Clinical and Experimental Medicine (2019) 19:439–447
https://doi.org/10.1007/s10238-019-00569-4

ORIGINAL ARTICLE

TNFA -308G>A and -238G>A polymorphisms and risk to systemic sclerosis: impact on TNF-*α* serum levels, *TNFA* mRNA expression, and autoantibodies

José Alvaro Lomelí-Nieto[1,2] · José Francisco Muñoz-Valle[1,2] · Christian Johana Baños-Hernández[3] · José Eduardo Navarro-Zarza[4] · María Guadalupe Ramírez-Dueñas[5] · Pedro Ernesto Sánchez-Hernández[5] · Andrea Carolina Machado-Sulbaran[5] · Isela Parra-Rojas[3] · Mariel García-Chagollán[1] · Jorge Hernández-Bello[1,2]

Received: 22 April 2019 / Accepted: 8 July 2019 / Published online: 29 July 2019
© Springer Nature Switzerland AG 2019

Abstract

Systemic sclerosis (SSc) is a rare autoimmune disease with high mortality, characterized by chronic inflammation and fibrosis, which are processes associated with higher serum tumor necrosis factor-*α* (sTNF-*α*) levels. *TNFA* -308G>A and -238G>A polymorphisms have been associated with higher sTNF-*α* levels. In this study, we genotyped the *TNFA* -308G>A and -238G>A polymorphisms in 53 SSc patients and 115 unrelated control subjects (CS) from southern Mexico. The *TNFA* mRNA expression and sTNF-*α* levels were also quantified by qPCR and enzyme-linked immunosorbent assays, respectively. *TNFA* -308GA genotype was associated with disease susceptibility according to a codominant genetic model (OR = 3.2, 95% CI 1.05–9.75, $p = 0.03$), and with higher anti-fibrillarin antibodies ($p = 0.01$), and higher skin thickening ($p = 0.006$). *TNFA* -238GA was not associated with SSc risk. *TNFA* mRNA expression and sTNF-*α* levels were similar between SSc patients and CS and were not statistically associated with the *TNFA* polymorphisms; however, a correlation (rho = 0.362, p = 0.009) between sTNF-*α* levels with anti-RNA polymerase III antibodies was observed in the SSc patients. In conclusion, the -308G>A polymorphism is a genetic marker of SSc susceptibility in population from southern Mexico, and it is associated with skin thickening and anti-fibrillarin antibodies. In addition, sTNF-*α* levels correlate positively with the anti-RNA pol III antibodies levels.

Keywords Systemic sclerosis · *TNFA* gene polymorphisms · *TNFA* mRNA expression · TNF-*α* · Autoantibodies

Introduction

Systemic sclerosis (SSc) is an autoimmune and inflammatory disease, characterized by sclerosis and fibrosis of the skin and other tissues or organs [1, 2]. It may have limited involvement (lcSSc) on the face, forearms, and legs or diffuse involvement (dcSSc) on internal organs, arms, thighs, and trunk [1, 3]. The prevalence (0.05%) and incidence (0.002%) per year of SSc are low, and it has been reported similar in Northern and Southern Europe and the USA [4–6]. In Mexico, no accurate data of prevalence and incidence have been reported for SSc; however, the overall prevalence of scleroderma has been reported at 0.02% [7].

SSc on its first stages has vascular abnormalities such as secondary Raynaud's phenomenon and the development of pulmonary arterial hypertension (PAH) [8, 9]. The pathogenic molecular mechanisms underlying SSc are not completely but may be due to the deregulation of transcription

✉ Jorge Hernández-Bello
jorge89_5@hotmail.com

1 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

2 Instituto Transdisciplinar de Investigación y Servicios, Universidad de Guadalajara, Zapopan, Jalisco, Mexico

3 Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, Mexico

4 Departamento de Medicina Interna/Reumatología, Hospital General de Chilpancingo "Dr. Raymundo Abarca Alarcón", Chilpancingo de los Bravo, Guerrero, Mexico

5 Laboratorio de Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico

factors controlling growth factors and proinflammatory cytokines synthesis in endothelial cells [1].

Tumor necrosis factor-α (TNFα) is an inflammatory cytokine involved in inflammation, apoptosis, and the signaling of stimulant interleukins of the fibrotic process [10]. It has been reported that serum levels of TNF-α (sTNF-α) are elevated in patients with SSc and favor the development of pulmonary fibrosis and pulmonary arterial hypertension [11]; thus, TNF-α could be a key cytokine in the pathogenesis of SSc [12].

In the promoter region of *TNFA* gene (which encode TNF-α), the -308G>A (*rs1800629*) and -238G>A (*rs361525*) polymorphisms have been associated with increased transcription of *TNFA* [13] and with susceptibility to autoimmune diseases such as psoriatic arthritis [14]. In Mexican population, these polymorphisms have been associated with some autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, and systemic lupus erythematosus, in which elevated sTNF-α has been observed [10, 15, 16]; however, there are no reports about the involvement of these *TNFA* polymorphisms in the susceptibility to SSc.

Based on this knowledge, we decided to evaluate the -308G>A and -238G>A polymorphisms of the *TNFA* gene as risk markers for SSc and its relationship with *TNFA* mRNA expression and sTNF-α levels in the population from southern Mexico.

Materials and method

Subjects

From February 2015 to May 2018, 53 patients with SSc and 115 control subjects (CS) were enrolled in this study. SSc patients were diagnosed by a rheumatologist according to the ACR/EULAR 2013 classification criteria [1] and were collected from the Department of Internal Medicine/Rheumatology of the General Hospital of Chilpancingo "Dr. Raymundo Abarca Alarcón," in the state of Guerrero, Mexico. The CS were healthy subjects recruited from the general population in the same geographic region than SSc patients. The rheumatologist conducted a medical record and an evaluation of the modified Rodnan skin score (MRSS) [17], and the Spanish version of the health assessment questionnaire disability index (HAQ-DI) [18] in all SSc patients.

