Single Nucleotide Polymorphism of COL6A1 in Patients with Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** To investigate the genetic association between ankylosing spondylitis (AS) and single nucleotide polymorphisms (SNP) of collagen 6A1 gene (COL6A1), the candidate gene for ossification of the posterior longitudinal ligament.

Methods. One-hundred thirty Korean patients with AS (M: 116, F: 14, age: 29.0 ± 4.6) and 130 age- and sex-matched healthy subjects were recruited. The SNP of G365G, IVS15+39 C/T, IVS21+18 A/C by Snap shot assay and the SNP of IVS32-29T/C, IVS33+15G/A, IVS33+20A/G, and IVS33+55A/G by direct sequencing were genotyped and analyzed. Bonferroni correction was applied to multiple comparisons.

Results. The observed allelic frequencies for these SNP met Hardy-Weinberg equilibrium in all AS and controls. We also found an additional 2 SNP (R783Q and IVS33+88C/T) during direct sequencing. Therefore, a total of 9 SNP were analyzed in this study. There were no significant associations of allelic and genotype variations between AS and controls. The presence of uveitis was marginally associated with a haplotype (CC in G365G + IVS15+39 C/T). The variation of allele or haplotype of COL6A1 is not significantly associated with "more ossified disease."

Conclusion. Because the genetic variations of COL6A1 could not be correlated with the occurrence of AS in Koreans, we conclude that despite common clinical features, AS and ossification of posterior longitudinal ligament are not genetically related, and the hyperostotic condition seen in the 2 diseases might be regulated differently. Further SNP of COL6A1 were not related to radiographic progression of AS. However, we found that the occurrence of uveitis might be related to the genetic variations of COL6A1 in patients with AS. (First Release July 15 2008; J Rheumatol 2008;35:1849–52)

Key Indexing Terms:
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Ankylosing spondylitis (AS) is characterized by inflammatory back pain, peripheral arthritis, uveitis, and an association with HLA-B27. However, the most distinct feature is spinal involvement, which includes syndesmophytosis and ectopic ossification of spinal ligaments. AS shares common features of heterotopic ossification with other relatively common diseases characterized by calcification of spinal ligaments, such as ossification of posterior longitudinal ligament (OPLL)[1]. AS is sporadically complicated with OPLL, and recently we reported that the frequency of OPLL in 544 Korean patients with AS was 3.5%[2]. A single nucleotide polymorphism (SNP) of the collagen 6A1 gene (COL6A1) has been revealed to be strongly associated with OPLL[3] and diffuse idiopathic skeletal hyperostosis (DISH)[4].

We hypothesized that these SNP of COL6A1 were implicated in development of AS and ectopic ossification in spinal ligaments.

MATERIALS AND METHODS

Subjects. One-hundred thirty AS patients, who met modified New York criteria[5], were consecutively recruited (116 men, mean age: 29.0 ± 4.6 yrs). One-hundred thirty age- and sex-matched controls were recruited from health professionals and college students (mean age: 28.6 ± 3.4 yrs).

Seventy-seven patients with ≥ 10 years of disease duration and available cervical spine radiographs were selected to examine the relationship between radiographic progression and COL6A1 SNP. Lateral views were scored using the Bath Ankylosing Spondylitis Radiology Index (BASRI)[6] and were examined for the existence of OPLL[7] and syndesmophytosis. We classified AS patients as having "more ossified disease" if they had a BASRI score of 3 or 4, or had OPLL of any type. Our research work was approved by the Institutional Review Board.

Polymerase chain reaction (PCR) and genotyping. Genomic DNA was prepared from peripheral blood with written consent, and PCR was performed by a standard method. Genotyping was done with a single base primer extension assay (Table 1) using SNaPShot assay, and direct sequencing using electrophoresis. Results were analyzed using Gene Mapper software (Applied Biosystems, Foster City, CA, USA).

We investigated 7 SNP that are reportedly related to OPLL or DISH. An

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Table 1. Primer sequences of SNP of COL6A1 gene.

Character	Method	Strand	Primer	Sequence (5'→ 3')
G365G	SnaPshot	Reverse	F	TTT TCT CCA GGG CAT TCA
			R	AGG GGC CGC AAG TCA CTC
			G	CCT TTT CTC CTT TCA GTC CAA AGG C
IVS15+39 C/T	SnaPshot	Forward	F	TTC TCC AGG GCA TTC AAG G
			R	GAC TTG CTT CCA GGA GAG G
			G	GGC CCC TGG AGG ACC AGG GCC TTC A
IVS21+18 A/C	SnaPshot	Forward	F	TAA AGG CTA CCG AGG CGA
			R	AGA CAG TGG GTT CTG GAT CC
			G	GGT CCG AGG TGA GTC CCA CTC CCC A
IVS32-29 T/C	Sequencing	Reverse	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA
R783Q	Sequencing	Reverse	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA
IVS33+15 G/A	Sequencing	Reverse	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA
IVS33+20 A/G	Sequencing	Reverse	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA
IVS33+55 A/G	Sequencing	Forward	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA
IVS33+88 C/T	Sequencing	Reverse	F	ATG CAG AGC CAT CAA GAG
			R	CCC TGG AGG TAA CAG TGA

F: PCR forward primer, R: PCR reverse primer, G: genotyping primer.

additional 2 SNP (SNP No. 5 and 9) were detected during direct sequencing. Therefore, 9 SNP (Table 2) were chosen for genotyping.

