Interleukin-10 promoter polymorphisms in patients with systemic lupus erythematosus from the Canary Islands

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Summary

The purpose of this study was to examine whether several allelic variants in the polymorphic IL-10 promoter region were related with an increased risk of developing SLE in Spanish patients from Canary Islands. Microsatellites (MS) at positions -4000 and -1200 (IL10R and IL10G, respectively) and single nucleotide polymorphisms (SNPs) at positions -1082, -819 and -592 of the IL-10 promoter were analysed in SLE patients and healthy controls from Canary Islands (Spain). We found that SNPs but not MS were associated with SLE. The GCC haplotype frequency was significantly higher in SLE patients (0.43) than in healthy donors (0.33) [P = 0.02; OR = 1.50 (95% CI = 1.06-2.14)], whereas the ACC haplotype was less represented in patients (0.28 versus 0.37) [P = 0.02; OR = 0.64 (95% CI = 0.44-0.92)]. To assess the functional role of genotypes, serum IL-10 levels from patients and controls were quantified by ELISA. Also, the LPS-induced IL-10 secretion by monocytes from healthy controls was evaluated in vitro. Serum IL-10 levels were higher in patients [median (IQR) = 2.8 pg/ml (1.8-4.2)] than in controls [0.9 pg/ml (0-3.5)] (P = 0.02), but no association was observed between serum IL-10 levels or LPS-induced IL-10 secretion and the IL-10 promoter haplotypes. . These data suggest that the IL-10 promoter haplotype that produces higher levels of cytokine is associated with SLE in patients from Canary Islands.

Introduction

Systemic lupus erythematosus (SLE) is a disorder of immune regulation manifested by a polyclonal B cell activation, and the presence of autoantibodies and immune complexes leading to tissue damage. The pathogenesis of SLE is multifactorial, with multiple susceptibility genes and environmental factors involved in its initiation (Mok & Lau, 2003, Tsao, 2004).

Interleukin-10 (IL-10) is an important pleiotrophic cytokine with anti-inflammatory and stimulatory activities (Moore *et al.*, 2001). This anti-inflammatory activity is mediated by the inhibition of the effector functions of T cells, macrophages and monocytes (de Waal *et al.*, 1991, Dokka *et al.*, 2001). On the other hand, IL-10 induces survival, proliferation, differentiation, and antibody isotype switching in B cells (Itoh & Hirohata, 1995). Thus, this cytokine plays an important role in the regulation of both cellular and humoral immune responses, explaining the two main features of the immune deregulation of SLE (high autoantibody production and decreased cellular immune responses). Several studies have shown that SLE patients spontaneously produce high levels of IL-10 (Llorente *et al.*, 1993) and an increased secretion is also found in some unaffected family members of SLE patients (Llorente *et al.*, 1997). The precise mechanisms engaged in the regulation of IL-10 production remain undetermined, although inherited factors appear to play an important role (Westendorp *et al.*, 1997). The implication of a heritable genetic basis for IL-10 production is supported by the concordance of IL-10 production in monozygotic twins, which suggests that genetic factors could account for up to 75% of IL-10 production.

The human IL-10 gene is located on chromosome 1 (1q31-1q32) and encodes for five exons (Eskdale *et al.*, 1997a, Kim *et al.*, 1992). The IL-10 promoter is highly polymorphic containing, among others, two microsatellites (MS), IL10R and IL10G, at 4

