Study of DNASE I gene polymorphisms in systemic lupus erythematosus susceptibility

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterised by a broad spectrum of autoantibodies directed to ubiquitous intracellular antigens. DNase I is a key enzyme implicated in the clearance of extracellular DNA,¹ and several studies point to defective DNase I as an important player in development of SLE.^{2–5} Yasutomo *et al* described two Japanese girls with SLE bearing a non-sense mutation, leading to a non-functional protein.⁴ We have described two Spanish patients with SLE with different mutations in the *DNASE I* gene and very low serum DNase I activity.⁵ Overall these mutations are very rare and do not explain the low DNase I activity found in most patients with SLE.

We have sequenced the *DNASE I* gene, including 2900 bp of the 5'upstream region in 16 patients with SLE with very low DNase I serum activity (<3 ng/ml) and in 3 controls with normal DNase I activity (median 6.2 ng/ml, interquartile range: 5.2–6.9).⁶

We identified 21 single-nucleotide polymorphisms (SNPs) and selected 4 SNPs for large-scale genotyping, three of which were in the 5' regulatory region (–1799G→A, –637G→A, –16C→T) and the fourth was in the 7th intron (+2035C→G). We analysed the frequency of the 4 SNPs in 287 patients with SLE (92.4% women) and 452 healthy controls (69.5% women) in the Spanish population. Patients with SLE fulfilled the

American College of Rheumatology criteria⁷ and gave informed, written consent. Genotypes of controls and patients were concordant with the Hardy–Weinberg equilibrium.

The analysis of allele frequencies showed that the G allele of the +2035C→G SNP was more common in patients with SLE than in controls (table 1; $p = 0.014$, after adjusting for sex). The allele A of –1799G→A had a higher frequency in patients, although the difference from controls was not significant (table 1, $p = 0.087$).

Next we explored the influence of the genotypes of these SNPs on DNase I activity and the production of anti-dsDNA, anti-ssDNA, anti-nucleosome or anti-RNP antibodies in sera from 101 patients with SLE. We did not find any evidence of a relationship (data not shown).

We analysed the linkage disequilibrium between the SNPs studied here and +2373A→G, which has been described previously,⁶ and observed a strong linkage disequilibrium, as shown by a mean D' value of 0.992 in pairwise analysis. Analysis of the haplotype frequency distributions showed significant differences (Omnibus test $p = 0.02$) (table 2). The distribution of frequencies suggested that disease susceptibility

Abbreviations: SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphisms

Table 1 Allele frequencies of four single-nucleotide polymorphisms (SNPs) of *DNASE I* in patients with SLE and controls

SNP	Alleles	Controls*	SLE	OR (95% CI)	p Value	Sex-adjusted OR
–1799G→A	G	875/896 (97.7)	542/564 (96.1)	1.69 (0.92 to 3.1)	0.087	1.58 (0.84 to 2.96)
	A	21/896 (2.3)	22/564 (3.9)			
–637G→A	G	844/902 (93.6)	532/560 (95)	0.76 (0.48 to 1.22)	0.25	0.82 (0.52 to 1.33)
	A	58/902 (6.4)	28/560 (5)			
–16C→T	C	849/902 (94.1)	540/564 (95.7)	0.71 (0.43 to 1.17)	0.17	0.78 (0.47 to 1.28)
	T	53/902 (5.9)	24/564 (4.3)			
+2035C→G	C	286/902 (31.7)	145/564 (25.7)	1.34 (1.06 to 1.69)	0.014	1.41 (1.03 to 1.92)
	G	616/902 (68.3)	419/564 (74.3)			

SLE, systemic lupus erythematosus.

*Values are number of alleles, number of chromosomes studied (%).

