HMOX1 promoter (GT)n polymorphism is associated with childhood-onset systemic lupus erythematosus but not with juvenile rheumatoid arthritis in a Mexican population

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Abstract

Objectives

The heme oxigenase 1 (HO-1), a rate-limiting enzyme for heme degradation, is an important cytoprotective protein. Transcriptional activity of HO-1 coding gene (HMOX1) can be regulated by the presence of a dinucleotide repeat polymorphism (GT)n at its promoter region. Accordingly, length of (GT)n repeat has been associated with susceptibility to several diseases. We investigated whether the HMOX1 (GT)n polymorphism was associated with childhood-onset systemic lupus erythematosus (SLE) and juvenile rheumatoid arthritis (JRA) susceptibility.

Methods

We studied 207 and 333 unrelated Mexican patients with JRA and childhood-onset SLE, respectively. The control population consisted of 653 individuals ethnically matched with cases. The HMOX1 (GT)n polymorphism was genotype by PCR and fluorescence technology.

Results

We found 27 different alleles, with the 22 and 29 repeats as the most common alleles. Distribution of short allele (n<25) and SS genotype was not statistically associated with JRA subjects. Interestingly, the frequency of both short allele and SS genotype was significantly associated with SLE susceptibility (OR=1.47, 95%CI [1.14–1.89], p=0.002; and OR=2.79, 95%CI [1.24–6.24], p=0.01, respectively).

Conclusion

The distribution pattern of HMOX1 (GT) alleles was different in the Mexican population than those reported elsewhere. Our results suggest that HMOX1 (GT)n polymorphism was associated with susceptibility to childhood-onset SLE but not with JRA in Mexican individuals.

Key words

HMOX1, polymorphism, paediatric SLE, arthritis

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Introduction

The inducible protein heme oxigenase 1 (HO-1) is one of the rate-limiting enzymes in the heme catabolism to biliverdin/bilirubin, free iron and carbon monoxide (1). HO-1 is capable of responding to several exogenous and endogenous factors, including oxidative stress, heavy metals, inflammatory molecules, hypoxia and several xenobiotics (2).

Mouse models have shown that HO-1 induction ameliorates diverse inflammatory events, while its inhibition increases chronic inflammation (3, 4). For instance, in experimental models of collagen- and (TNF)-mediated arthritis, the induction of HO-1 results in a decrease of pro-inflammatory cytokine secretion and inhibition of cartilage and bone erosion (5, 6). Likewise, pharmacologic induction of HO-1 in lupus nephritis in MRL/lpr mouse model reduces pathological injury of glomeruli and inhibited deposition of immune complex (7). These results suggest that HO-1 plays an important role in protection against chronic inflammation associated with these autoimmune diseases. Interestingly, rheumatoid arthritis (RA) patients showed elevated levels of HO-1 protein in synovial tissue and peripheral blood monocytes (8, 9). The increasing levels of HO-1 in RA could be a cellular response against the inflammatory processes, typical of this illness.

The (GT)n repeats polymorphism located in the promoter region of HO-1 encoding gene (*HMOX1*) has been shown to regulate its transcriptional activity (10). Thus, induced levels of HO-1 mRNA is higher in lymphoblast cell lines carrying the homozygous genotype for the short fragment (GT)n repeat (<25), than those with the homozygous genotype for the long fragment repeat (≥25) (11). In accordance with the cytoprotective function of HO-1, the (GT)n repeats polymorphism has been associated with susceptibility to several pathologic events, including RA, restenosis and nephritis (12-14). Therefore, in the present study we investigated whether the *HMOX1* functional (GT)n polymorphism is associated with childhood-onset SLE and JRA susceptibility in a Mexican-Mestizo population.