kb (Eskdale *et al.*, 1996) and 1.2 kb (Eskdale & Gallagher, 1995) upstream of the transcription start site, and three single nucleotide polymorphisms (SNPs): a G to A substitution at position -1082, a C to T at -819 and a C to A at -592. From all the possible haplotypes resulting from the combination of these three SNPs, only three have been described in Caucasoid populations ACC, ATA and GCC (Turner *et al.*, 1997b). As these MS and SNPs are in the proximity of putative transcription factor binding sites and regulatory regions, they are believed to affect innate IL-10 production at the transcriptional level. Several reports have analysed the influence of these polymorphisms on lupus disease showing conflicting results. Whereas the IL-10G microsatellite has been associated with SLE incidence in Scottish (Eskdale *et al.*, 1997b), Mexican-American (Mehrian *et al.*, 1998) and Italian (D'Alfonso *et al.*, 2000) populations, this has not been confirmed in Mexican (Alarcon-Riquelme *et al.*, 1999) Caucasoid (Schotte *et al.*, 2004) and Taiwanese (Ou *et al.*, 1998) populations. Concerning the SNPs, it has been described the frequency of -1082G allele is increased in SLE and related to synthesis of Ssa antibodies in British Caucasians (Lazarus *et al.*, 1997), while the ATA haplotype is overrepresented among Dutch Caucasians with neuropsychiatric lupus (Rood *et al.*, 1999) and Chinese patients suffering from renal disorder (Mok *et al.*, 1998). However, these haplotypes have not been found to be strong determinants of susceptibility to lupus disease in Caucasian populations. In the present study we investigated the possible relationship between those five IL-10 gene polymorphisms and the occurrence of SLE in Canary Islanders.

Materials and methods

Subjects

SLE patients comprised women admitted to the Rheumatology Unit at the Dr. Negrin Hospital (Canary Islands, Spain). All patients analysed fulfilled at least four of the American College of Rheumatology criteria for SLE (Tan *et al.*, 1982). An individual informed consent was obtained from each SLE patients. From a total of 116 patients, clinical and analytical characteristics were available in 80 and are resumed in Table 1. One hundred and fifty one control subjects with no autoimmune history and matched to patients by age, gender and geography were recruited among healthy blood donors. To the best of our knowledge samples in this group were unrelated (as indicated by no deviations of Hardy-Weinberg equilibrium). All patients and controls were of Canary origin without discernible ethnic variation (Citores *et al.*, 2004). Population of Canary Islands is genetically composed of a major European component, admixtured with a minor aborigine component of North Western African origin and from Subsaharian populations. According to the literature, this population is rather homogeneous among the islands (Fregel *et al.*, 2005).

Genotyping analyses

Genomic DNA was isolated from peripheral blood mononuclear cells (PBMCs) using a standard proteinase K (Roche Diagnostics GmbH, Mannheim, Germany) digestion and phenol/chloroform extraction method (Blin & Stafford, 1976). Genotyping at the MS and SNPs in 80 SLE patients and 79 healthy controls was performed as previously described in detail (Eskdale *et al*, 1996;Mehrian *et al*, 1998;Turner *et al*, 1997b) by polymerase chain reaction (PCR) on a Perkin Elmer 2400 thermal cycler (Perkin Elmer Applied Biosystems, Foster City, CA). A DNA polymerase (Pwo Roche Diagnostics

GmbH, Mannheim, Germany) with proofreading activity was used for MS amplification. Amplified products of both MS were separated by electrophoresis on an 15% polyacrylamide gels (GeneGel Clean 15/24 Kit, Amersham Biosciences AB, Uppsala, Sweden) in denaturing conditions and the DNA bands were visualized with a silver staining kit (Amersham Bioscences AB). PCR products resulting from the amplification of the gene region containing the three SNPs (Turner *et al.*, 1997a) were digested with the restriction enzyme *MnlI* (New England BioLabs®, Beverly, MA) for the -1082 polymorphism, *MaeIII* (Roche Diagnostics GmbH) for the -819 and *RsaI* (Roche Diagnostics GmbH) for the –597. Fragments generated by *MaeIII* and *RsaI* were separated on an 2% and 3% agarose Metaphor® (Biowhittaker Molecular Applications, Rockland, ME) gel, respectively, and then stained with ethidium bromide. Fragments generated by *MnlI* digestion were separated on a 15% polyacrylamide gel and silver stained. Allele assignment was performed using the Quantity-One® software (Bio-Rad Laboratories, Hercules, CA). The length of the amplified fragments or the restriction profile with the MS was estimated by reference to the standards of a known length.

