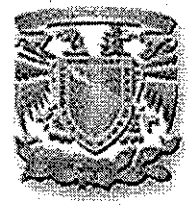
UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
FACULTAD DE MEDICINA



INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN

Ausencia de asociación entre el polimorfismo genético de la proteína de choque térmico (HSP70-2) y el lupus eritematoso generalizado, en pacientes mestizos mexicanos

TESIS PARA OBTENER EL GRADO DE ESPECIALISTA EN MEDICINA INTERNA PRESENTADA POR LA DRA. TATIANA SOFÍA RODRÍGUEZ REYNA

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Lack of association between the polymorphism at the heat-shock protein (HSP70-2) gene and systemic lupus erythematosus (SLE) in the Mexican Mestizo population

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Major histocompatibility complex (MHC) alleles have been recognized as genetic factors for developing systemic lupus erythematosus (SLE). In the present study we analyzed whether a heat-shock protein gene (HSP70-2) is involved in determining susceptibility to develop SLE in a Mexican Mestizo population. A HSP70-2 Pst I polymorphism was detected by a restriction fragment length polymorphism analysis of polymerase chain reaction (PCR-RFLP) in 107 SLE patients and 158 healthy controls. No statistically significant differences were observed in the HSP70-2 allele distribution between patients and healthy controls. HLA-DR analysis showed an increased frequency of HLA-DR3 allele in the patients group ($P < 0.05$, OR = 2.26, EF = 6.0%). On the other hand, when we analyzed HSP70-2 polymorphism in relation to HLA-DR3 allele, we could only detect an increased frequency of AB genotype in the DR3 negative patients ($pC < 0.05$, RR = 2.6, EF = 11.3%). Linkage disequilibrium was observed for three haplotypes: HLA-DR3-HSP70-2A ($D = 0.03$, $D' = 0.67$, $P < 0.01$); HLA-DR1-HSP70-2A ($D = 0.03$, $D' = 0.86$, $P < 0.01$) and HLA-DR8-HSP70-2B ($D = 0.02$, $D' = 0.46$, $P = 0.02$). Our data indicate that HSP70-2 gene polymorphism as opposed to the other ethnic groups does not appear to be relevant in SLE susceptibility in Mexican patients and that the distribution of the different alleles depend on the frequency of HLA alleles associated with them. Genes and Immunity (2000) 1, 367–370

Keywords: *genetic susceptibility; heat shock protein; systemic lupus erythematosus*

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder in which the genetic factors play an important role. Some major histocompatibility complex (MHC) genes have been associated with susceptibility to develop SLE. Among these HLA-B8, DR2, DR3, homozygous C4 and C4A and/or C4B deficiency,[1] are potential susceptibility loci. The mechanism by which these genes determine susceptibility is not completely understood. However, the hypothesis of unknown susceptibility genes in linkage disequilibrium with those currently detected is being suggested. Thus, other MHC genes detected in the class III region have been studied.[2] The heat-shock protein (HSP70-2) gene, encodes a highly inducible stress protein fundamentally important for protein transport and folding.[3] Several studies have proposed the HSP's loci as possible susceptibility genes for the development of autoimmune diseases; ie, Grave's disease,[4] insulin-dependent diabetes mellitus,[5] celiac disease[6] and SLE.[7,8] In Spanish patients with SLE, the association was due to linkage disequilibrium between HSP70 and HLA-DR3,[7] whereas in African American patients the association was independent of this disequilibrium.[8] Since the linkage disequilibrium between HLA and HSP70-2 alleles has been described in Caucasian populations, studies in other populations are mandatory. To expand the knowledge of this association, we have investigated by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, the distribution of the HSP70-2 alleles in Mexican Mestizo SLE patients.

