The Association of LRP5 Gene Polymorphisms with Ankylosing Spondylitis in a Chinese Han Population

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ABSTRACT. ***Objective.*** To clarify the association between polymorphisms of low-density lipoprotein receptor-related protein 5 (LRP5) with ankylosing spondylitis (AS) in a Chinese Han population.

Methods. Sixteen patients with AS were recruited for preliminary screening through gene sequencing. Then 14 single-nucleotide polymorphisms (SNP) of LRP5 were followed up in 296 patients and 170 controls.

Results. Sequencing the LRP5 showed 24 SNP including 3 novel SNP [LRP5SNP1 (c.-1596T > C), LRP5SNP2 (c.3764-30G > A), and LRP5SNP3 (c.4488+74G > A)]. Validation of SNP showed that the LRP5SNP3 were associated with AS after multiple testing correction (allele P_c = 0.0087, genotype P_c = 0.0316, haplotype AGA, P_c = 0.0051, OR = 2.54 and haplotype AGG, P_c = 0.048, OR = 0.63, respectively). The SNP rs686921 was associated with male predominance in both patients with AS (p = 0.032, OR 1.54) and controls (p = 0.014, OR 1.94).

Conclusion. LRP5 may be involved in the pathogenesis of AS. Further study will be required to clarify the effect of LRP5 on the pathogenic mechanism of AS. (First Release Sept 1 2011; J Rheumatol 2011;38:2616–18; doi:10.3899/jrheum.111117)

Key Indexing Terms:
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Ankylosing spondylitis (AS) is a seronegative spondyloarthritis with a high degree of familiality (λ_s = 82) and heritability (> 90%) that primarily affects spinal, sacroiliac joints, and entheses followed by pathological bone formation[1]. Wnt (wingless) signaling has expanded to embryogenesis, oncogenesis, stem cell research, and now bone biology. LRP5 is a transmembrane protein that functions as a coreceptor in canonical Wnt signaling[2].

We explored the association of AS with LRP5. We hypothesized that LRP5 regulates the bone mass density (BMD), osteoblast proliferation, and bone formation[2]; patients with AS manifest not only osteoporosis but also pathological bone formation[3], therefore, LRP5 would be involved in the variance of BMD and/or bone formation in AS. We also hypothesized that the Wnt signaling pathway plays a role in the pathogenesis of RA[4], and that LRP5 would serve as a switch of inflammation/ossification and play a pathologic role independent of Dickkopf-1 (DKK-1) in AS. LRP5 would cause increased osteoprotegerin (OPG), inhibiting bone resorbing. Concurrently, LRP5 would facilitate osteoblast proliferation and differentiation.

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MATERIALS AND METHODS

Subjects. Sixteen unrelated patients with AS were recruited for the discovery of mutation or novel polymorphisms in LRP5. Our study enrolled 300 patients and 180 controls for validation of SNP. All study subjects were of Han Chinese origin, from Shandong Province, China. Diagnosis of AS met the modified New York criteria[5]. The patients' pelvic and lumbosacral spine radiographs were read by a radiologist. Information was collected systematically (Table 1). The study was approved by the ethics committee, and written informed consent was obtained.

SNP selection. Tag SNP covering LRP5 (including 3000 bp upstream of the first exon) were selected using Tagger in Haploview 4.0. Five SNP (LRP5SNP1, LRP5SNP2, LRP5SNP3, rs41494349, rs3736228), which were discovered in the initial screening, and 9 tag SNP (rs312016, rs312015, rs4988300, rs312024, rs638051, rs4930573, rs686921, rs471966, rs531163) were validated in 300 patients and 180 controls.

Gene sequencing and variant typing. We sequenced the LRP5 in 16 patients with AS, including the promoter region, exonic region, and exon-intron junctions with an ABI3130 sequencer. We validated 14 SNP using the Illumina GoldenGate gene typing platform.

Splice-site prediction programs (SSPP). The splice-site analysis tools NetGene 2[6], Automated Splice-Site Analysis[7], and MaxEntScan[8] were used to predict the possible effect of a variant on RNA splicing.

Statistical analysis. The Hardy-Weinberg equilibrium test was performed on controls. Allelic association tests and logistic regression analysis were conducted using PLINK1.07, and genotype and haplotype analysis were performed using SHEsis (http://analysis.bio-x.cn/myAnalysis.php)[9]. Multiple testing corrections were conducted using SHEsis, and the number of permutations was 10,000. We carried out multivariate logistic regression

Table 1. Basic characteristics of 466 subjects (296 cases, 170 controls). Numbers do not add up to the total number of cases because some test results were not recorded.