An additional group of frozen DNA samples from 36 SLE patients and 72 controls were genotyped for the three SNPs by 5' nuclease allelic discrimination assays (TaqMan®MGB; Applied Biosystems). Genotypes were determined by using the same primer pair used in the RFLP-based method and probes C_1747360_10, C_1747363_10 and C_1747363_10 for –1082, -819 and –592 positions respectively. The polymerase chain reaction (PCR) was performed in an ABI Prism 7700 system in duplicate samples with a total volume of 5 μl under conditions recommended by the manufacturer (Applied Biosystems). Also, five samples previously analyzed by the PCR-RFLP-based method were included in this new group to control for coincidence between the two methods used, resulting in 100% replication of the results.

Cell Cultures

Peripheral blood was drawn into heparinized tubes and PBMCs were obtained by density gradient centrifugation. PBMCs were enriched in monocytes using a monocyte-cell-negative isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The resulting cell suspension was cultured at a concentration of 5 x 10^5 monocytes/ml in 24-well plates for 24 h in the presence or in the absence of 0.5 μg/ml of LPS (from *Escherichia coli* 055:B5, Sigma-Aldrich) for IL-10 induction in vitro. Supernatants were harvested for immediate freezing at -80 ºC until use.

Determination of IL-10 levels

Five ml of peripheral venous blood was collected from each patient and control in anticoagulant-free tubes, and centrifuged for 10 min at 800 g after being allowed to clot at room temperature for 30 minutes. The serum recovered was stored in small aliquots at -80 ºC until used. Serum IL-10 levels and LPS-induced IL-10 secretion were measured in duplicates by immunoassay with Quantikine HS and Quantikine Il-10 (R&D Systems Inc, Minneapolis, MN). The detection limit of the test was 0.78 pg/ml.

Statistical analysis

The association of allele or genotype frequencies with SLE was assessed with the χ^2 test and Yates correction or the two-tailed Fisher's exact test when required. The strength of association was then assessed by calculating odds ratios (OR) and the exact limits for the 95% confidence intervals (95% CI). Estimation of the statistical power for the comparison of allele frequencies was performed with the STPLAN software. The arcsin approximation of the binomial distributions of allele frequencies was used with a two-sided test and with α fixed at 0.05. The *P* values for multiple significance tests were

always corrected using the Bonferroni method (P_c). Those statistical tests with P value of less than 0.05 were considered significant. Hardy-Weinberg equilibrium and pairwise linkage disequilibrium between polymorphisms were assessed using the ARLEQUIN v.2 software (Schneider S, 2000).

Serum IL-10 levels and LPS-induced IL-10 secretion were not distributed normally, and so non-parametric testing was used throughout (Kruskal-Wallis test or Mann-Whitney U test). IL-10 levels are described by median and interquartile range (IQR), which represents the 25th and 75th percentiles of the distribution. All calculations were performed by using the SPSS v.11 software (SPSS Inc, Chicago, IL).

Results

IL-10 promoter polymorphisms in Canary patients

We examined the distribution of IL-10R, IL-10G polymorphisms in Canary healthy females to determine whether any of them was associated with SLE in this population. First we identified three distinct alleles (12, 13 and 14 CA repeat number) for the IL10R MS and nine allelic variants (ranging from 17 to 25 CA repeat number) for the IL10G MS, as previously described by Eskdale *et al*. (1997b) for Caucasian population. Hardy-Weinberg analysis of the genotype data from both MS indicated a random distribution (data not shown). We did not find any difference in allelic and genotypic frequencies of IL-10R between SLE patients (n = 80) and controls (n = 79), being the allele with 12 CA repeat the most frequent in both groups. No association between IL10G MS and SLE was found either, being the most frequent alleles those of 19, 21 and 23 CA repeats.