Genotyping of *TNFA* -308 G>A and -238 G>A polymorphisms

Extraction of total genomic DNA from peripheral blood leukocytes was performed by the salting out method [19]. The *TNFA* -308G>A and -238G>A polymorphisms were analyzed and genotyped by a PCR–RFLP method; we used the primers sequence and conditions reported by Oregon-Romero et al. [20].

Quantification of TNF-*α* serum levels

sTNF-α levels were quantified using a conventional ELISA kit (LEGEND MAX Human Active TNF-α ELISA Kit, BioLegend) following the manufacturer's instructions. The sensitivity limit of the assay was 1.7 pg/mL.

TNFA mRNA expression analysis

Total RNA was extracted from 5 mL of peripheral blood of 15 SSc patients and 15 CS matched by age, according to Chomcyznski and Sacchi technique [21]. Patients of both study groups were selected taking account a representative genotype for each polymorphism, and similar gender and age. For mRNA analysis, 1 ng of total RNA was transcribed reversely using oligo-dT and M-MLV reverse transcriptase as indicated by the manufacturer (Promega, Madison, WI, USA). The quantification of *TNFA* mRNA was conducted by real-time PCR, using FastStart Essential DNA Green master kit (Roche Applied Science). The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as a reference gene. All samples were run in triplicate using the conditions indicated in the Gene Expression Assay protocol in a LightCycler 96 System (Roche Applied Science). The mRNA analysis expression was performed through $2^{-\Delta\Delta Cq}$ after validation of reaction efficiency; on the other hand, $2^{-\Delta Cq}$ method was used as a complementary analysis to determine differences between the study groups.

Statistical analysis

Statistical analysis was performed using STATA Software v11.1 and GraphPad Prism v6.0. The Shapiro–Wilk normality test was applied to verify the normal distribution of the data; depending on the results obtained, parametric or nonparametric tests were used for the analysis. Mann–Whitney *U* test was used to evaluate differences between two groups. Kruskal–Wallis test was used to analyze differences between three or more groups (for variables distributed non-normally) followed by Dunn's adjustment for multiple comparisons. A probability (p) value of less than 0.05 was considered significant.

Results

Clinical and demographic characteristics

The clinical and demographic characteristics of the study groups are described in Table 1. There was no significant

Table 1 Clinical features of SSc patients and CS

	SSc ($n=53$)	CS ($n=115$)	p value
Demographics data			
Age (years)[a]	50 (39–57)	46 (35–56)	0.242
Male/female (n)	13/87	9/91	0.367
BMI (kg/m^2)[a]	24 (21–26)	27 (24–29)	< 0.0001
Type of SSc and clinical record			
dSSc[b]	85 (44)	–	
lcSSc[b]	15 (8)	–	
Age of disease onset (years)[c]	41 ± 17	–	
Disease evolution (years)[a]	5.0 (0.6–20.3)	–	
Signs and symptoms			
Raynaud's phenomenon[b]	75 (40)	–	
Skin thickening[b]	92 (49)	–	
Telangiectasia[b]	58 (31)	–	
Digital ulcers[b]	43 (23)	–	
Puffy fingers[b]	72 (38)	–	
Arthritis[b]	89 (47)	–	
Clinical evaluation			
Mouth opening (cm)[a]	5 (3–7)	–	
MRSS score[a]	6 (0–26)	–	
HAQ-DI, 0–3 score[a]	0.3 (0.0–1.3)	–	
Laboratory findings			
ESR (mm/h)[a]	28 (19–41)	25 (18–27)	0.092
CRP (mg/dL)[a]	12 (5–29)	11 (6–18)	0.855
ANAs[b]	75 (40)	0 (0)	
Treatment %(n)			
NSAIDs[b]	19 (10)	–	
Prednisone[b]	34 (18)	–	
DMARDs[b]		–	
Methotrexate[b]	43 (23)	–	
Chloroquine[b]	13 (7)	–	
No treatment[b]	40 (21)	–	

[a]Data provided in medians (p5–p95); [b]data provided in percentages and "n"; [c]data provided in mean ± SD. *BMI* body mass index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *ANAs* antinuclear antibodies, *ACAs* anti-centromere antibodies, *ATAs* anti-topoisomerase antibodies, *NSAIDs* nonsteroidal anti-inflammatory, *DMARDs* disease-modifying antirheumatic drugs, *MRSS* modified Rodnan skin score

difference in age or gender between SSc patients and CS; however, significant differences were observed between both groups in the body mass index (BMI) (median BMI = 24 in SSc vs. 27 in CS, respectively, $p < 0.0001$).

In SSc patients, a greater percentage of individuals with a classification of lcSSc (85%) compared to dcSSc (15%) was observed. The average onset of the disease was 41 years and a time of evolution of approximately 5 years. In the signs and symptoms, most patients had sclerodactyly (92%), followed by arthritis (89%), Raynaud's phenomenon (75%), and puffy fingers (72%). In the clinical evaluation, the patients showed an average mouth opening of 5 cm, and the MRSS and HAQ-DI indexes were on average 6 and 0.3, respectively.

Regarding laboratory tests, the presence of antinuclear antibodies (75%) was highlighted, of which anti-centromere antibodies (ACAs) were the most prevalent (26%). Regarding treatment, most of the patients were treated with methotrexate (43%). On the other hand, 40% of patients had no treatment at the time of sampling because they were diagnosed that same day.

