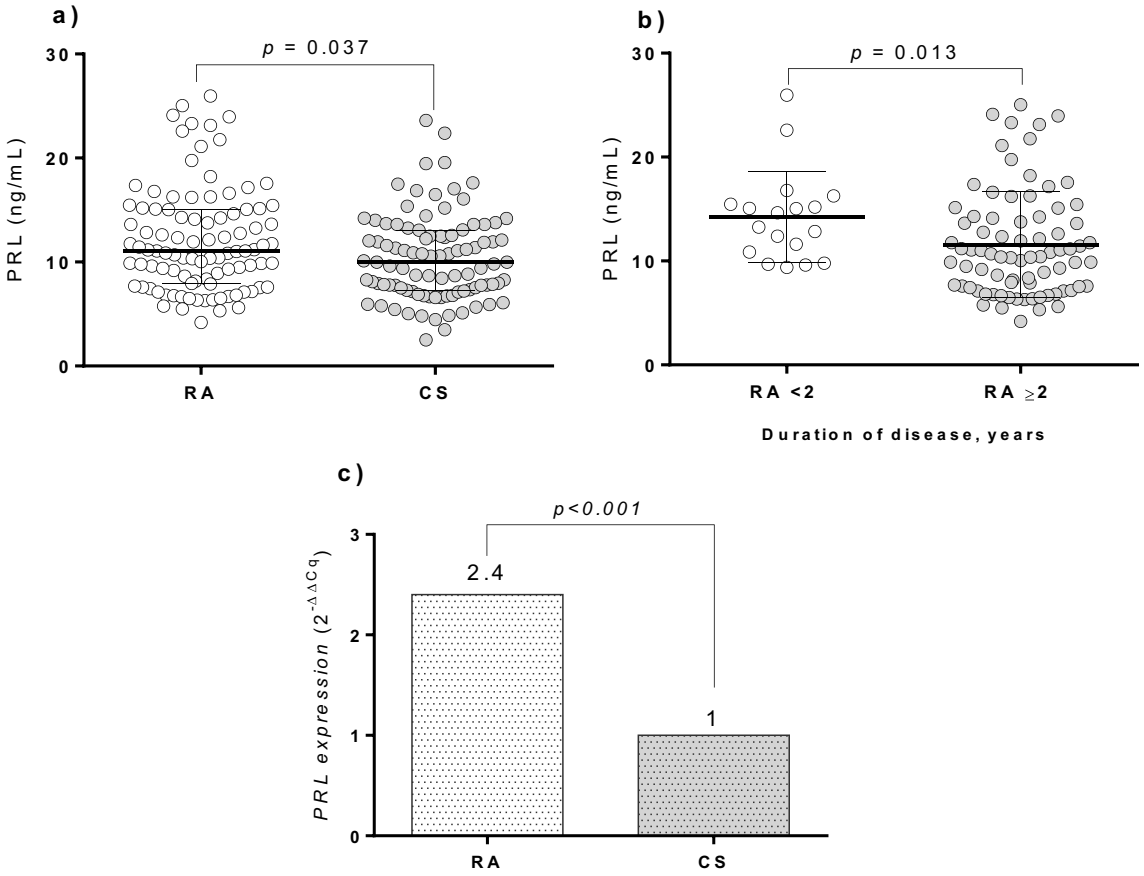

Fig. 1 Serum levels and mRNA expression of PRL in RA and CS. **a** Prolactin serum levels in RA patients and CS; **b** PRL serum levels in RA according to the duration of disease; **c** Comparison of mRNA expression of *PRL* in RA patients and CS. Relative *PRL* gene expression was determined by the $2^{-\Delta\Delta Cq}$ method using *GAPDH* as a reference gene; *p* value was calculated by comparisons of individuals relative expression using $2^{-\Delta Cq}$ method. The concentrations of serum PRL levels were expressed in scatter plots with median (p25–p75) values indicated by horizontal lines. Data were compared using the Mann–Whitney *U* test

Promoter sequences are potential sources of polymorphism affecting gene expression (at mRNA and protein levels) attributable to altered transcription factor binding, and this can contribute to the risk of disease. The *PRL* -1149G>T polymorphism in the distal *PRL* promoter has been associated with RA in Caucasians from European ancestry [30] and individuals from Western Mexico [17]. In both populations, this polymorphism was in HWE.