Concerning SNPs, we first checked that each polymorphic site fitted Hardy-Weinberg equilibrium and linkage disequilibrium, and then we constructed the haplotypes with the Arlequin software. From all the possible haplotypes resulting from the combination of the three SNPs, we only observed three (ACC, ATA and GCC), as previously described by Turner *et al*. (1997b) for Caucasian population, and the genotypes more represented in our population were similar to those described for Spaniards (Suarez *et al.*, 2003). When comparing the haplotype and genotype distributions between 80 SLE patients and 79 controls, we observed significant differences in the overall haplotype frequencies (P_c = 0.04). If we compared each haplotype with all the others we observed that the GCC haplotype frequency was significantly higher in SLE patients (0.44) than in healthy controls (0.32) [P = 0.03; OR = 1.68 (95% CI = 1.06-2.66); Power = 0.60] and the ACC haplotype frequency was

significantly lower in SLE patients (0.24) than in healthy subjects (0.35) [P = 0.04; OR = 0.6 (95% CI = 0.36-0.95)] (Table 2). In contrast, we did not found significant differences in the overall genotype frequencies when Bonferroni correction for multiple tests was applied, but the proportion of the GCC/GCC genotype was higher in SLE patients when compared with normal controls [20% vs. 6%; P = 0.02; OR = 3.7 (95% CI = 1.28-10.66); Power = 0.75] (Table 2).

An additional group of 36 SLE patients and 72 healthy females was genotyped for the three SNPs to provide a more reliable statistical analysis of the association found with the GCC haplotype. The results were similar after the inclusion of these new samples. Significant differences in the overall haplotype frequencies between patients and controls were conserved (P_c = 0.03), as well in the GCC haplotype frequency [0.43 vs 0.33; P = 0.02; OR = 1.50 (95% CI = 1.06-2.14); Power = 0.66] and in the ACC haplotype frequency [0.28 vs 0.37; P = 0.02; OR = 0.64 (95% CI = 0.44-0.92)] (Fig. 1). And, although no differences in overall genotype frequencies were found (P_c = 0.08), the proportion of the GCC/GCC genotype was higher in patients than in controls [0.18 vs. 0.09; P = 0.03; OR = 2.2 (95% CI = 1.05-4.47); Power = 0.58] (Table 2).

IL-10 promoter polymorphisms and clinical presentation of SLE

Once studied the association with SLE, we examined the IL-10 genotype in those SLE patients with known phenotype (n = 80) and stratified according to the clinical parameters showed in Table 1. As shown in Table 3, we observed that patients homozygous for the IL10R-12 allele showed a lower incidence of pericarditis (9%) and vasculitis (15%) than those patients with another genotype (33% and 50%, respectively), and a significantly higher proportion of the IL10R 12/13 genotype was observed in patients who presented vasculitis (53%). Regarding to IL-10G, we observed that the

19/23 genotype frequency was increased in the SLE patients with Anti-Sm (53% vs. 21%) and seizures (40% vs. 9%).

In contrast to expected, we could not find an association of GCC/GCC homozygous genotype with any disease feature. However, the heterozygous genotypes such as ACC/GCC were associated with a significantly higher incidence of discoid rash (40% vs. 11%) and the ATA/GCC genotype with pericarditis (37% vs. 11%) (Table 1).

Influence of genotype on circulating IL-10 protein levels

Initially we determined the serum IL-10 levels in 27 healthy subjects and 50 SLE patients. Serum IL-10 levels were higher in patients [median (IQR) = 2.8 pg/ml (1.8-4.2)] than in controls [0.9 pg/ml (0-3.5)] (P = 0.02), which was consistent with previous reports (Grondal *et al.*, 2000, Llorente *et al.*, 1994). Due to the fact that we found an association of IL-10 SNPs with SLE, we considered that these polymorphisms might be related to IL-10 protein levels in SLE patients. Thus, in order to analyse the effect of the haplotypes on the serum IL-10 levels, SLE patients were classified according to their haplotype-carrier status. As shown in Fig. 2, the concentration of serum IL-10 was higher in patients carrying the GCC haplotype [2.98 pg/ml (1.77-5.10)] than in non-carriers [2.54 pg/ml (1.76-3.48)], although these differences were not significant. A possible explanation for these results could be that IL-10 serum levels might be influenced by the clinical disease activity, as previously described (Miret *et al.*, 2001, Waszczykowska *et al.*, 1999). For this reason, we next analysed the association between IL-10 genotypes and LPS-induced levels of IL-10 in healthy individuals.