Distribution of *TNFA* -308G>A and -238G>A polymorphisms in SSc patients and CS

The genotypic and allelic frequencies of the *TNFA* -308G>A and -238G>A polymorphisms in SSc patients and CS are shown in Table 2. Both polymorphisms agreed with the Hardy–Weinberg equilibrium ($p > 0.05$, data not shown). The allele and genotype frequencies for the -238G>A polymorphism did not show statistical differences between SSc patients and CS. Regarding -308G>A polymorphism, a significant difference in the genotype frequencies was observed between both study groups under a genetic codominant model. The -308GA genotype was associated with an increased SSc susceptibility (OR = 3.200, IC = 1.050–9.750; $p = 0.033$).

TNFA -308G>A and -238G>A polymorphisms and clinical parameters in SSc

According to the codominant genetic model, we analyzed the relationship between both *TNFA* polymorphisms with the clinical parameters of SSc patients. For -308G>A polymorphism, GA genotype carriers showed higher MRSS ($p = 0.006$) and higher levels of anti-fibrillarin autoantibodies ($p = 0.018$) in comparison with GG genotype carriers (Table 3). However, the -238G>A polymorphism had no significant associations with the clinical parameters of SSc patients ($p > 0.05$, data not shown).

sTNF-α levels and *TNFA* mRNA expression in SSc patients and CS

sTNF-α levels were similar between SSc patients and CS ($p = 0.248$, Fig. 1a), but a tendency of higher sTNF-α levels in comparison with the CS group (median 2.63 pg/mL vs. 1.94 pg/mL, respectively) was observed. Moreover, a correlation between the sTNF-α levels with the anti-RNA polymerase III autoantibodies (rho = 0.362, $p = 0.009$, Fig. 1b) was found. On the other hand, the relative expression of *TNFA* mRNA was 2.6-fold higher in SSc patients than in CS (Fig. 1c); however, when these data were analyzed by

Table 2 Distribution of the genotypic and allelic frequencies of the *TNFA* -238 G>A y -308 G>A polymorphisms

SNPs	Allele/genotype	SSc ($n=53$) % (n)	CS ($n=115$) % (n)	OR (CI 95%); p
-238 G>A (*rs361525*)				
Alleles	G	97 (103)	92 (212)	1.0
	A	3 (3)	8 (16)	0.324 (0.093–1.125); 0.062
Assessed association model				
Codominant	GG	94 (50)	84 (97)	1.0
	GA	6 (3)	16 (18)	0.305 (0.086–1.083); 0.054
	AA	0 (0)	0 (0)	–
Dominant	GG	94 (50)	84 (97)	1.0
	GA + AA	6 (3)	16 (18)	0.305 (0.086–1.083); 0.054
Recessive	GG + GA	100 (53)	100 (115)	–
	AA	0 (0)	0 (0)	
-308 G>A (*rs1800629*)				
Alleles	G	92 (98)	97 (222)	1.0
	A	8 (8)	3 (8)	2.265 (0.826–6.210); 0.104
Assessed association model				
Codominant	GG	85 (45)	94 (108)	1.0
	GA	15 (8)	5 (6)	**3.200 (1.050–9.750); 0.033**
	AA	0 (0)	1 (1)	0.795 (0.032–19.880); 0.519
Dominant	GG	85 (45)	94 (108)	1.0
	GA + AA	15 (8)	6 (7)	2.743 (0.939–8.015); 0.057
Recessive	GG + GA	100 (53)	99 (114)	–
	AA	0 (0)	1 (1)	

Significant p values are shown in bold

OR odds ratio, *CI* confidence interval, *SSc* systemic sclerosis, *CS* control subjects. The p value was calculated by a Chi-squared test (χ^2). *SNPs* single-nucleotide polymorphisms

Table 3 Clinical parameters of SSc patients according to the *TNFA* -308 G>A polymorphism

Clinical variables	GG $n=45$	GA $n=8$	p
BMI (kg/m^2)[a]	24 (21–26)	24 (19–27)	0.944
lSSc[b]	84 (38)	86 (6)	0.931
dcSSc[b]	16 (7)	14 (1)	
Mouth opening (cm)[a]	5 (5–6)	5 (4–6)	0.844
MRSS score[a]	6 (3–6)	12 (6–22)	**0.006**
HAQ-DI, 0–3 score[a]	0.2 (0.0–0.7)	1.0 (0.2–1.2)	0.108
VAS score[a]	30 (10–50)	20 (20–70)	0.552
ESR (mm/h)[c]	28 ± 13	31 ± 11	0.663
CRP (mg/dL)[a]	8 (5–25)	20 (0–35)	0.902
ANAs[b]	77 (33)	88 (7)	0.497
ACAs (U/mL)[a]	0.7 (0.4–1.3)	14 (0.4–90)	0.300
ATAs(U/mL)[a]	0.4 (0.3–0.6)	0.3 (0.3–0.4)	0.511
Anti-RNA Pol III (U/mL)[a]	3.7 (2.4–6.2)	3.8 (3.1–5.7)	0.853
Anti-fibrillarin (U/mL)[a]	1.2 (1.0–2.0)	3.5 (1.6–7.0)	**0.018**

Significant p values are shown in bold

[a]Data provided in medians (p5–p95); [b]data provided in percentages; [c]data provided in mean ± SD. BMI: body mass index. *MRSS* modified Rodnan skin, *HAQ-DI* health assessment questionnaire disability index, *VAS* visual analog scale, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *ANAs* antinuclear antibodies, *ACAs* anti-centromere antibodies, *ATAs* anti-topoisomerase antibodies

the $2^{-\Delta Cq}$ method, these differences were not statistically significant ($p=0.138$, data not shown).