In Mexico, most of the populations (> 90%) are Mestizos, because of admixture after the European contact between Spaniards, Amerindians, and African slaves, principally. The Amerindian ancestry distribution displays an opposite pattern regarding the European ancestry, increasing gradually to the Southeast and reaching maximum values in Oaxaca and Guerrero and decreasing northwards [31]. Given the genetic structure demonstrated among Mestizos from different regions, the Mexican population harbors rich amounts of genetic variation that may underlie important biomedical phenotypes [32].

In this studied population (CS from Guerrero), the *PRL* -1149G>T polymorphism was not in HWE, which agrees with the reported by the HapMap-MEX (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1341239). A departure from HWE can be explained by natural selection, population admixture, inbreeding, experimental errors, and duplication [33]; conventionally, SNPs that are significantly deviated from HWE are discarded before further analysis. However, it has been estimated that in about 10% of case-control studies in the biomedical field, the genotype distributions in the healthy control group violates HWE, thus HWE departures do not necessarily mean that postulated associations should be dismissed, but they provide hints at the need for more evidence and validation [34].

As previously described in the methods section, we re-genotyped some patients by PCR-RFLPs using internal controls and by sequencing of DNA to exclude genotyping error; therefore, it seems unlikely that genotyping error is responsible for the HWE deviation observed in our study. Interestingly, previous reports including populations from Guerrero do not report endogamy or consistent Hardy–Weinberg disequilibrium analyzing Ancestry informativity markers (AIMS) [35, 36] neither with 15 forensic STR markers [32].