Different IL-10 promoter genotypes express different levels of protein

Monocytes from 17 healthy individuals with different IL-10 genotypes at the positions -1082 A/G, -819 C/T and -592 A/C of the promoter were cultured in medium alone or in the presence of LPS (0.5 μg/ml). After 24 h, culture supernatants were harvested and IL-10 concentration was measured by ELISA. The analysis of IL-10 protein in supernatants of cultures revealed that healthy individuals carrying the GCC haplotype produced more IL-10 [median (IQR) = 2726 pg/ml (1918-3107)] than those non-GCC carriers [1901 pg/ml (1618-2092)], although these differences were not significant, probably due to the low number of samples studied (Fig. 3A). When we classified the carriers of the GCC haplotype in homozygous and heterozygous and compared the three resulting genotypes (X/X, X/GCC and GCC/GCC), we observed that subjects homozygous for the GCC haplotype produced more IL-10 [3107 pg/ml (2408-3417)] than heterozygous [2443 pg/ml (1702-2885)] and homozygous for non-GCC haplotype [1901 pg/ml (1618-2092)], although the differences were not statistically significant (Fig. 3B). These findings were in accordance with previous reports in Caucasoid population and Spaniards (Edwards-Smith *et al.*, 1999, Turner *et al.*, 1997b, Suarez *et al.*, 2003).

Discussion

IL-10 production in SLE patients is increased and it is known to be released from both B cells and monocytes (Houssiau *et al.*, 1995, Llorente *et al.*, 1994, Richaud-Patin *et al.*, 1995). It has been proposed that IL-10 production is under strong genetic influence (Westendorp *et al.*, 1997) and several polymorphisms have been described in the promoter region of IL-10 that might be related with transcriptional regulation of this gene (Eskdale *et a.l*, 1997a, Kube *et al.*, 1995). In the present work, we have characterised a cohort of SLE patients from Canary Islands (Spain) according to their IL-10 promoter genotypes at positions -4000, -1200, -1082, -819 and - 592 upstream of the transcription start site.

A number of studies have been published regarding the association of IL-10 polymorphisms with SLE, with discordant results. Alleles in the IL-10 G microsatellite have been found to be associated in Mexican and Caucasian populations (D'Alfonso *et al.*, 2000, Eskdale *et al.*, 1997b, Mehrian *et al.*, 1998), but these findings were not replicated in independent studies (Alarcon-Riquelme *et al.*, 1999, Schotte *et al.*, 2004). In the present study no association has been found in the IL-10 G microsatellite. However, the reliability of the comparisons is low because this microsatellite is highly polymorphic, greatly reducing sample numbers for individual allele comparisons. Regarding the SNPs, we initially found association of the GCC haplotype and the GCC/GCC genotype with SLE. An additional group of patients and controls was analysed for SNPs distribution and added to the previous samples to increase the consistency of the comparisons. Association found in the first group remained the same in the final extended group. Distribution of the SNP haplotypes in controls and SLE patients are similar to that previously reported in Spain (Suarez *et al.*, 2005) although in this work statistical differences are found only in a subgroup of discoid lupus patients.

Also, another study of association with Sjögren syndrome in Spaniards (Font *et al.*, 2002) reports similar SNP frequencies. However, again discordant results have been published regarding the association of the SNPs with SLE in several populations.

Distribution of the SNPs was also analysed in relation to clinical features among SLE patients. Several associations have been found, but because of the limited sample numbers these comparisons lack statistical power to be taken as consistent results.

Based on the association found with the GCC haplotype, we investigated whether GCC haplotype exerted any influence on serum IL-10 levels, thus explaining the association with SLE. No influence of the genotype on serum IL-10 levels was found in patients but, as the disease activity in our patients was variable, we could not exclude an important influence in serum IL-10 levels, as previously reported (Miret *et al.*, 2001, Waszczykowska *et al.*, 1999). Although the contribution of IL-10 promoter haplotypes has been proposed, the identification of the highest IL-10 producer haplotype is controversial. Several authors have associated the highest levels with the ATA and others with ACC haplotype (Huizinga *et al.*, 2000, Temple *et al.*, 2003), in contrast, Crawley *et al*. (1999) exposed that individuals with ATA genotypes produced significantly less IL-10 than non-ATA carriers. When we evaluated the relation of IL-10 promoter genotypes with LPS-induced IL-10 production in healthy individuals, we detected that the highest production of IL-10 after LPS stimulation corresponded to the GCC haplotype, as previously described in Caucasian and Spanish population (Suarez *et al.*, 2003, Turner *et al.*, 1997b).