Association of *TNFA* -308G>A and -238G>A genotypes with sTNF-*α* levels and *TNFA* mRNA expression

In both study groups and for the two polymorphisms evaluated, carriers of GA genotype showed a slight increase in the sTNF-*α* levels (Fig. 2) in comparison with the GG genotype carriers. However, these results were not statistically significant ($p>0.05$).

According to the $2^{-\Delta\Delta Cq}$ method, *TNFA* mRNA expression (Fig. 3) showed differences by genotypes of the *TNFA* -308G>A and -238G>A polymorphisms. In SSc patients, -308GA genotype carriers showed 2.331-fold higher *TNFA* mRNA expression than GG genotype carriers. Similarly, in CS, GA and AA genotype carriers had higher *TNFA* mRNA expression than those carrying the GG genotype (1.285-fold and 2.088-fold, respectively).

Regarding -238G>A polymorphism, SSc patients carrying the *TNFA* -238GA genotype had 4.45-fold higher *TNFA* mRNA expression than GG genotype carriers. Likewise, in CS, GA genotype carriers had higher *TNFA* mRNA

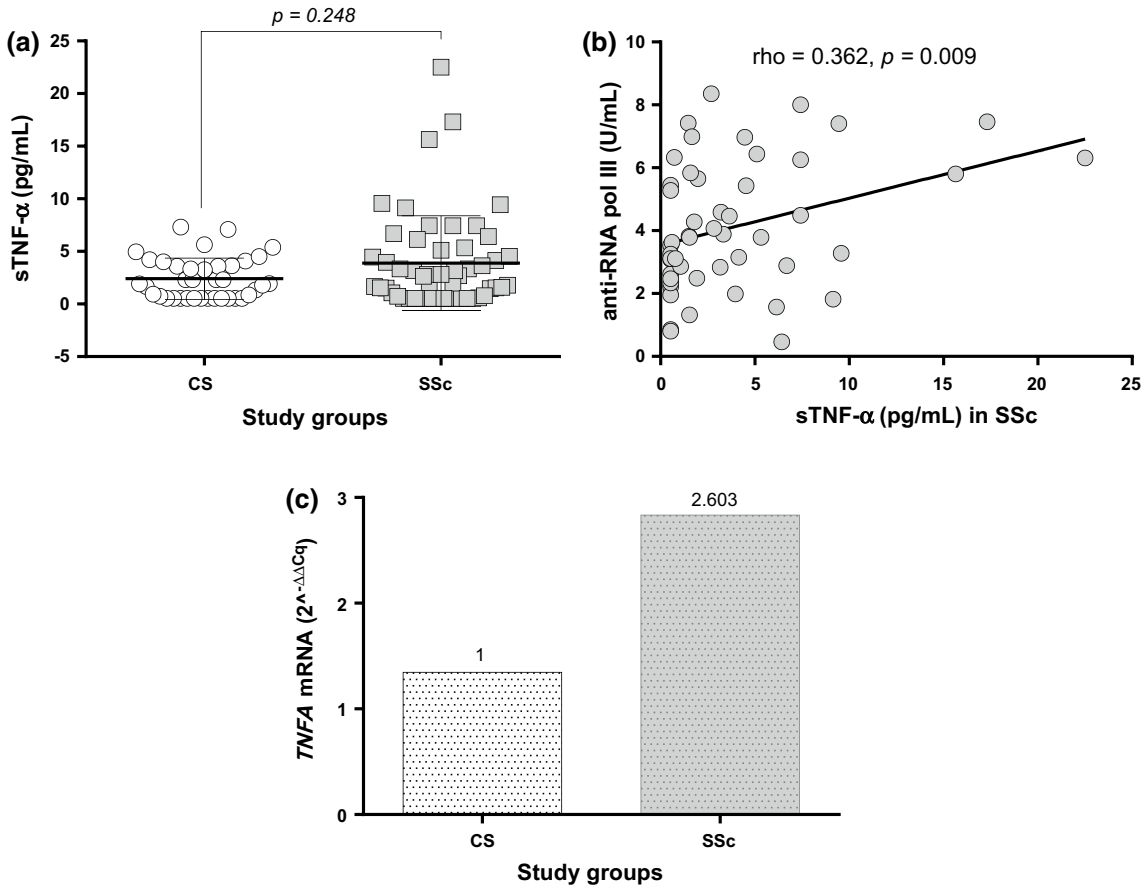

Fig. 1 sTNF-α levels and *TNFA* mRNA expression between SSc patients and CS. **a** Comparison of the sTNF-α levels between SSc patients and CS; data provided in medians and 5–95 percentile. **b** Correlation between sTNF-α levels and anti-RNA pol III antibodies in SSc patients; data analyzed with a Spearman's correlation. **c** Comparison of *TNFA* mRNA expression between SSc patients and CS. *P* value was calculated by the Mann–Whitney U test. SSc: systemic sclerosis. CS: control subjects. Graphics were made with GraphPad Prism v6.0

expression than those carrying the GG genotype (7.936-fold). The $2^{-\Delta Cq}$ analysis showed no association between the *TNFA* -308 and -238 genotypes with the *TNFA* mRNA expression ($p > 0.05$, data not shown).

Discussion

TNF-α participates in activation of vascular endothelium, regulation of immune response and metabolism of the connective tissue by modulation of fibroblastic function. Thus, it has been suggested as a major contributor to SSc progression [22].