Table 2 Haplotype frequencies of five single-nucleotide polymorphisms of *DNASE I* in patients with systemic lupus erythematosus (SLE) and controls

Haplotype	–1799G→A	–637G→A	–16C→T	+2035C→G	+2373A→G	Frequency controls (%)	Frequency SLE (%)
ht1	G	G	C	G	G	65.8	69.9
ht2	G	G	C	C	A	23.3	18
ht3	G	A	T	C	A	5.8	4
ht4	A	G	C	G	G	2.3	3.9
ht5	G	G	C	C	G	2.0	2.7

SLE, systemic lupus erythematosus.

will be more dependent on +2373A→G than on +2035C→G, as this latter SNP was discordant in haplotype 5.

This study confirms the association of common SNPs in the DNASE I gene with SLE, although it fails to show a correlation with low DNase I activity or production of antinuclear antibodies in patients' sera. Therefore, other mechanisms should be explored to explain the SLE susceptibility related to DNASE I variation and the low DNase I activity observed in most patients.

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High disease activity of ankylosing spondylitis in the community before the introduction of tumour necrosis factor blockers

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Before the introduction of tumour necrosis factor (TNF) blocking agents, the management of ankylosing spondylitis was mainly limited to physical exercise and the administration of non-steroidal anti-inflammatory drugs.^{1–3} To estimate the ankylosing spondylitis disease status in conventionally treated patients, we performed a community-based cross-sectional postal survey before TNF blocking agents became available in Austria.

Between January and April 2003, a questionnaire to assess ankylosing spondylitis disease activity, function and pain levels using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BASFI) and a Visual Analogue Scale (VAS) was distributed to all 1400 members of the Austrian ankylosing spondylitis patient's association treated in the community, to patients with ankylosing spondylitis treated in tertiary rheumatology centres and to consecutive sex-matched controls with coronary artery disease, diabetes mellitus or goitre without signs of inflammatory rheumatic pain attending a general internal medicine outpatient clinic.

In all, 665 (47.6%) members of the ankylosing spondylitis patient's association (469 men and 196 women, mean (standard deviation (SD)) age 52 (12) years); 129 patients from rheumatology outpatient clinics (92 men and 37 women, mean (SD) age 40 (11) years); and 150 controls (89 men and 61 women, mean (SD) age 54 (14) years) returned an assessable questionnaire. Diagnosis of ankylosing spondylitis was confirmed in 97 of 100 randomly selected members of the ankylosing spondylitis patient's association by re-evaluating their medical records. Disease activity evaluated by the BASDAI, and the percentage of

patients with BASDAI ≥ 4 and >7 were equally high in patients in the community and in tertiary centres, but significantly lower in patients without inflammatory back pain (table 1). No correlation was found between the BASDAI and the age of patients ($r = 0.03$; $p = 0.42$). Functional impairment measured by the BASFI was significantly lower in patients treated in tertiary centres than in patients in the community and was lowest in controls (table 1). The BASFI increased significantly with age in all three groups and was highest in patients >60 years treated in tertiary centres (median (range) 7.6 (2.5–8.8)). The BASFI correlated with the BASDAI ($r = 0.67$; $p < 0.01$) and weakly with patient's age ($r = 0.23$; $p < 0.01$). The median pain score and the percentage of patients with a pain score >7 was equally high in patients treated in the community and in tertiary centres, but significantly lower in patients with non-inflammatory back pain (table 1). Pain levels correlated with the BASDAI ($r = 0.80$; $p < 0.01$) and the BASFI ($r = 0.61$; $p < 0.01$), but were independent of age ($r = -0.07$).

In summary, we have reported high disease activity, functional impairment and pain levels in 75% of conventionally treated patients with ankylosing spondylitis in the community, bearing in mind that the BASDAI may not be an appropriate measure to determine ankylosing spondylitis disease activity in a postal questionnaire. Conceivably, the population of organised patient's groups answering the questionnaire may be biased to report more severe disease than the total cohort of all patients with ankylosing spondylitis, but the disease status in our study was not different from a population of 246 patients with ankylosing spondylitis queried with a postal questionnaire between 2001 and 2003 in the UK.⁴