Materials and methods

Patients

We studied 207 unrelated patients with JRA (59% females and 41% males) and 333 with childhood-onset SLE (83% females and 17% males). In both groups, patients were Mexican-Mestizo with less than 16 years of age at onset of the disease and were recruited from four tertiary level institutions located in Mexico City. The diagnosis of both JRA and SLE was made based on the American College of Rheumatology (ACR) criteria. Evaluation of SLE-related nephritis was available in 258 SLE patients, whom were assigned to Classes I–VI according to the severity of disease, following the ACR classification (15).

We also included 653 unrelated healthy individuals ethnically matched with cases (58% females and 42% males). All controls were enrolled from a blood bank in Mexico City and were >18 years of age. Local ethics and research committees approved this study and an informed written consent was obtained from all controls. Parents provided consent for the children's participation and the children assented.

Genotyping analysis

Genomic DNA was isolated from whole blood samples using the QIAamp DNA Blood Maxi kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Genotyping analysis of the (GT)n polymorphism was performed using a fluorescent-labelled forward primer (FAM 5'-GCTCTGGAAGGAGCAAAATCACACC-3') and an unlabelled reverse primer (5'-TATGACCCTTGGGAAACAAAGTCTGG-3'). DNA amplification was carried out with an initial denaturing step at 94°C by 10 min, followed by 30 PCR cycles 94°C for 1 min, 60°C for 45 sec and 72°C for 1 min. A final extension step at 72°C for 60 min completed the reaction. For analysis, aliquots containing 0.5µl of PCR product were mixed with 0.1µl of GeneScan-500 LIZ Size standard solution as an internal lane size and 9.4µl of Hi-Di Formamide (Applied Biosystems, Foster City, CA). The length of the PCR products was determined using an automated DNA cap-

