Letters to the editor

Heme oxygenase-1 promoter polymorphisms do not influence susceptibility to systemic sclerosis and its clinical phenotypes

Sirs,

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease leading to extensive fibrosis of skin and internal organs (1). One of the phenomena of interest in SSc pathogenesis is hypoxia (2). A protein strongly involved in hypoxia responses is the cytoprotective enzyme heme oxygenase 1 (HMOX1), a stress-response protein catalysing the degradation of heme into biliverdin, carbon monoxide (CO) (3). Both biliverdin and CO are known for their anti-oxidant and anti-inflammatory effects (4). We hypothesised that polymorphisms in HMOX1 may influence susceptibility to SSc by determining the levels of HMOX1 following transcriptional activation. Individuals with short $(GT)_n$ repeats have been shown to induce HMOX1 faster than people with long $(GT)_n$ repeats, which is associated with protection against a wide range of pro-inflammatory diseases (5). To address this hypothesis we investigated this (GT) n repeat and a single nucleotide polymorphism (SNP) (*HMOX1 -413A>T)* in the HMOX1 promoter region using a large cohort of clinically well-documented SSc patients.

The study population was composed of 604 SSc patients and 1378 healthy controls from the Netherlands, Spain and Germany. The characteristics and classification criteria of this cohort have been published previously (Table I) (6).

Custom Taqman assays were constructed for both genotyping the SNP at position *-413* in the *HMOX1* promoter region and the HMOX1 repeat, both construction and genotyping have been previously described (7). Association analysis was performed by Chi-square tables and Fisher's exact test. Pooled OR was performed with Mantel-Haenszel meta-analysis. Survival analysis was performed using Kaplan-Meier curves and Log Rank (Mantel-Cox) statistics. For the power calculation of the combined analyses we considered a minor allele frequency of 0.42 as displayed in the control population, under a multiplicative model our study than reaches a power of 75% to detect an OR of 1.2 and 97% to detect an OR of 1.3.

No significant heterogeneity between the three European populations was detected. We did not observe any differences in the allele distribution of the *HMOX1 -413A>T* SNP when comparing SSc patients with their healthy counterparts nor when comparing cinical phenotypes of SSc (Table I). Moreover, no association was found when scrutinising dominant and negative effects of this polymorphisms in SSc. Likewise, no differences were present comparing $(GT)_n$ repeat length. There was no combined effect of the $(GT)_n$ repeat and the *HMOX1 -413A>T* SNP nor in a multi-linear regression model consisting of the SNP and $(GT)_n$ repeat (SSc p=0.761, lcSSc p=0.672, dcSSc p=0.443).

Table I. Characteristics of cohort and accompanying results.

Population	Netherlands	Spain	Germany	Genotype			Allele	Mean repeat length
				AA M-H p	AT M-H p	AT M-H p	T M-H p	GT
Number SSc	200	189	215	0.34	0.49	0.17	0.42	28.1 (2,7)
Number HC	273	214	891	0.35	0.47	0.18	0.41	27.8 (3,9)
LcSSc n (%)	139 (69)	121 (56)	123 (65)	0.34	0.47	0.19	0.43	27.9 (2,7)
dcSSc n (%)	57 (31)	87	56	0.34	0.49	0.17	0.41	28.5 (2,7)
Age (years, (SD))	58 (13)	58 (13)	57 (12)					
Disease duration (months; (SD))	131 (82)	144 (90)	113 (109)					
Female % (controls %)	81 (84)	82 (78)	76 (76)					
Positivity anti-topo %	23	23	26					
Positivity ACA %	58	39	46					
Pulmonary fibrosis CT scan	32.3	30.7	37.2					
Low FVC (<70% predicted) %	26.1	29.1	18.5					
Low DLCO (<70% predicted) %	33	45.1	50.2					

SSc: systemic sclerosis; lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; HC: healthy controls; M-H p: Mantel-Haenszel p-value; ACA: anti-centromere antibodies; anti-topo: anti-topoisomerase antibodies; FVC forced vital capacity; DLCO; diffusion capacity of the lung for carbon monoxide; HRCT; high resolution computed tomography; SD: standard deviation.

When studying the possible effect of the $(GT)_n$ length or the *HMOX1 -413A>T* SNP on development of PAH and/or pulmonary fibrosis in 15 years from the development of the first non-Raynaud's SSc symptom no effects were observed. There was no correlation between the $(GT)_n$ repeat length and the time to develop pulmonary fibrosis (p=0.064) and PAH (p=0.889) neither was there a significant difference in the Kaplan-Meier survival curves for pulmonary fibrosis (log rank p=0.198, Breslow p=0.295) and PAH (log rank p=0.693, Breslow p=0.496). In this study we show that two functional variants within the HMOX1 promotor do not influence susceptibility to SSc nor its clinical phenotype. However, based on the observations that HMOX1 is aberrantly expressed in SSc models, pulmonary fibrosis and PAH (8-10) the regulation, either epigenetically or post-translational, of HMOX1 stays an potentially important molecular pathway in SSc.

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