Some studies on *TNFA* polymorphisms have reported an association between the -308 G>A and -238 G>A polymorphisms with SSc risk in different populations [23–25]; nevertheless, its role in the Mexican population was unknown. In this study, we evaluated the relationship between *TNFA* -308G>A and -238G>A polymorphisms with the SSc susceptibility, *TNFA* mRNA expression, sTNF-α levels, and clinical variables in SSc patients from southern Mexico.

We found that the GA genotype of the -308G>A polymorphism is a genetic marker of SSc susceptibility according to a codominant genetic model, but we did not find an association between -238G>A polymorphisms with the disease risk. These results agree with those reported in a Turkish population [25]; however, it is different to that reported in the Japanese population, where no association between any of these two *TNFA* polymorphisms with SSc risk was found [23]. In contrast to our findings, a study performed in Italy reported an association between the -238GA genotype polymorphisms with SSc susceptibility, particularly with dSSc phenotype [24]. These heterogeneous associations between *TNFA* polymorphisms and SSc risk can be explained by the genetic structure of each evaluated population, which it is well known that it contributes to the differences in disease incidence between populations. Moreover, genetic variants

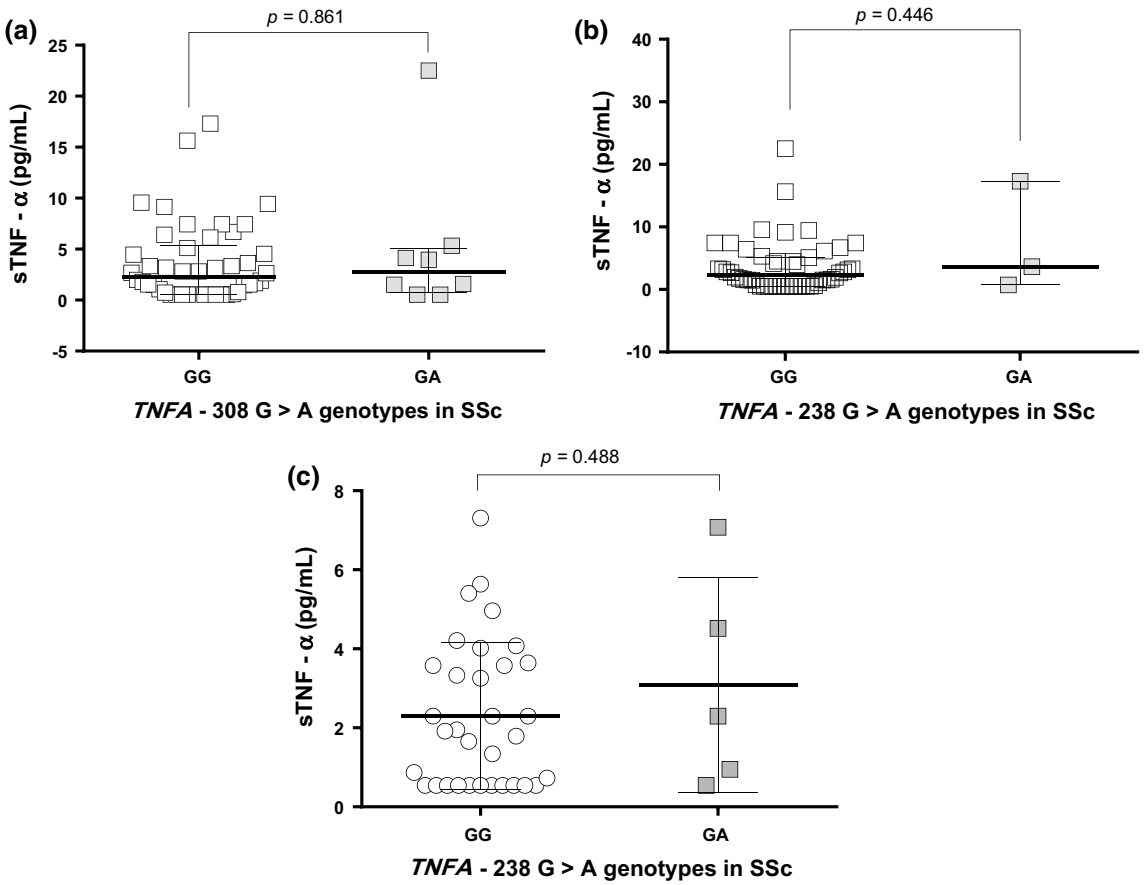

Fig. 2 sTNF-α levels according to the *TNFA* -308 G>A and 238 G>A genotypes. sTNF-α levels were compared according to the **a** *TNFA* -308 G>A and **b** -238 G>A genotypes in patients with SSc. **c** sTNF-α levels according to the *TNFA* -238 G>A genotypes in CS; -308 G>A analysis was not performed in CS because serum levels were only determined in one GA genotype carrier. Data provided in median with interquartile ranges. *p* values were calculated by Mann–Whitney *U* test. Graphics were made with GraphPad Prism v6.0

may also have different effects among populations because of unmeasured (and perhaps unknown) environmental risk factors, for which ethnicity may be the major factor, which modifies the genetic variant penetrance [26].

The most common autoantibodies detected in SSc patients are anti-topoisomerase I (ATAs) and anti-centromere (ACAs), while the least frequent are the anti-RNA polymerase III and anti-fibrillarin autoantibodies [27]. In some studies reported in Japan [28] and Italy [24], *TNFA* polymorphisms have been associated with ACAs and ATAs antibodies. In the present study, an association for the -308GA genotype with anti-fibrillarin antibodies was found; this finding has not been reported before in any population, so it is a novel finding from our study; however, due to the low prevalence of this polymorphism in our population, this finding should be interpreted with caution and it should be proved in different populations.