The current study shows that the presence of the GCC haplotype of the IL-10 gene is associated with an increased susceptibility to SLE in Spanish population from Canary Islands. This effect is probably mediated by the increased capability to produce

IL-10 among carriers of the GCC haplotype. We also suggest an association of IL-10 gene polymorphism with the clinical phenotypic diversity observed in these patients.

Acknowledgements

This project was supported by Fundacion LAIR (8108) and Ministerio de Educacion y Ciencia (AP2000-2160). We would like to thank Alberto Durantez, Beatriz Contreras-Martin, Sandra Cuni, Isabel Martin-Larrauri, for their continuous help.

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Table 1. Clinical and analytical features of SLE patients studied

Clinical feature	n/N[(1)] (%)	Analytical feature	n/N (%)
Malar rash	43/80 (53.8)	Proteinuria	35/80 (43.8)
Discoid rash	13/80 (16.3)	Urinary cellular casts	7/80 (8.8)
Photosensitivity	45/80 (56.3)	Glomerulonephritis	10/16 (62.5)
Oral ulcers	32/80 (40)	Haemolytic anaemia	6/80 (7.5)
Vasulitis	17/67 (25.4)	Thrombocytopenia	20/80 (25)
Raynaud's phenomenon	32/70 (45.7)	Leukopenia	55/80 (68.8)
Arthritis	66/80 (82.5)	Lymphopenia	9/80 (11.3)
Serositis	23/80 (28.8)	Low complement levels	62/80 (77.5)
Renal disorder	36/80 (45)	Anti-DNA	71/80 (88.8)
Neurologic disorder	13/80 (16.3)	Anti-Sm	20/73 (27.4)
Haematological disorder	65/80 (81.3)	Anti-phospholipid	36/73 (49.3)
Immunologic disorder	72/80 (90)		
Antinuclear antibody	80/80 (100)		
Thrombosis	62/80 (77.5)		

[(1)]n = number of SLE patients presenting the analytical feature / N = Total SLE patients evaluated for the mentioned analytical feature. The percentage is indicated in parentheses.

Table 2. SNPs genotype frequencies of IL-10 promoter in SLE patients and controls

Genotypes	Controls		SLE		*P* value OR (95% CI)[2]	*P*' value OR (95% CI)[3]
	n = 79	n = 151	n = 80	n = 116		
ACA/ACA	0(0)[1]	0(0)	1(0.01)	1(0.01)	ns	ns
ACA/GCC	0(0)	0(0)	3(0.04)	3(0.03)	ns	ns
ACC/ACC	6(0.08)	15(0.10)	5(0.06)	9(0.08)	ns	ns
ACC/ATA	19(0.24)	37(0.25)	13(0.16)	18(0.16)	ns	ns
ACC/ATC	0(0)	0(0)	1(0.01)	3(0.03)	ns	ns
ACC/GCC	25(0.32)	45(0.30)	15(0.19)	25(0.22)	ns	ns
ATA/ATA	9(0.11)	13(0.09)	5(0.06)	6(0.05)	ns	ns
ATA/ATC	0(0)	0(0)	1(0.01)	1(0.01)	ns	ns
ATA/GCC	15(0.19)	27(0.18)	16(0.20)	25(0.22)	ns	ns
ATC/GCC	0(0)	0(0)	2(0.02)	2(0.02)	ns	ns
GCC/GCC	5(0.06)	14(0.09)	16(0.20)	21(0.18)	3.7 (1.28-10.66)	2.2 (1.05-4.47)
GCC/GTC	0(0)	0(0)	2 (0.02)	2(0.02)	ns	ns

[1]Number of times that each genotype was observed. In parenthesis the frequencies.

[2]*P* value from comparison of 79 controls and 80 SLE patients. The strength of association was evaluated as odds ratio (OR) with the exact limits for the 95% confidence intervals (CI).