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Results

Table 1 summarizes the HSP70-2 genotype and allele frequencies of 107 SLE patients and 158 controls. In both groups, the most common genotype was heterozygous AB (83.1% in patients and 72.2% in controls) and the most frequent allele was A (58.4% in patients and 58.9% in controls). The BB homozygous individuals were only detected in the control group and showed an increased frequency when compared with patients (5%, $pC < 0.05$ *vs* patients). No statistically significant differences were observed when the HSP70-2 alleles distribution between patients and healthy controls was compared. Complete analysis of HLA-DR alleles in Mexican patients and controls showed an increased frequency of HLA-DR3 ($P < 0.05$, pC = NS, OR = 2.26, EF = 6.0%) and decreased frequencies of HLA-DR7 ($P < 0.05$, pC = NS) and HLA-DR8 ($P < 0.05$, pC = NS) alleles in the patient's group (Table 2). Because the HLA-DR3 is in strong linkage disequilibrium with the HSP70 alleles, we analyzed HSP70–2 polymorphism in relation to HLA-DR3 in 107 patients (20 positives and 87 negatives for DRB1*03) and 78 controls (six positives and 72 negatives for DRB1*03) (Table 3). In this case, we did not find significant differences in the HSP70-2 alleles distribution between SLE patients and controls. An increased frequency of AB genotype was observed in the DR3 negative patient's group (81.6% in patients *vs* 62.5% in controls, $pC < 0.05$, RR = 2.6, EF = 11.3%). Linkage disequilibrium between HSP70-2 and HLA-DR alleles was noted: HLA-DR3-HSP70-2A ($D = 0.03$, $D' = 0.67$, $P < 0.01$); HLA-DR1-HSP70-2A ($D = 0.03$, $D' = 0.86$, $P < 0.01$) and HLA-DR8-HSP70-2B ($D = 0.02$, $D' = 0.46$, $P = 0.02$) (data not shown).

Table 1 HSP70-2 genotype and allele frequencies (%) in SLE patients and controls

	SLE (n=107)	*Controls (n=158)*
Genotype	*n (%)*	*n (%)*
AA	18 (16.8)	36 (22.8)
AB	89 (83.1)	114 (72.2)
BB	0 (0.0)	8 (5.0)*
Alleles		
A	125 (58.4)	186 (58.9)
B	89 (41.5)	130 (41.1)

The P values were corrected by multiplying them by the number of comparisons.
*$pC < 0.05$.

Table 2 Gene frequencies (gf) of HLA-DR alleles in Mexican patients with systemic lupus erythematosus (SLE) and controls

	SLE (n=107)		*Controls (n=78)*				
	n	*gf*	*n*	*gf*	*P**	*RR*	*EF (%)*
DR1	17	0.079	6	0.038	NS	—	—
DR3	22	0.102	7	0.044	<0.05	2.43	6.0
DR4	57	0.266	44	0.282	NS	—	—
DR7	10	0.046	16	0.102	<0.05	0.42	—
DR8	16	0.074	24	0.153	<0.05	0.44	—
DR9	2	0.009	2	0.012	NS	—	—
DR10	1	0.004	1	0.006	NS	—	—
DR11	15	0.070	17	0.108	NS	—	—
DR12	3	0.014	2	0.012	NS	—	—
DR13	19	0.088	8	0.051	NS	—	—
DR14	16	0.074	16	0.102	NS	—	—
DR15	24	0.112	9	0.057	NS	—	—
DR16	12	0.056	4	0.025	NS	—	—

This analysis included 78 controls typed for HLA-DR and HSP70-2. *pC not significant. NS, not significant; RR, relative risk; EF, etiologic fraction.

Discussion

SLE is an autoimmune disorder with a large spectrum of clinical manifestations and a variety of immunological features. In Mexican Mestizo patients the disease has been associated principally with the HLA-DR3 allele.[9] However, other susceptibility genes could be located either close by or outside of the HLA class II region. The chromosomal mapping of the three members of the HSP70 gene family in the HLA class III region[10] and their linkage disequilibrium with other relevant HLA genes[6] has led to investigation of the possible role of HSP70 genes in the genetic susceptibility to autoimmune diseases. Our results suggest that there is no significant association of HSP70-2B allele with the susceptibility to develop SLE. The presence of BB homozygous individuals in the control group could only reflect HSP70-2B allele linkage disequilibrium with some frequent HLA alleles in this group. The present results do not support the findings in previous studies which have implicated HSP70-2 gene polymorphism in SLE and other autoimmune diseases.[4–8] However, in most of the cases, the associations were due to linkage disequilibrium between HSP70-2B and DR3 alleles[5–7] which are included in the A1-B8-DR3 and A30-B18-DR3 extended haplotypes and thus, probably secondary to, the DR/DQ associations.