Overall, our finding and the data reported in the Italian and Japanese population suggest a prominent role for *TNFA* promoter polymorphisms on the synthesis of autoantibodies in SSc. This association between the *TNFA* -308G>A polymorphism with anti-fibrillarin antibodies could not be totally explained by the effect of these variants on the sTNF-α levels since there are many discrepant results in this regard. However, it cannot be ruled out that this association may be the result of an indirect effect of some *HLA* alleles (*HLA-DRB1 * 01* and *HLA-DQB1 * 0501*), as they have been associated with seropositivity to autoantibodies and they are in linkage disequilibrium with some promotor variants of the *TNFA* gene [28].

Another interesting finding in our study was the association of the -308GA genotype with a higher MRSS, which consists of a higher patient's skin thickness. This may be grounded by the fact that TNF-α, via NF-kB, stimulates fibroblast to secrete matrix metalloproteinase 2 (MMP-2) which is involved in fibrosis development [29]. However, a study found no association between sTNF-α levels with skin thickness in SSc patients [30]. Until today, there are no reports between -308 GA genotype positive associations with skin thickness in SSc disease. The closest studies

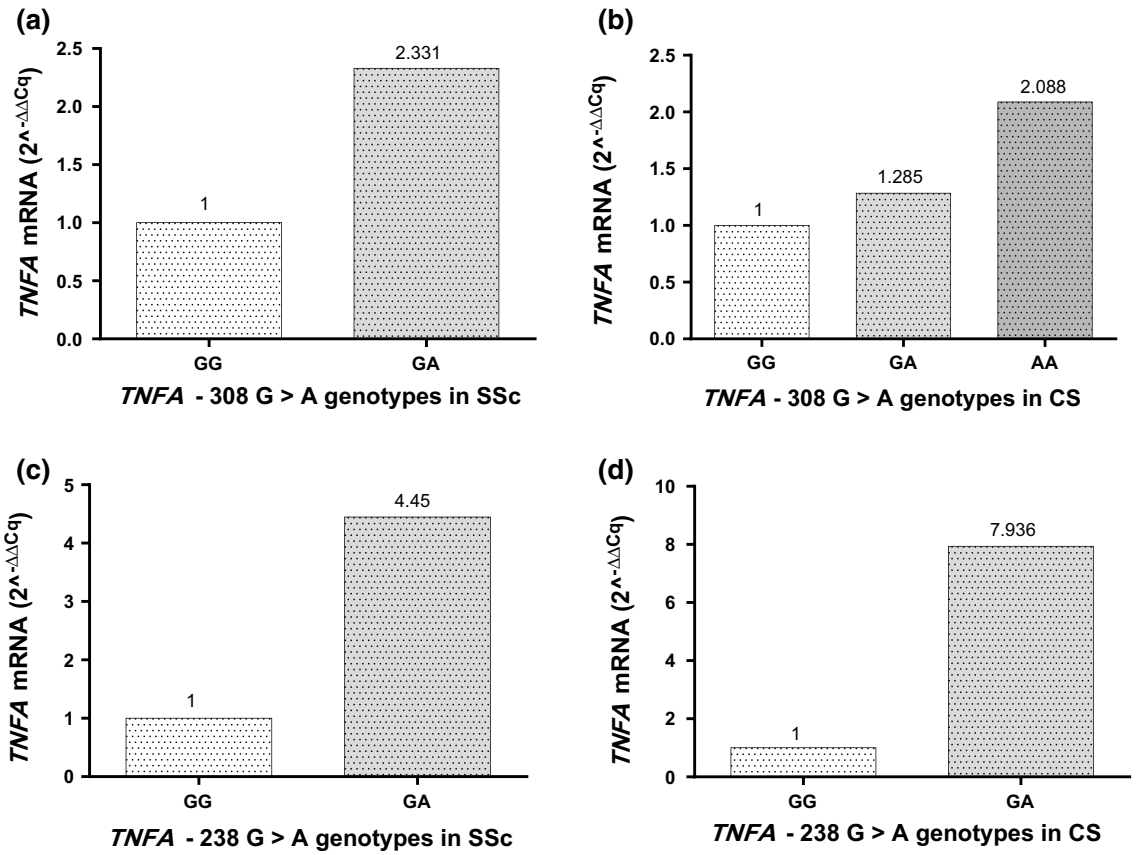

Fig. 3 *TNFA* mRNA expression according to the *TNFA* -308 G>A and -238 G>A genotypes. *TNFA* mRNA expression ($2^{-\Delta\Delta Cq}$ method) was compared according to the -308 G>A (**a**, **b**) and -238 G>A genotypes (**c**, **d**) in SSc patients and CS, respectively. The values at the top of the bars represent the change folds of the relative expression. *SSc* systemic sclerosis, *CS* control subjects. *p* values were calculated by Mann–Whitney *U* test, using the relative units of expression calculated by the $2^{-\Delta Cq}$ method (data not shown). Graphic were made with GraphPad Prism v6.0

about the relationship between TNF-α and skin alterations are described in psoriatic arthritis, where they have been reported high *TNFA* mRNA expression in patients [10], and anti-TNFA agents have a good response at skin affectation treatment [31]. In this respect, the -308GA genotype has been proposed to explain the *TNFA* overexpression in an Indian population with psoriatic arthritis [32].