[3]*P* value from comparison of 151 controls and 116 SLE patients.

ns = not significant.

Table 3. Clinical manifestations associated with polymorphisms of IL-10 gene promoter

Polymorphisms	Clinical feature	With genotype (n/N)(%)[2]	Without genotype (n/N)(%)[3]	*P* value OR (95% CI)[4]
		12/12		
IL10R	***Pericarditis*** (13/79)[1]	5/55 (9)	8/24 (33)	0.02 0.2 (0.06-0.7)
	Vasculitis (17/67)	7/47 (15)	10/20 (50)	0.007 0.2 (0.05-0.6)
		12/13		
	Vasculitis (17/67)	9/17 (53)	8/50 (16)	0.007 5.9 (1.7-19.9)
		19/23		
IL10G	***Seizures*** (12/80)	6/15 (40)	6/65 (9)	0.01 6.55 (1.73-24.8)
	Anti-Sm (20/73)	8/15 (53)	12/58 (21)	0.03 4.38 (1.32-14.5)
		ACC/GCC		
SNPs	***Discoid rash*** (13/80)	6/15 (40)	7/65 (11)	0.02 5.5 (1.5-20.2)
		ATA/GCC		
	Pericarditis (13/79)	6/16 (37)	7/63 (11)	0.03 4.8 (1.33-17.3)

[1]Patients presenting the indicated clinical manifestation/Total of patients evaluated for the mentioned clinical feature.

[2]n = number of patients with or without the indicated genotype presenting the mentioned clinical feature /N = Total of patients evaluated for the mentioned clinical feature. The percentage is indicated in parentheses.

[3]Like [2] but in patients with other genotype.

[4]The strength of association was evaluated as odds ratio (OR) with the exact limits for the 95% confidence intervals (CI).

Figure 1. SNPs haplotype frequencies in SLE patients and healthy controls. Significant differences were found in haplotype distribution between SLE patients and controls (corrected *P* value (*Pc*) for multiple significance tests using the Bonferroni method was 0.03). ACC haplotype was reduced in the SLE patients while GCC haplotype was increased.

*$P < 0.05$, when each haplotype was compared with all the others by χ^2 analysis.

Figure 2. Median values for serum IL-10 in 50 SLE patients classified in non-carriers (X/X) and carriers (X/GCC) of the IL-10 GCC haplotype. The box represents the 25th-75th quartile divided by the median, the whiskers the range.

n = number of patients with the indicated genotype.

Figure 3. LPS-induced IL-10 levels *in vitro* in healthy donors. Monocytes from 17 healthy donors were cultured for 24 h in the presence of absence of 0.5 μg/ml LPS. Then, IL-10 secreted was measured by ELISA. (A) Carriers and non-carriers of IL-10 GCC haplotype were compared. (B) It is shown the comparison among genotypes. The medians and the 25th-75th quartile of increment in IL-10 levels after stimulation are shown for each genotype.

n = number of patients with the indicated genotype.

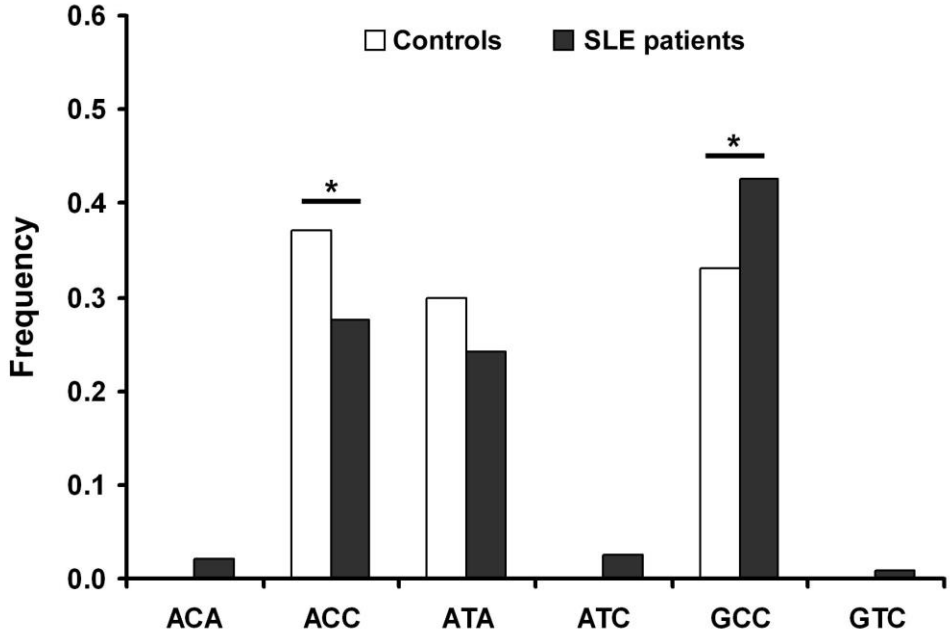
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Frequency
Controls
SLE patients
*
*
ACA
ACC
ATA
ATC
GCC
GTC