On the other hand, previous studies have failed to show significant relevance of HSP70 polymorphism in the susceptibility to several HLA-associated diseases.[11,12] The HSP70-2 polymorphism analysis, together with a previous one in which no association was observed

Table 3 Distribution of HSP70-2 allele and genotypes in patients and controls considering the HLA-DR3 allele

	DR3 (–)		*DR3 (+)*	
	SLE (n=87)	*Controls (n=72)*	*SLE (n=20)*	*Controls (n=6)*
Genotype				
AA	16 (18.3)	27 (37.5)*	2 (10.0)	2 (33.3)
AB	71 (81.6)**	45 (62.5)	18 (90.0)	4 (66.6)
Alleles				
A	103 (59.1)	99 (68.7)	22 (55.0)	8 (66.6)
B	71 (40.8)	45 (31.2)	18 (45.0)	4 (33.3)

This analysis included 78 controls typed for HLA-DR and HSP70-2. None of them were BB homozygous. The P values were corrected by multiplying them by the number of comparisons.
**,*$pC < 0.05$. In the patients group, 20 individuals were DR3 positives: 19 were DRB1*0301 and one was DRB1*0302, whereas in the control group only six were DR3 positives: four were DRB1*0301, one was DRB1*0302 and one was DRB1*0303.

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The mechanism by which the HSP70 genes could deter-ine the susceptibility to SLE and other autoimmune dis-ases is largely unknown. Recent information in rheuma-oid arthritis[14] suggests that HLA-DR4 and DR10 motifs ssociated with the disease bind a 70 Kb heat-shock pro-in. This could influence the processing of antigenic pep-des and their inclusion in the HLA-DRB1 chain groove owever, this would be expected in the case of HSP70-m variation which lies within the predicted peptide-nding domain,[15] but not in the case of HSP70-2 in hich polymorphism depends only on a silent change \→G) in the coding region. Thus, any contribution of e HSP70-2 polymorphism to SLE susceptibility could e attributed to a neighboring gene yet unidentified.

In conclusion, our results appear to rule out any rel-ance of the HSP70-2 locus in the susceptibility to SLE Mexican Mestizo individuals

Material and methods

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ne-hundred and seven unrelated Mexican Mestizo atients with SLE, who fulfilled the American College of heumatology criteria, were studied. As controls, 158 ealthy Mexican Mestizo individuals, without a case his-ry of connective tissue disorders, were included HSP70-2 polymorphism for this group has been pub-shed previously).[16] We considered Mexican Mestizo ose individuals whose last two generations were born Mexico. This group is genetically well characterized d has shown a proportion of 56% Indian genes, 40% Caucasian genes and 4% of Black genes.[17–19]

NA extraction

enomic DNA from whole blood containing EDTA was tracted by standard techniques.[20]

eneric HLA-DR typing

eneric DR typing was performed by PCR-SSO reverse ot blot using the Amplicor Kit (Hoffmann La Roche, asel, Switzerland).

yping for DR3 alleles

ubtypes of DR3 specificity were detected by a direct equence strategy. DNA of each individual (patient or ontrol) that was positive for DR3, was amplified using e primers: DRBAMP-B (5'-CCGCTGCACTGTGAAG TCT-3') and DRBAMP-3 (5'-CACGTTTCTTGGAG ACTCTAC-3'). Templates were purified using Qiaquick el Extraction Kit (QIAGEN, Hilden, Germany) and equenced by the Sanger dideoxy chain terminator ethod, with dye-labeled dideoxy terminator chemistry. NA samples were analyzed in a Perkin-Elmer 310 auto-ated DNA sequencer (Applied Biosystems, CA, USA).