In the present study, we did not find differences in the sTNF-α levels between SSc patients and CS, but there was a tendency of higher sTNF-α levels in SSc patients. On the other hand, *TNFA* mRNA expression was 2.603-fold higher in SSc patients than in CS. In agreement with our results, other studies have shown higher *TNFA* mRNA expression and sTNF-α levels in patients with SSc [33, 34]. This increase seems to be due in part to genetic variants of the *TNFA* gene; the A allele of the *TNFA* -308A>G polymorphism increases *TNFA* transcription activity and the sTNF-α level [35]. In our study, a tendency of higher *TNFA* mRNA expression and sTNF-α levels in SSc patients carrying at least one polymorphic allele (GA or AA genotypes) of the *TNFA* -308G>A and -238G>A polymorphisms was observed. Particularly, carriers of -308GA genotype had 2.331-fold higher *TNFA* mRNA expression than GG genotype carriers in SSc patients. Similarly, SSc patients carrying the -238GA genotype had 4.45-fold higher *TNFA* mRNA expression than GG genotype carriers. Although the subsequent analysis of mRNA expression with the $2^{-\Delta Cq}$ method did not show significant differences between groups, it is evident that both polymorphisms affect the expression of *TNFA*, and the lack of statistical association does not necessarily indicate that there is no biological effect; however, a weakness of our study is that the effect of minor allele was tested in a low number of subjects due to the low frequency observed in our population. Therefore, more studies are needed to verify the effect of these polymorphisms on transcriptional and translational alterations in the *TNFA* gene.

Supporting that observed for the -308GA genotype, a positive correlation between sTNF-α levels with anti-RNA polymerase III antibodies was observed. This is important because anti-RNA pol III autoantibodies have

been reported in association with increased renal affection (which is observed in 10–40% of SSc patients) and mortality in SSc patients [36, 37]; thus, high sTNF-α levels could be a serum marker with a potential prognostic application of renal damage in SSc patients. In accordance with these findings, a study performed in SSc patients from the USA found higher sTNF-α levels in patients with renal crisis [38].

The association of sTNF-α levels with anti-RNA polymerase III antibodies is not clear since TNF-α is not characterized by having effects on the B cells activation and/or immunoglobulin secretions. However, it is possible that this association could be explained because sTNF-α is a potent inductor of IL-6 [39], which increases B cell IgG production [40] and it has been associated with autoantibodies production in autoimmune diseases [41].

Conclusions

This study shows that -308GA genotype is a genetic marker for SSc susceptibility in population from southern Mexico, and it is also associated with higher skin thickness and higher anti-fibrillarin antibodies levels. Both minor alleles of the polymorphisms evaluated (*TNFA* -308G>A and -238G>A) seem to upregulate the transcriptional rate of the *TNFA* gene. On the other hand, sTNF-α levels are correlated positively with the anti-RNA polymerase III antibodies levels. Taken together, these data suggest a prominent role of TNF-α in SSc susceptibility via the production of autoantibodies and fibrosis, so future studies should be focused on this topic.

Funding This work was supported by funding from the PRODEP- SEP Mexico under Grant (No. UDG-PTC-1433) assigned to HBJ and by the University of Guadalajara—Strengthening Research and Postgraduate 2018 under Grant (No. 244159) assigned to MVJF. Part of this work was supported by PROFIDES-SEP for research networks, 2017 (511-6/18-11870).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Local Bioethical Committee at the Hospital General de Chilpancingo "Dr. Raymundo Abarca Alarcón" and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Elhai M, Avouac J, Kahan A, Allanore Y. Systemic sclerosis: recent insights. Jt Bone Spine Rev Rhum. 2015;82:148–53.
2. Brembilla NC, Dufour AM, Alvarez M, et al. IL-22 capacitates dermal fibroblast responses to TNF in scleroderma. Ann Rheum Dis. 2016;75:1697–705.
3. Denton CP. Advances in pathogenesis and treatment of systemic sclerosis. Clin Med Lond Engl. 2015;15(Suppl 6):s58–63.
4. Andréasson K, Saxne T, Bergknut C, Hesselstrand R, Englund M. Prevalence and incidence of systemic sclerosis in southern Sweden: population-based data with case ascertainment using the 1980 ARA criteria and the proposed ACR-EULAR classification criteria. Ann Rheum Dis. 2014;73:1788–92.
5. Allanore Y, Simms R, Distler O, et al. Systemic sclerosis. Nat Rev Dis Primer. 2015;1:15002.
6. Royle JG, Lanyon PC, Grainge MJ, Abhishek A, Pearce FA. The incidence, prevalence, and survival of systemic sclerosis in the UK Clinical Practice Research Datalink. Clin Rheumatol. 2018;37:2103–11.
7. Peláez-Ballestas I, Sanin LH, Moreno-Montoya J, 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.
8. Bongi SM, Del Rosso A, Passalacqua M, Miccio S, Cerinic MM. Manual lymph drainage improving upper extremity edema and hand function in patients with systemic sclerosis in edematous phase. Arthritis Care Res. 2011;63:1134–41.
9. Alba MA, Velasco C, Simeón CP, et al. Early- versus late-onset systemic sclerosis: differences in clinical presentation and outcome in 1037 patients. Medicine (Baltimore). 2014;93:73–81.
10. Bautista-Herrera LA, De la Cruz-Mosso U, Morales-Zambrano R, et al. Expression of MIF and TNFA in psoriatic arthritis: relationship with Th1/Th2/Th17 cytokine profiles and clinical variables. Clin Exp Med. 2018;18:229–35.
11. Murdaca G, Spanò F, Contatore M, Guastalla A, Magnani O, Puppo F. Pharmacogenetics of etanercept: role of TNF-α gene polymorphisms in improving its efficacy. Expert Opin Drug Metab Toxicol. 2014;10:1703–10.
12. Murdaca G, Spanò F, Contatore M, Guastalla A, Puppo F. Potential use of TNF-α inhibitors in systemic sclerosis. Immunotherapy. 2014;6:283–9.
13. Zúñiga J, Vargas-Alarcón G, Hernández-Pacheco G, Portal-Celhay C, Yamamoto-Furusho JK, Granados J. Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with systemic lupus erythematosus (SLE). Genes Immun. 2001;2:363–6.
14. Murdaca G, Gulli R, Spanò F, Lantieri F, Burlando M, Parodi A, et al. TNF-α gene polymorphisms: association with disease susceptibility and response to anti-TNF-α treatment in psoriatic arthritis. J Investig Dermatol. 2014;134:2503–9.
15. De la Cruz-Mosso U, Bucala R, Palafox-Sánchez CA, et al. Macrophage migration inhibitory factor: association of -794 CATT5-8 and -173 G>C polymorphisms with TNF-α in systemic lupus erythematosus. Hum Immunol. 2014;75:433–9.
16. Brennan-Bourdon LM, De la Cruz-Mosso U, Reyes-Castillo Z, et al. MIF and TNFα serum levels in rheumatoid arthritis patients treated with disease-modifying antirheumatic drugs: a cross-sectional study. Immunopharmacol Immunotoxicol. 2015;37:207–13.
17. Moniuszko A, Gińdzieńska-Sieśkiewicz E, Pancewicz SA, Czupryna P, Zajkowska J, Sierakowski S. Evaluation of skin thickness lesions in patients with Lyme disease measured by modified Rodnan total skin score. Rheumatol Int. 2012;32:3189–91.
18. De la Corte-Rodriguez H, Rodriguez-Merchan EC. The health assessment questionnaire disability index (HAQ-DI) as a valid