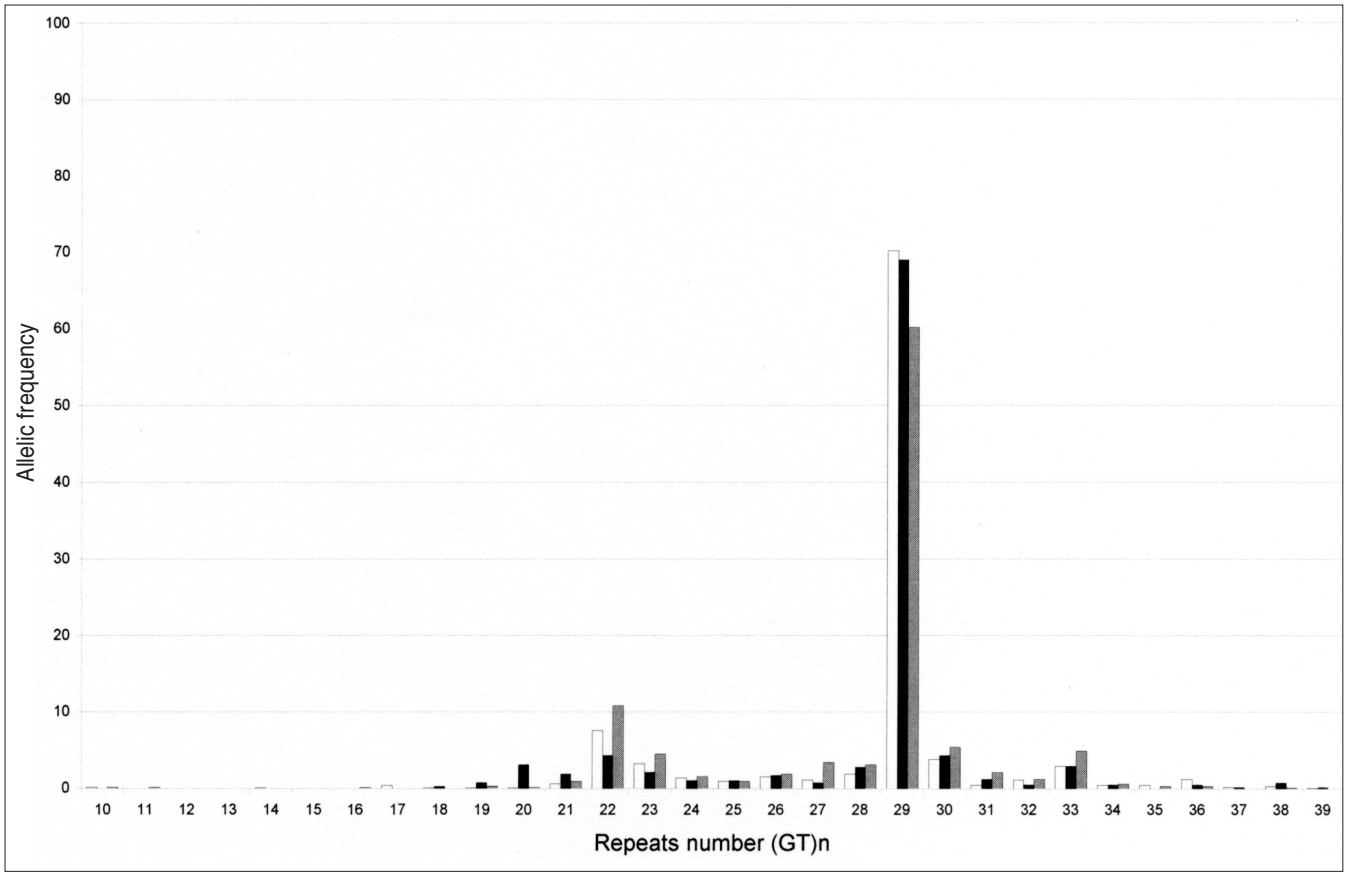

Fig. 1. Frequency distribution of (GT)n repeats in patients: controls (white bar, n=653), juvenile rheumatoid arthritis (black bar, n=207) and systemic lupus erythematosus (grey bar, n=333).

illary sequencer and the Peak Scanner software v1.0 (FAL3730xl DNA Analyzer, Applied Biosystems, Foster City, CA). Each (GT)n repeats number was calculated using as reference the PGL3 vector containing a 30-repeat fragment validated by sequencing. To validate genotyping, 30 random samples were sequenced. On the basis of previous reports, we considered as small alleles (S) those with less than 25 (GT)n repeats and as long alleles (L) those with equal or more than 25 (GT)n repeats (12-14).

Statistical analysis

Significant differences between cases and controls were determined using χ^2 analysis. Odds ratio (OR) with 95% confidence intervals (95% CI) was calculated using allele frequencies for cases and controls. All statistical calculations and Hardy-Weinberg equilibrium were performed using Stat-Calc (Epi Info 2005 v3.3.2; Centers of Disease Control and Prevention, Atlanta, GA, USA) and the FINETTI software with the Fisher's exact test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). *P*-values less than 0.05 were considered statistically significant.

Results

A total of 1,193 Mexican individuals were included. The 207 JRA (59% females and 41% males) and 333 SLE (83% females and 17% males) cohorts included patients from less than 16 years of age at onset of disease and fulfilled the ACR criteria. The mean (±SD) age at onset of JRA and SLE were 8.7±2.46 and 11.62±2.46 years, respectively. The 653 healthy, ethnically matched controls were older than 18 years of age. Since differences between the genders in healthy individuals were not observed, when allelic frequencies of *HMOX1* (GT)n polymorphism were compared (p=0.119), we used the same control group for analyses of both SLE and JRA cohorts.

Genotype frequencies of (GT)n polymorphism were in Hardy-Weinberg equilibrium in the three evaluated populations (JRA, SLE and controls) (p<0.87). In all the Mexicans studied, the number of (GT)n repeats of the human *HMOX1* polymorphism ranged from 10 to 39 repeats, with 27 different alleles showing a bimodal distribution, peaking at 22 and 29 repeats in all three groups (Fig. 1). The GT repeats range was from 18 to 39 in JRA patients, 10 to 38 in SLE individuals and 10 to 39 in healthy subjects. In all three groups, the most frequent genotype was the homozygous LL, followed by the heterozygous LS and the homozygous SS (Tables I, II). When allele and genotype frequencies were compared between JRA subjects and healthy individuals, no significant differences were found (Table I).