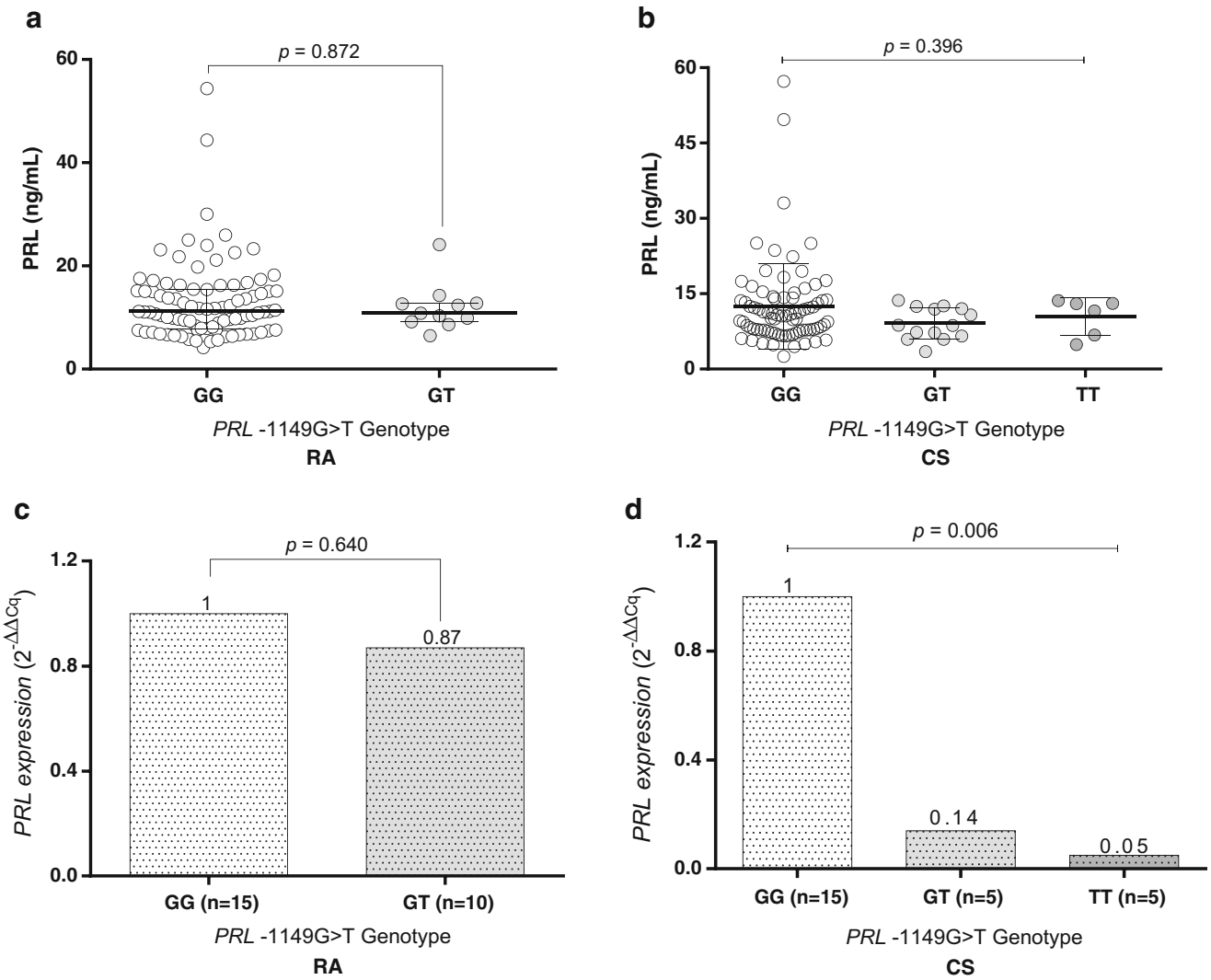

Fig. 2 Serum levels and mRNA expression of PRL according to the *PRL* -1149G>T polymorphism. PRL serum levels in RA and CS (**a**) according to *PRL* -1149G>T genotypes (**b**); data were expressed in scatter plots with median (p25–p75) values indicated by horizontal lines. *PRL* relative expression was evaluated in RA patients and CS (**c**) according to *PRL* -1149G>T genotypes (**d**), using the $2^{-\Delta\Delta Cq}$ method, and the *p* value was calculated by comparisons of individuals relative expression using $2^{-\Delta Cq}$ method. Statistical analysis was performed using the Kruskal–Wallis test or Mann–Whitney *U* test when appropriate

Generally, the increased homozygosity is related to inbreeding [37]; although we recruited unrelated individuals, it is probably an unnoticed kinship in the population sample due to the presence of individuals from rural and isolated communities. In brief, this result suggests inbreeding in some rural communities of Guerrero, which should be confirmed including more markers and a wider population sample.

This study supports evidence from previous reports [17, 30] suggesting associations between the *PRL* -1149T allele and decreased RA risk. We found significant differences between genotypic and allelic frequencies from RA patients and CS, with a higher frequency of the T allele in the CS. When the association between a genetic marker and a trait is evaluated in a population-based study, there is rarely a priori biologic evidence supporting a genetic model of inheritance for the risk/protective allele [38], for this reason, we analyze our results applying various genetic models of inheritance. The results were consistent under dominant, recessive, and additive genetic models ($p < 0.05$); however, the risk in carriers of two copies of the T allele was lower than heterozygotes; therefore, we propose an additive model (per-allele) for the genetic effect of this polymorphism in RA.