alternative for measuring the functional capacity of people with haemophilia. Thromb Res. 2017;153:51–6.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
20. Oregón-Romero E, Vázquez-Del Mercado M, Ruiz-Quezada SL, et al. Tumor necrosis factor alpha-308 and -238 polymorphisms in rheumatoid arthritis. Association with messenger RNA expression and sTNF-alpha. J Investig Med Off Publ Am Fed Clin Res. 2008;56:937–43.
21. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162:156–9.
22. Alekperov RT, Timchenko AV, Nasonov EL. Tumor necrosis factor alpha in systemic scleroderma. Klin Med (Mosk). 2003;81:4–7.
23. Pandey JP, Takeuchi F. TNF-alpha and TNF-beta gene polymorphisms in systemic sclerosis. Hum Immunol. 1999;60:1128–30.
24. Tolusso B, Fabris M, Caporali R, et al. 238 and +489 TNF-alpha along with TNF-RII gene polymorphisms associate with the diffuse phenotype in patients with systemic sclerosis. Immunol Lett. 2005;96:103–8.
25. Ates O, Müsellim B, Ongen G, Topal-Sarikaya A. Analysis of TNF polymorphisms in Turkish systemic sclerosis patients with interstitial lung involvement. Biochem Genet. 2008;46:696–701.
26. Haiman CA, Stram DO. Exploring genetic susceptibility to cancer in diverse populations. Curr Opin Genet Dev. 2010;20:330–5.
27. Liaskos C, Marou E, Simopoulou T, et al. Disease-related autoantibody profile in patients with systemic sclerosis. Autoimmunity. 2017;50:414–21.
28. Sato H, Lagan AL, Alexopoulou C, et al. The TNF-863A allele strongly associates with anticentromere antibody positivity in scleroderma. Arthritis Rheum. 2004;50:558–64.
29. Han YP, Tuan TL, Wu H, Hughes M, Garner WL. TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. J Cell Sci. 2001;114:131–9.
30. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. J Dermatol Sci. 2001;27:140–6.
31. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic arthritis. N Engl J Med. 2017;376:957–70.
32. Moorchung NN, Vasudevan B, Chatterjee M, Grewal RS, Mani NS. A comprehensive study of tumor necrosis factor-alpha genetic polymorphisms, its expression in skin and relation to histopathological features in psoriasis. Indian J Dermatol. 2015;60:345–50.
33. Young V, Ho M, Vosper H, Belch JJF, Palmer CNA. Elevated expression of the genes encoding TNF-alpha and thromboxane synthase in leucocytes from patients with systemic sclerosis. Rheumatol Oxf Engl. 2002;41:869–75.
34. Pehlivan Y, Onat AM, Ceylan N, et al. Serum leptin, resistin and TNF-α levels in patients with systemic sclerosis: the role of adipokines in scleroderma. Int J Rheum Dis. 2012;15:374–9.
35. Li Y. Tumor necrosis factor-alpha G308a gene polymorphism and essential hypertension: a meta-analysis involving 2244 participants. PLoS ONE. 2012;7:e35408.
36. Faucher B, Stein P, Granel B, et al. Low prevalence of anti-RNA polymerase III antibodies in a French scleroderma population: anti-RNA polymerase III scleroderma. Eur J Intern Med. 2010;21:114–7.
37. Guerini S, Cavazzana I, Venturelli C, et al. Renal involvement in systemic sclerosis. G Ital Nefrol Organo Uff Della Soc Ital Nefrol. 2007;24:295–310.
38. Gourh P, Arnett FC, Assassi S, et al. Plasma cytokine profiles in systemic sclerosis: associations with autoantibody subsets and clinical manifestations. Arthritis Res Ther. 2009;11:R147.
39. Tanabe K, Matsushima-Nishiwaki R, Yamaguchi S, Iida H, Dohi S, Kozawa O. Mechanisms of tumor necrosis factor-alpha-induced interleukin-6 synthesis in glioma cells. J Neuroinflamm. 2010;7:16.
40. Maeda K, Mehta H, Drevets DA, Coggeshall KM. IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood. 2010;115:4699–706.
41. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6:a016295.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.