Haplotype estimation and defining haplotype blocks. Haplotypes and haplotype blocks were defined by Haploview. There were 2 blocks: the first included the first 2 SNP and the second included the others. Seven haplotypes with a frequency ≥ 5% were submitted to further analysis.

Statistical analysis. Differences in frequency were determined using a 2-tailed test or multiple logistic regression analysis. Odds ratios were calculated with 95% confidence interval for the relative risk for the development of AS and its clinical manifestations related to the specific genotypes by logistic regression analysis, including age and sex. Haplotype frequencies were calculated by using an expectation-maximization algorithm. Bonferroni correction was applied to multiple comparisons. Statistical analyses were performed with SAS (version 9-1-3, SAS Institute, Cary, NC, USA).

Table 2. Individual SNP and haplotype studied.

SNP	Character	Location	NCBI dbSNP ID	Nucleotide Substitution
1	G365G	Exon 15 (+39)	rs1980982	T/C
2	IVS15+39 C/T	Intron 15 (+39)	Non-dbSNP	C/T
3	IVS21+18 A/C	Intron 21 (+18)	rs2276254	A/C
4	IVS32-29 T/C	Intron 32 (–29)	Non-dbSNP	T/C
5*	R783Q	Exon 33 (+98)	Non-dbSNP	G/A
6	IVS33+15 G/A	Intron 33 (+15)	rs2236485	G/A
7	IVS33+20 A/G	Intron 33 (+20)	rs2236486	A/G
8	IVS33+55 A/G	Intron 33 (+55)	rs2236487	A/G
9*	IVS33+88 C/T	Intron 33 (+88)	rs2236488	C/T
Block	Haplotype	Selected SNP	Alleles	
Haplotype block 1	Haplotype 1	SNP 1 + 2	TC	
	Haplotype 2		CC	
	Haplotype 3		CT	
Haplotype block 2	Haplotype 4	SNP 3+4+6+7+8	ATGAA	
	Haplotype 5		CCGGG	
	Haplotype 6		CCAGG	
	Haplotype 7		ATGGG	

SNP 5 and 9: detected by chance during direct sequencing.

RESULTS

Allele and genotype frequency. All allele and genotype frequencies met Hardy-Weinberg equilibrium. There were no significant associations of allelic and genotype variations between AS and controls. Uveitis was associated with SNP No. 1 (p = 0.03, OR = 3.222, 95% CI = 1.119–9.278, recessive model) and SNP No. 6 (p = 0.027, OR = 2.654, 95% CI = 1.114-6.326, co-dominant model), but these associations did not show statistical significance after Bonferroni correction. No SNP was associated with peripheral arthritis and HLA-B27.

Haplotype analysis. Within the block, no haplotype frequencies in all haplotypes except Haplotype 1 showed statistical differences. There was a significant difference of haplotype distribution in patients according to presence of uveitis after Bonferroni correction for 3 haplotypes and 3 models (Table 3). Haplotype 1 occurred at a lower frequency in patients with uveitis compared to patients without uveitis (p = 0.032 before Bonferroni correction, OR = 0.315, 95% CI = 0.109–0.906, dominant model), but no significance remained after Bonferroni correction. In contrast, Haplotype 2 showed at a higher frequency in patients with uveitis (p = 0.04978 after Bonferroni correction, OR = 3.331, 95% CI = 1.001–11.086, co-dominant model). No haplotype had a significant correlation with peripheral arthritis.

"More ossified disease." Among 77 patients, 27 patients were classified as having more ossified disease. There was no significant variation of alleles/genotypes or haplotypes of COL6A1 between the control disease and the more ossified disease (data not shown).

DISCUSSION

We found no significant associations of allelic and genotype variations of COL6A1 between AS and controls. Thus, COL6A1 may not have a role in development of spinal manifestations of AS. Especially SNP No. 4 (association with OPLL, p = 0.000003), and SNP No. 4, 6, and 3 (association with DISH, p = 0.0005, p = 0.022, p = 0.024, respectively) showed no genetic significance[3,4]. Further, OPLL-specific haplotype[3], constructed with 3 SNP (No. 3, 4, and 7), did not show any association with the development of AS.

Interestingly, uveitis was marginally associated with Haplotype 2. Although there have been some reports on the association between cytokine and chemokine gene polymorphism and uveitis[8,9], we do not have an explanation for association of COL6A1 with uveitis.

COL6A1 region has been reported to be associated with congenital heart defects of Down syndrome[10], muscle disorders such as Ullrich muscular dystrophy[11], Bethlem myopathy[12], and fibronectin organization[13]. The functional role of SNP of COL6A1 is still unknown, but several authors have suggested that these genetic variations of collagen genes may contribute to the membranous or enchondral ossification of the spinal ligaments[3,14].

In conclusion, although some SNP of COL6A1 have been reported to be strongly related to OPLL and DISH, COL6A1 gene may not be the gene responsible for development of AS. Further, SNP of COL6A1 did not seem to influence radiographic progression of AS. However, the association of Haplotype 2 (CC) of COL6A1 with uveitis in AS patients requires further investigation; the power of our study is not strong due to limited sample size and absence of non-AS uveitis controls.

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Table 3. Haplotype analysis of SNP in COL6A1 gene in patients with AS according to uveitis.

	Dominant		Recessive		Co-dominant	
	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
Haplotype 2						
Uveitis	0.075	3.280	1.000	∞	0.04978	3.331
No uveitis		(0.941, 11.439)		(0.000, ∞)		(1.001, 11.086)

∞: infinite. p value: after Bonferroni correction for 9 tests (3 haplotypes and 3 models).

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