Moreover, we observed that carriers of the GT or TT *PRL* genotype had lower mRNA expression and PRL serum levels than carriers of the GG genotype; however, these differences were only significant at the mRNA expression in the CS group ($p = 0.006$). These data agree with that reported by previous studies [17, 18] who observed an association of *PRL* -1149T allele with lower *PRL* mRNA expression in healthy subjects. The lack of correlation of these findings in the RA group could

be due to the anti-rheumatic treatment, disease activity, and/or pathological condition of this group, which could be minimizing or masking its effect.

It has been reported that the -1149 position at *PRL* could be part of the consensus sequence for a GATA-related transcriptional factor, thus, the T allele could be altering protein-DNA binding characteristics and acting in an inhibitory mode or modifying the magnitude of the *PRL* transcription activation [18]. Since the high levels of PRL are associated with autoimmunity and the clinical parameters in RA, this fact would also explain why the T allele can be a genetic marker of lower risk to the development of RA.

In conclusion, this study showed that the *PRL* -1149T allele is associated with decreased susceptibility to RA in population from south of Mexico which extends previous findings reported for the Mexican population. The findings of increased *PRL* mRNA expression and PRL serum levels in RA patients lead to the assumption that this hormone may play a role in the process of joint damage in RA and may favor autoimmunity at early stages of RA, as the PRL serum levels were higher in patients with < 2 years of disease evolution. The association of *PRL* -1149T allele as a protective marker for RA could be via downregulation of *PRL* promoter, which was reflected in the lowest *PRL* expression observed in the T allele carriers.