However, when *HMOX1* allele and genotype frequencies were compared between SLE patients and healthy controls, an evidence of association was observed (Table II). The frequency of the S allele in our SLE patients was signifi-

cantly higher in cases than in controls (18.8% *vs*. 13.6%) (p=0.002; OR=1.47, 95%CI [1.14–1.89]). This significance was also observed when SS+SL *versus* LL genotypes were compared. Furthermore, the SS genotype conferred a 2-fold larger odds ratio for childhood-onset SLE (p=0.01, OR=2.79, 95%CI [1.24–6.24]) than that observed in the heterozygous SL (p=0.03, OR=1.37, 95%CI [1.01–1.85]). We also investigated the possible association between the (GT)n repeats and lupus nephritis, however, no significant difference among patients with or without nephritis was observed (Table III).

Table I. *HMOX-1* (GT)n distribution in juvenile rheumatoid arthritis and controls.

	Controls	JRA		OR (95% CI)	p-value
Genotyping	n=653 (%)	n=207 (%)			
LL	487 (74.6)	154 (74.4)			
LS	155 (23.7)	50 (24.1)	LS *vs*. LL	1.02 (0.70–1.47)	0.91
SS	11 (1.7)	3 (1.5)	SS *vs*. LL	0.82 (0.23–3.13)	0.82
			SS+SL *vs*. LL	1.01 (0.32–4.21)	0.81
Allele					
L	1129 (86.4)	358 (86.5)			
S	177 (13.6)	56 (13.5)	S *vs*. L	0.99 (0.72–1.3)	0.98

JRA: juvenile rheumatoid arthritis; OR: odds ratio; 95% CI: 95% confidence interval.

Table II. Systemic lupus erythematosus and *HMOX-1* (GT)n polymorphism.

	Controls	SLE		OR (95% CI)	p-value
Genotyping	n=653 (%)	n=333 (%)			
LL	487 (74.6)	222 (66.6)			
LS	155 (23.7)	97 (29.1)	LS *vs*. LL	1.37 (1.01–1.85)	0.03
SS	11 (1.7)	14 (4.3)	SS *vs*. LL	2.79 (1.24–6.24)	0.01
			SS+SL *vs*. LL	1.46 (1.10–1.95)	0.009
Allele					
L	1129 (86.4)	541 (81.2)			
S	177 (13.6)	125 (18.8)	S *vs*. L	1.47 (1.14–1.89)	0.002

SLE: systemic lupus erythematosus; OR: odds ratio; 95% CI: 95% confidence interval.

Discussion

The inducible HO-1 enzyme encoded by the *HMOX1* gene, exerts important anti-inflammatory and anti-oxidative properties. The transcriptional activity of this antioxidant gene is influenced by a GT repeats polymorphism, located at 526 bp upstream of the transcription site. Previous studies described more than 30 different alleles of the (GT)n polymorphism, ranging from 10 to 44 dinucleotide repeats with a bimodal distribution (16-18). The most frequent allele worldwide is that one with 30 (GT)n repeats, followed by the alleles with 22 or 23 GT repeats (17-19). In our study, we found 27 different (GT)n repeats alleles, ranging between 10 and 39 repeats showing a bimodal distribution. We found that the frequency distribution of these repeated alleles has a bimodal fashion in the three groups (control, JRA and SLE), but with the highest peaks at positions of 22 and 29 (GT)n repeats. This bimodal pattern has been found only in a Korean population (19), but interestingly, in our population the frequency of the 29 (GT)n repeats was significantly higher (71% *vs*. 35%). In addition, the 22 (GT)n repeats allele showed a significantly lower frequency (10%) than that reported in other studies (20–30%). It would be important to determine whether these differences could be due to the high level of admixture of the Mexican population (20).

