Association of the Intergenic Single-Nucleotide Polymorphism rs10865331 (2p15) with Ankylosing Spondylitis in a Spanish Population

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ABSTRACT. ***Objective.*** A recent genome-wide association study has identified 2 single-nucleotide polymorphisms (SNP) associated with ankylosing spondylitis (AS), rs10865331 (2p15) and rs2242944 (21q22). We assessed the association of these SNP with AS in a Spanish population.

Methods. Four hundred fifty-six patients with AS fulfilling the modified New York Criteria and 300 healthy donors were analyzed.

Result. SNP rs10865331 (allele A: $p = 0.039$; genotype: $p = 0.016$) was significantly associated with AS, while no association was found for rs2242944.

Conclusion. This is the first study that replicates in an independent cohort the association of the intergenic SNP rs10865331 with susceptibility to AS. (First Release September 1 2010; J Rheumatol 2010;37:2345–7; doi:10.3899/jrheum.100211)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS SINGLE-NUCLEOTIDE POLYMORPHISM
GENETIC STUDIES

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterized by sacroiliitis, ankylosis of the spine, and enthesitis. Although the exact cause of AS is unknown, genetic factors have long been implicated in AS, beginning with the discovery of the association of HLA-B27 with the disease in the early 1970s[1]. However, although around 90% of white patients with AS are B27-positive, only < 5% of the approximately 8% of B27-positive individuals in the general population develop the disease. Over the last few years, the development of high-throughput microarray-based single-nucleotide polymorphism (SNP) genotyping techniques and genome-wide association studies (GWAS) have helped to highlight non-HLA genetic risk factors associated with AS, involving genes such as *IL23R*, *ERAP1*, *CYP2D6*, and *IL1* gene complex, among others[2,3,4,5,6]. A recent GWAS confirmed the association of *ERAP1*, *IL23R*, and proinflammatory genes (*IL1* complex and *TNFR1*) with AS and found a new association for 2 intergenic SNP: rs2242944, on chromosome 21q22, and rs10865331, on chromosome 2p15[7]. It was the first time that an association for these intergenic regions with AS was reported, so we selected these 2 intergenic SNP for replication. Later, the same authors published extended results reporting an association with AS for the intergenic SNP rs4672495 and for SNP in the *ANTXR2* gene[8]. Our purpose was to evaluate the association of the intergenic SNP rs2242944 and rs10865331 with AS in a Spanish population.

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Supported by the Ministerio de Ciencia e Innovación: Proyect PSE-01000-2006-1.

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Accepted for publication July 6, 2010.

MATERIALS AND METHODS

Study population. The study population consisted of 456 patients with AS from the national registry of spondyloarthropathies of Spain (REGISPONSER)[9], who fulfilled the modified New York criteria[10], and 300 spondylarthritis-free individuals from the National DNA Bank of Spain. Both cases and controls were of Spanish white origin. The case cohort comprised 348 men and 108 women aged 51 ± 11 years. Controls were selected to have a similar sex ratio as cases, 225 men and 75 women, and to be over 50 years old, with a mean age of 55 ± 4.5 years. All patients gave written informed consent to participate, and the Ethics Committee of the Puerta de Hierro University Hospital centrally approved the study. The ethics approval for the control cohort was obtained from the Ethics Committee of the National DNA Bank of Spain.

SNP genotyping and HLA-B27 typing. The 2 SNP, rs10865331 and rs2242944, were genotyped using KASPar chemistry (KBioscience, Hertfordshire, UK), which is a competitive allele-specific polymerase chain reaction (PCR) SNP genotyping system using FRET quencher cassette oligonucleotides. HLA-B27 carriage was tested by conventional

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PCR[11] using the amplification of exon 8 of p53 as an internal control[12] (Table 1).

Statistical analysis. A test for deviation from Hardy-Weinberg equilibrium (HWE) was performed for each SNP in the control population using Helix Tree software v.6.4.2 (Golden Helix Inc., Bozeman, MT, USA). Association tests for allele and genotype frequencies for each SNP were performed by chi-squared test, using Helix Tree software v.6.4.2. OR were calculated with 95% CI. Statistical significance was set at $p < 0.05$.

Assuming a population prevalence of 0.2% and minor allele frequencies of 0.35–0.45, the study had 80% power to detect an additive association with OR of 1.35, a dominant association with OR of 1.6, and a recessive association with OR of 1.65, at the 5% significance level (Quanto software v.1.2.4, University of Southern California, Los Angeles, CA, USA).

RESULTS

From the 456 cases and 300 controls, 387 (85%) and 20 (7%), respectively, were found to be HLA-B27-positive.

Both SNP, rs10865331 and rs2242944, were in HWE or close to HWE in controls ($p = 0.38$ and 0.88, respectively) and cases ($p = 0.02$ and 0.83, respectively). The genotyping call rate was 0.98, with 449 and 295 genotypes obtained for rs10865331 and 454 and 290 for rs2242944, in cases and controls, respectively.

Results of allele and genotype comparisons are given in Table 2. A significant association was found for rs10865331 at both allele ($p = 0.039$) and genotype ($p = 0.016$) levels (Table 2). Specifically, the A allele was found to confer susceptibility to AS with an OR of 1.25 (95% CI 1.01–1.54). We observed a significant genotype association under the recessive model (OR 1.74, 95% CI 1.18–2.54; $p = 0.004$), but not under the dominant model (OR 1.10, 95% CI 0.80–1.51; $p = 0.53$). Neither the allele ($p = 0.105$) nor the genotype ($p = 0.274$) frequencies of rs2242944 were significantly different between patients and controls. In this case, we did not find significant associations under either the dominant model (OR 1.24, 95% CI 0.92–1.68; $p = 0.16$) or the recessive model (OR 1.3, 95% CI 0.83–2.02; $p = 0.24$).