Further studies are needed to examine more closely the functional effect of the *PRL* -1149T allele to support our findings and additional research regarding this topic.

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Compliance with ethical standards

The protocol for the study was approved by the Local Bioethical Committee at the Hospital General de Chilpancingo "Dr. Raymundo Abarca Alarcón" and was conducted according to the Helsinki Declaration guidelines.

Disclosures None.

References

1. Neumann E, Frommer K, Diller M, Müller-Ladner U (2018) Rheumatoid arthritis. Z Rheumatol 77:769–775. https://doi.org/10.1007/s00393-018-0500-z
2. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z (2013) Genetics of rheumatoid arthritis — a comprehensive review. Clin Rev Allergy Immunol 45:170–179. https://doi.org/10.1007/s12016-012-8346-7
3. Muñoz-Valle JF, Valle Y, Padilla-Gutiérrez JR, Parra-Rojas I, Rangel-Villalobos H, del Mercado MV, Ledezma-Lozano IY, Villafan-Bernal JR, Armendáriz-Borunda J, Pereira-Suárez AL (2010) The +49A>G CTLA-4 polymorphism is associated with rheumatoid arthritis in Mexican population. Clin Chim Acta 411:725–728. https://doi.org/10.1016/j.cca.2010.02.001
4. Llamas-Covarrubias MA, Valle Y, Bucala R, Navarro-Hernández RE, Palafox-Sánchez CA, Padilla-Gutiérrez JR, Parra-Rojas I, Bernard-Medina AG, Reyes-Castillo Z, Muñoz-Valle JF (2013) Macrophage migration inhibitory factor (MIF): genetic evidence for participation in early onset and early stage rheumatoid arthritis. Cytokine 61:759–765. https://doi.org/10.1016/j.cyto.2012.12.032
5. Reyes-Castillo Z, Palafox-Sánchez CA, Parra-Rojas I, Martínez-Bonilla GE, del Toro-Arreola S, Ramírez-Dueñas MG, Ocampo-Bermudes G, Muñoz-Valle JF (2015) Comparative analysis of autoantibodies targeting peptidylarginine deiminase type 4, mutated citrullinated vimentin and cyclic citrullinated peptides in rheumatoid arthritis: associations with cytokine profiles, clinical and genetic features. Clin Exp Immunol 182:119–131. https://doi.org/10.1111/cei.12677
6. Reyes-Castillo Z, Muñoz-Valle JF, Llamas-Covarrubias MA (2018) Clinical and immunological aspects of anti-peptidylarginine deiminase type 4 (anti-PAD4) autoantibodies in rheumatoid arthritis. Autoimmun Rev 17:94–102. https://doi.org/10.1016/j.autrev.2017.11.023
7. Ruiz-Noa Y, Padilla-Gutiérrez JR, Hernández-Bello J, Palafox-Sánchez CA, Valle Y, Oregón-Romero E, Pereira-Suárez AL, Bernard-Medina AG, Muñoz-Valle JF (2017) Association of PTPN22 haplotypes (−1123G>C/+1858C>T) with rheumatoid arthritis in Western Mexican population. Int J Genomics 2017:1–5. https://doi.org/10.1155/2017/8753498
8. Alpízar-Rodríguez D, Pluchino N, Canny G, Gabay C, Finckh A (2017) The role of female hormonal factors in the development of rheumatoid arthritis. Rheumatology 56:1254–1263. https://doi.org/10.1093/rheumatology/kew318
9. Costenbader KH, Manson JE (2008) Do female hormones affect the onset or severity of rheumatoid arthritis? Arthritis Care Res 59:299–301. https://doi.org/10.1002/art.23324
10. Borba VV, Zandman-Goddard G, Shoenfeld Y (2018) Prolactin and autoimmunity. Front Immunol 9:. https://doi.org/10.3389/fimmu.2018.00073
11. Tang MW, Gerlag DM, Codullo V, de Sousa ECV, Reuwer AQ, Twickler M, Tak PP (2012) The role of prolactin, as sex hormone, and its receptor involved in rheumatoid arthritis. Ann Rheum Dis 71:A72. https://doi.org/10.1136/annrheumdis-2011-201238.1
12. Fojtíková M, Tomasová Studýnková J, Filková M et al (2010) Elevated prolactin levels in patients with rheumatoid arthritis: association with disease activity and structural damage. Clin Exp Rheumatol 28:849–854
13. Saha S, Gonzalez J, Rosenfeld G, Keiser H, Peeva E (2009) Prolactin alters the mechanisms of B cell tolerance induction. Arthritis Rheum 60:1743–1752. https://doi.org/10.1002/art.24500
14. Gellersen B, Kempf R, Telgmann R, DiMattia GE (1994) Nonpituitary human prolactin gene transcription is independent of Pit-1 and differentially controlled in lymphocytes and in endometrial stroma. Mol Endocrinol 8:356–373. https://doi.org/10.1210/mend.8.3.8015553
15. Li B-Y, Yang Y-M, Liu Y, Sun J, Ye Y, Liu XN, Liu HX, Sun ZQ, Li M, Cui J, Sun DJ, Gao YH (2017) Prolactin rs1341239 T allele may have protective role against the brick tea type skeletal fluorosis. PLoS One 12:e0171011. https://doi.org/10.1371/journal.pone.0171011
16. Hernández-Bello J, Palafox-Sanchez CA, García-Arellano S, Reyes-Castillo Z, Pereira-Suárez AL, Parra-Rojas I, Navarro-Zarza JE, cruz-Mosso UD, Torres-Carrillo NM, Muñoz-Valle JF (2018) Association of extrapituitary prolactin promoter polymorphism with disease susceptibility and anti-RNP antibodies in Mexican patients with systemic lupus erythematosus. Arch Med Sci 14:1025–1032. https://doi.org/10.5114/aoms.2016.62138