Figure 1

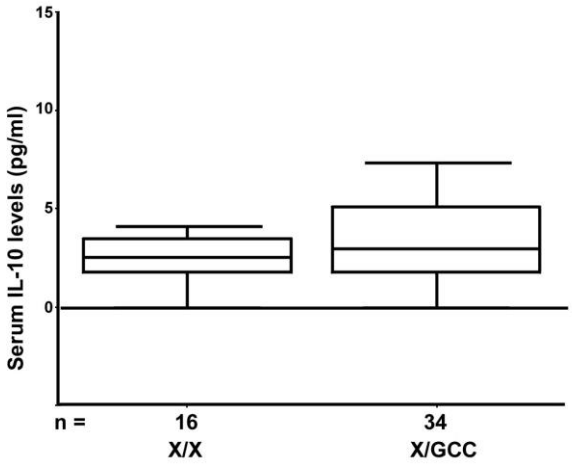

Figure 2

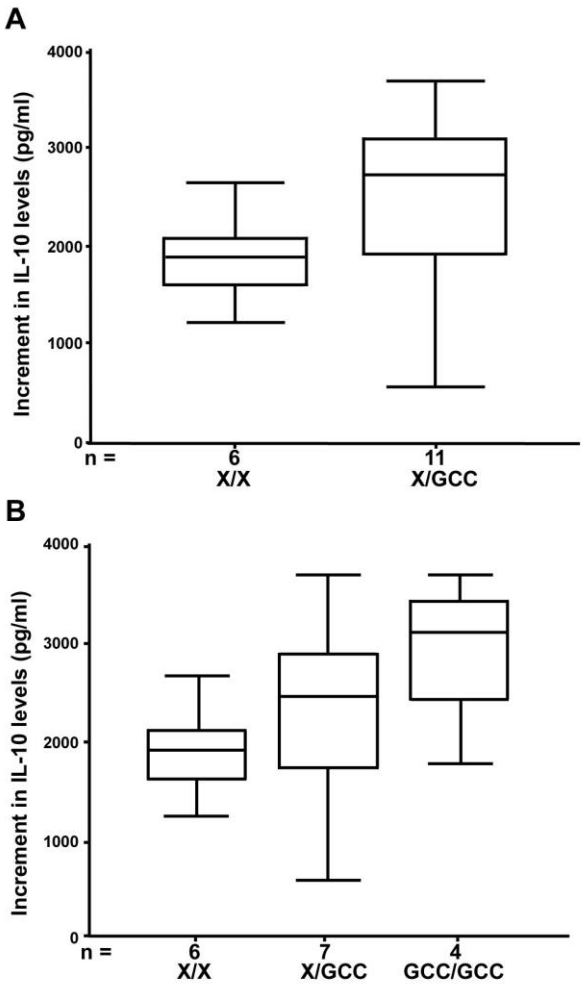
A
Increment in IL-10 levels (pg/ml)
4000
3000
2000
1000
0
n =
6
11
X/X
X/GCC
B
Increment in IL-10 levels (pg/ml)
4000
3000
2000
1000
0
n =
6
7
4
X/X
X/GCC
GCC/GCC

Figure 3