In two studies involving Japanese patients with juvenile idiopathic arthritis or RA, elevated levels of HO-1 in serum was observed in both diseases. These data suggest the contribution of this cytoprotective enzyme in counteracting the chronic inflammation associated with those diseases (8, 21). In addition, in mouse models of arthritis as well as in arthritis-derived cell cultures, the pharmacologic up-regulation of HO-1 reduces the inflammatory response (5, 8, 22, 23).

The short allele (n<25) of this polymorphism induces a greater expression of RNA and protein than the long allele (n>25) (11). Thus, the short allele of the (GT)n polymorphism has been associated with protection against several diseases such as emphysema, ischaemic stroke and RA (10, 12, 24), although its participation in the last entity remains controversial. In accordance with the proposed role of HO-1 in RA, in a previous report (n=736), the (GT)n short allele was found significantly associated with protection to RA in a Spanish population (OR=0.8) (12). Nevertheless, a study involving a sample of Dutch patients (n=325) failed to find association between RA and HMOX1 (GT)n short allele, although they found that SS genotype is associated with protection against joint damage and activity disease (25). Similarly, we did not find any association between (GT)n S allele and JRA, but it is necessary to increase the sample size and stratify by severity to understand better the role of the *HMOX1* (GT)n polymorphism in JRA susceptibility.

On the other hand, it is worth noting that the same (GT)n short allele have also been associated with susceptibility to develop several diseases such as cerebral malaria, type 2 diabetes and hyperbilirubinaemia (26-28). Actually, we found a significant association of the *HMOX1* (GT)n S allele with an increased risk of childhood-onset SLE, in an additive model. It has been suggested that genetic association of the *HMOX1* (GT)n S allele increasing risk for disease could be the consequence of an increase level of secondary metabolites of HO-1 activity (free-iron and bilirubin). It is possible that the association found in this study between *HMOX1* (GT)n S allele and SLE susceptibility could be due to the increase in HO-1 metabolites. In fact, it has been described that hyperferritinaemia plays an important role in the pathogenesis of SLE (29). In order to assess this hypothesis it is necessary to analyse the relationship among HO-1 metabolite levels, *HMOX1* (GT)n genotypes and clinical manifestations.

Table III. *HMOX-1* (GT)n polymorphism and lupus related nephritis.

	No nephritis	Nephritis		OR (95% CI)	*p*-value
Genotyping	n=91 (%)	n=167 (%)			
LL	58 (63.6)	110 (65.9)			
LS	27 (29.2)	52 (31.2)	LS *vs*. LL	1.01 (0.57–1.78)	0.95
SS	6 (7.2)	5 (2.9)	SS *vs*. LL	0.43 (0.12–1.51)	0.17
			SS+SL *vs*. LL	0.91 (0.53–1.55)	0.73
Allele					
L	143 (78.6)	272 (81.4)			
S	39 (21.4)	62 (18.6)	S *vs*. L	0.83 (0.54–1.31)	0.43

OR: odds ratio; 95% CI: 95% confidence interval.

It is possible that polymorphisms located in antioxidant genes may determine which individuals are more prone to develop some clinical manifestations in SLE. In a previous study we found that the *NFE2L2-653G/A* SNP was associated with lupus nephritis, suggesting that this gene could have a modifier role in lupus (30). In addition, HO-1 activity was showed as a protective factor against nephritis in a mouse model of lupus (7). However, in spite of several evidences for the participation of the antioxidant and detoxification pathways in lupus nephritis (14, 19), we did not find association between *HMOX1* (GC)n polymorphism and this clinical manifestation in Mexican children. However, we cannot rule out that besides *NFE2L2*, other genes belonging to the antioxidant pathway are associated with lupus nephritis or that *HMOX1* could be associated with a particular clinical manifestation of lupus other than nephritis. Further studies are needed with detailed clinical data and information on disease activity and organ involvement, to evaluate if there is an association between clinical manifestations of the disease other than nephritis and genetic polymorphisms on *HMOX1*.

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