CR analysis of *HSP70-2* alleles

HSP70-2 restriction fragment length polymorphism at osition 1267 was characterized by a PCR procedure.[7] his analysis was performed considering the polymor-hic *Pst* I site at position 1267 of these alleles. We selected e following primers spanning the polymorphic *Pst* I site (nucleotide 1267) according to the previously described sequence,[7] sense (nucleotide 1083–1102 within the coding region) 5'-CATCGACTTCTACACGTCCA-3' and antisense (nucleotide 2180–2199 within the 3' untranslated end to avoid HSP70–1 homology) 5'-CAAAGTCC TTGAGTCCCAAC-3' Genomic DNA from peripheral blood leukocytes of patients and controls was amplified under the following conditions: 200 μM each dNTP, 2 mM $MgCl_2$, 1X PCR buffer, 25 pmol of each primer and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI, USA) in 25 μl of reaction. The PCR was carried out by 35 cycles of denaturing at 94°C for 1 min., annealing at 56°C for 1 min., and extension at 72°C for 2 min. The products of PCR were cleaved with *Pst* I (Pharmacia, Uppsala, Sweden) and electrophoresced on ethidium bromide-stained 1.5% agarose. The DNA lacking polymorphic *Pst* I site within the HSP70-2 gene generates a product of 1117 bp after restriction (allele A), whereas the *Pst* I site produced two fragments of 936 and 181 bp (allele B).

Statistical analysis

Significance in genotype and allele frequencies was analyzed by using contingency 2×2 tables and the chi-square test. The *P* values were corrected by the Bonferroni method multiplying the *P* values for the number of comparisons. The strength of the association was estimated by the relative risk (RR) and etiologic fraction (EF).[21] Also, linkage disequilibrium (D = disequilibrium values; D' = normalized disequilibrium values, D/Dmax) between HSP70 and HLA-DR alleles was evaluated using the Arlequin v. 1.1 software kindly provided by L. Excoffier and M Slatkin.[22]

Acknowledgements

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DIVISION DE ESTUDIOS DE POSGRADO E INVESTIGACION

SUBDIVISION DE ESPECIALIZACIONES MEDICAS

OFICIO FMED/SEM/1898/2002

ASUNTO: Autorización del trabajo de investigación de la Dra. Tatiana Sofía Rodríguez Reyna.

DR. CESAR AUGUSTO COLINA RAMÍREZ
SECRETARIO DE SERVICIOS ESCOLARES
DE LA FACULTAD DE MEDICINA
Presente.

Estimado Dr. Colina Ramírez:

Me permito informar a usted que la **Dra. Tatiana Sofía Rodríguez Reyna,** alumna del curso de especialización en **Medicina Interna** en el **Instituto Nacional de Ciencias Médicas y de la Nutrición "Dr. Salvador Zubirán"**, presenta el trabajo de investigación intitulado ***"Lack of association between the polymorphism at the heat-shock protein (HSP70-2) gene and systemic lupus erythematosus (SLE) in the Mexican Mestizo population".***

De conformidad con el artículo 21 capítulo 5º de las Normas Operativas del Plan Unico de Especializaciones Médicas (PUEM) se considera que cumple con los requisitos para validarlo como el trabajo formal de Investigación que le otorga el derecho de la diplomación como especialista.

Sin otro particular de momento, reciba un cordial saludo.

INCMNSZ
INSTITUTO NACIONAL
DE CIENCIAS MEDICAS Y NUTRICION
"DR SALVADOR ZUBIRAN"
DIRECCION DE ENSEÑANZA
México, D.F

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D. F. a 30 de septiembre de 2002

JEFE DE LA SUBDIVISION

DR. LEOBARDO C. RUIZ PEREZ

LRP*ajr.

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