We did not perform an association test based on carriage of HLA-B27, since this analysis was highly limited by the small number of B27-positive controls (n = 20) and B27-negative cases (n = 69).

DISCUSSION

We examined in a Spanish population the association with AS of 2 SNP recently identified in a GWAS, rs10865331 (2p15) and rs2242944 (21q22). We replicated for the first time in an independent cohort the association of rs10865331 with AS. No association was seen with rs2242944. This lack of association could be due to a sample size limitation, given the modest statistical power of our study, or to the different ethnic origins of the population, as seen for other SNP associated with AS[13].

Both SNP, rs2242944 (2p15) and rs10865331 (21q22), map to intergenic regions where no known protein-coding gene has been described. Several recent genetic studies have shown the involvement of intergenic genomic regions and SNP in autoimmune diseases[14,15]. According to HapMap CEU genotype data (HapMap #27, on NCBI B36 assembly), rs10865331 and rs2242944 are not contained within the linkage disequilibrium (LD) blocks of their neighboring

Table 1. Oligonucleotides used for genotyping of SNP rs10865331 and rs2242944 and for PCR detection of HLA-B27 and exon 8 of p53. The annealing temperature of the PCR was 62°C for HLA-B27 and p53 detection, and 57°C for rs10865331 and rs2242944 genotyping.

	Forward	Reverse
rs10865331 (2p15)	CTG ACT TTG GTG CCG TAT CTA CCA A TACT TTG GTG CCG TAT CTA CCA G	TGA GGC AAT GGC CAC TTT ACG ACT T
rs2242944 (21q22)	TGA TAA TTC TGT ATG TTT TAG TAT CAC TGG T ATAAT TCT GTA TGT TTT AGT ATC ACT GGC	GAG GTG GGG AGA GTT CCA AAG G
HLA-B27	GCT ACG TGG ACG ACA CGC T	CAG TCT GTG CCT TGG CCT TGC
p53	TAG GAC CTG ATT TCC TTA CTG CCT C	AAC TGC ACC CTT GGT CTC CTC CAC C

Table 2. Genotype and allele association analysis of SNP rs10865331 and rs2242944 in patients with AS and spondyloarthritis-free controls. Study population consisted of 456 white Spanish patients with AS who fulfilled the modified New York criteria and 300 spondyloarthritis-free Spanish controls. The 2 SNP, rs10865331 and rs2242944, were genotyped by competitive allele-specific PCR.

	Case					Control							
	Genotype			Allele		Genotype			Allele		Allele	Genotype	
SNP	AA	AG	GG	A	G	AA	AG	GG	A	G	OR (95% CI)	p	p
rs10865331, n (%)	109 (24)	199 (44)	141 (31)	417 (46)	481 (54)	46 (16)	150 (51)	99 (33)	242 (41)	348 (59)	1.25 (1.01–1.54)	0.039*	0.016*
rs2242944, n (%)	67 (15)	212 (47)	175 (38)	346 (38)	562 (62)	34 (12)	129 (44)	127 (44)	197 (34)	383 (66)	1.20 (0.96–1.49)	0.105	0.274

Statistical significance denoted by *$p < 0.05$. SNP: single-nucleotide polymorphism; AS: ankylosing spondylitis; PCR: polymerase chain reaction.

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genes. Thus, it appears unlikely that these SNP are associated with AS due to LD with a causal variant in a neighboring gene.

The closest protein-coding genes to rs10865331 are *B3GNT2* (UDP-GlcNAc: betaGal beta-1,3-N-acetylglucosaminyltransferase 1) and *COMMD1* (copper metabolism domain containing 1), which codify for a type II transmembrane protein involved in the biosynthesis of poly-N-acetyllactosamine chains and for a protein known to inhibit nuclear factor-κB activation, respectively. The nearest gene to rs2242944 is *PSMG1* (proteasome assembly chaperone 1).

We tested whether rs10865331 and rs2242944 were located in putative transcription factor binding sites (TFBS) using the MAPPER platform (http://mapper.chip.org) and whether the SNP could disrupt the TFBS. Regarding rs10865331, a putative TFBS for the transcription factor Tal1beta-E47S (score 6 and e-value 1.4) is predicted only if the G allele is present. If the susceptibility A allele is present, the binding of Tal1beta-E47S to this region is predicted to be disrupted. Since this putative TFBS is located downstream of *COMMD1* and *B3GNT2* genes, we hypothesize that it could be part of a long-range transcriptional regulatory element involved in the regulation of these neighboring genes or, alternatively, it could act as a proximal promoter element for a not yet identified protein coding gene in this region. We did not find any disruption of predicted TFBS for rs2242944, so we hypothesize that this SNP could be involved in AS pathogenesis-modifying gene expression through effects on noncoding RNA.

Our study provides evidence of the association of the rs10865331 intergenic variant with AS in a Spanish population, replicating the results recently obtained in a GWAS. Further research is necessary to unravel the functional significance and the biological processes altered by this intergenic AS-associated SNP.

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