17. Reyes-Castillo Z, Pereira-Suárez AL, Palafox-Sanchez CA, Rangel-Villalobos H, Estrada-Chávez C, Oregón-Romero E, Angel-Chávez LI, Muñoz-Barrios S, Bueno-Topete MR, Muñoz-Valle JF (2013) The extrapituitary prolactin promoter polymorphism is associated with rheumatoid arthritis and anti-CCP antibodies in Mexican population. Gene 525:130–135. https://doi.org/10.1016/j.gene.2013.04.068
18. Stevens A, Ray D, Alansari A, Hajeer A, Thomson W, Donn R, Ollier WER, Worthington J, Davis JRE (2001) Characterization of a prolactin gene polymorphism and its associations with systemic lupus erythematosus. Arthritis Rheum 44:2358–2366
19. Lee YC, Raychaudhuri S, Cui J, de Vivo I, Ding B, Alfredsson L, Padyukov L, Costenbader KH, Seielstad M, Graham RR, Klareskog L, Gregersen PK, Plenge RM, Karlson EW (2009) The PRL -1149 G/T polymorphism and rheumatoid arthritis susceptibility. Arthritis Rheum 60:1250–1254. https://doi.org/10.1002/art.24468
20. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159. https://doi.org/10.1006/abio.1987.9999
21. Emily M (2018) Power comparison of Cochran-Armitage trend test against allelic and genotypic tests in large-scale case-control genetic association studies. Stat Methods Med Res 27:2657–2673. https://doi.org/10.1177/0962280216683979
22. Au A, Feher A, McPhee L, Jessa A, Oh S, Einstein G (2016) Estrogens, inflammation and cognition. Front Neuroendocrinol 40:87–100. https://doi.org/10.1016/j.yfrne.2016.01.002
23. Clapp C, Adán N, Ledesma-Colunga MG, Solís-Gutiérrez M, Triebel J, Martínez de la Escalera G (2016) The role of the prolactin/vasoinhibin axis in rheumatoid arthritis: an integrative overview. Cell Mol Life Sci 73:2929–2948. https://doi.org/10.1007/s00018-016-2187-0
24. Costanza M, Binart N, Steinman L, Pedotti R (2015) Prolactin: a versatile regulator of inflammation and autoimmune pathology. Autoimmun Rev 14:223–230. https://doi.org/10.1016/j.autrev.2014.11.005
25. Ghule S, Dhotre A, Gupta M, et al (2009) Serum prolactin levels in women with rheumatoid arthritis. Biomed Res 20
26. Seriolo B, Ferretti V, Sulli A et al (2002) Serum prolactin concentrations in male patients with rheumatoid arthritis. Ann N Y Acad Sci 966:258–262
27. Legorreta-Haquet MV, Flores-Fernández R, Blanco-Favela F, Fuentes-Pananá EM, Chávez-Sánchez L, Hernández-González R, Tesoro-Cruz E, Arriaga-Pizano L, Chávez-Rueda AK (2013) Prolactin levels correlate with abnormal B cell maturation in MRL and MRL/lpr mouse models of systemic lupus erythematosus-like disease. Clin Dev Immunol 2013:287469. https://doi.org/10.1155/2013/287469
28. Salesi M, Sadeghihaddadzavareh S, Nasri P, Namdarigharaghani N, Farajzadegan Z, Hajalikhani M (2013) The role of bromocriptine in the treatment of patients with active rheumatoid arthritis. Int J Rheum Dis 16:662–666. https://doi.org/10.1111/1756-185x.12015
29. McMurray RW (2001) Bromocriptine in rheumatic and autoimmune diseases. Semin Arthritis Rheum 31:21–32. https://doi.org/10.1053/sarh.2001.25482
30. Lee YC, Raychaudhuri S, Cui J, de Vivo I, Ding B, Alfredsson L, Padyukov L, Costenbader KH, Seielstad M, Graham RR, Klareskog L, Gregersen PK, Plenge RM, Karlson EW (2009) Investigation of the PRL–1149 G/T polymorphism and rheumatoid arthritis susceptibility. Arthritis Rheum 60:1250–1254. https://doi.org/10.1002/art.24468
31. Moreno-Estrada A, Gignoux CR, Fernández-López JC et al (2014) The genetics of Mexico recapitulates native American substructure and affects biomedical traits. Science 344:1280–1285. https://doi.org/10.1126/science.1251688
32. Locia-Aguilar GJ, López-Saucedo B, Deheza-Bautista S, Salado-Beltrán OV, Martínez-Sevilla VM, Rangel-Villalobos H (2018) Forensic parameters for 15 autosomal STRs in Mestizo population from the state of Guerrero (South, Mexico). Legal Med 32:104–106. https://doi.org/10.1016/j.legalmed.2018.03.002
33. Cox DG, Kraft P (2006) Quantification of the power of Hardy-Weinberg equilibrium testing to detect genotyping error. Hum Hered 61:10–14. https://doi.org/10.1159/000091787
34. Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JPA (2006) Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. Am J Epidemiol 163:300–309. https://doi.org/10.1093/aje/kwj046
35. Bonilla C, Gutiérrez G, Parra EJ, Kline C, Shriver MD (2005) Admixture analysis of a rural population of the state of Guerrero, Mexico. Am J Phys Anthropol 128:861–869. https://doi.org/10.1002/ajpa.20227
36. Cahua-Pablo JÁ, Cruz M, Tello-Almaguer PV, del Alarcón-Romero LC, Parra EJ, Villerías-Salinas S, Valladares-Salgado A, Tello-Flores VA, Méndez-Palacios A, Pérez-Macedonio CP, Flores-Alfaro E (2017) Analysis of admixture proportions in seven geographical regions of the state of Guerrero, Mexico. Am J Hum Biol Off J Hum Biol Counc 29:. https://doi.org/10.1002/ajhb.23032
37. Sun S, Klebaner F, Tian T (2017) Mathematical modelling for variations of inbreeding populations fitness with single and polygenic traits. BMC Genomics 18:196. https://doi.org/10.1186/s12864-017-3492-1
38. Salanti G, Southam L, Altshuler D, Ardlie K, Barroso I, Boehnke M, Cornelis MC, Frayling TM, Grallert H, Grarup N, Groop L, Hansen T, Hattersley AT, Hu FB, Hveem K, Illig T, Kuusisto J, Laakso M, Langenberg C, Lyssenko V, McCarthy MI, Morris A, Morris AD, Palmer CNA, Payne F, Platou CGP, Scott LJ, Voight BF, Wareham NJ, Zeggini E, Ioannidis JPA (2009) Underlying genetic models of inheritance in established type 2 diabetes associations. Am J Epidemiol 170:537–545. https://doi.org/10.1093/aje/kwp145