Characteristics	Cases	Controls
Sex (men/women)	228/68	128/42
Average age, yrs, SD	29.19 ± 9.86	24.66 ± 6.66
No. patients juvenile onset/adult onset	114/170	
B-27 (negative/positive)	14/155	
Kyphosis (negative/positive)	169/120	
Bamboo spine (negative/positive)	195/94	
Radiographic grading of sacroiliitis (2/3–4)	123/167	

analysis through SPSS16.0 to show whether the association was due to linkage disequilibrium or was a true association with functionally relevant polymorphisms. We performed power calculations for detectable effect sizes using software Quanto 1.2.4.

RESULTS

Gene sequencing in the initial stage. We found 24 SNP, including 3 novel SNP: LRP5SNP1 (c.-1596T > C), LRP5SNP2 (c.3764-30G > A), and LRP5SNP3 (c.4488+74G > A), having the minor allele frequencies (MAF) 6.2%, 9.4%, and 12.5%, respectively. No mutations were found.

Quality control. To test the accuracy of genotyping, we regenotyped the randomly selected 10 samples, and the reproducibility was > 96%.

Validation of SNP in a large population. After being filtered with criteria, 296 cases, 170 controls (Table 1), and 12 SNP (Table 2) were retained for final analysis. The criteria were Hardy-Weinberg equilibrium for controls > 0.05, MAF > 0.05, callrate for individual sample > 0.9, and call frequency for individual SNP > 0.9.

Logistic regression analysis showed no significant difference in distribution of sex ratio between patients and controls (p = 0.37). A significant association was found for LRP5SNP3 at both allele (P_c = 0.0087) and genotype (P_c = 0.0316). The A allele was found to confer susceptibility to AS, with an OR of 1.86. The haplotype AGA, concerning rs531163, LRP5SNP2, LRP5SNP3 (P_c = 0.0051, OR 2.54, risk haplotype) and AGG (P_c = 0.048, OR 0.63, protective haplotype) showed significant association (Table 3). Multivariate logistic regression analysis showed that the association between LRP5 and AS was due to a true association with LRP5SNP3, not linkage disequilibrium.

We had 80% power to detect SNP with OR > 3.0 (recessive inheritance model) and OR > 1.7 (dominant inheritance model). In fact, for the LRP5SNP3 we had 95.93% and 62.17% power to detect risks at OR 3.98 (recessive inheritance model) and OR 1.56 (dominant inheritance model), respectively.

However, no association was found between LRP5 and the ossification phenotype (kyphosis, bamboo spine, radiology grade 3–4), as well as the age of onset in AS, and for these phenotypes, the statistical power to calculate the correlation was low due to small sample size. The rs686921 was associated with male predominance in both patients (p = 0.0323, OR 1.5414) and controls (p = 0.01428, OR 1.9396).

Using SSPP, we found no possible effect of the LRP5SNP3 on RNA splicing.

DISCUSSION

To our knowledge, we are the first researchers to investigate the association of LRP5 with AS. Our findings show that LRP5 is associated with susceptibility to AS. Loss-of-function mutations in LRP5 were associated with low bone mass in osteoporosis pseudoglioma syndrome[10]. LRP5$^{-/-}$ mice demonstrated decreased osteoblast proliferation and low bone mass[11]. In addition, LRP5 indicates susceptibility to osteoporosis. Further, the LRP5 gene plays an important role in the regulation of BMD in the general population. If LRP5SNP3 is a loss-of-function variant, LRP5 may be involved in BMD conditions in AS such as osteoporosis.

However, a single gain-of-function amino-acid substitu-

Table 2. Allelic and genotype association analysis of single-nucleotide polymorphism (SNP) in the LRP5 gene in case control.

		Allele						Genotype							
								Case Frequency			Control Frequency				
SNP	Allele	Freq (case)	Freq (control)	p	OR	P_c	95% CI	1/1	1/2	2/2	1/1	1/2	2/2	Fisher's p	P_c
LRP5SNP1	G	0.052	0.059	0.627	0.866	0.92	0.647–2.059	0.897	0.103	—	0.882	0.118	—	0.616	0.94
rs312016	G	0.377	0.406	0.399	0.888	0.86	0.854–1.485	0.372	0.502	0.126	0.335	0.518	0.146	0.681	0.96
rs312015	G	0.385	0.382	0.936	1.011	0.92	0.752–1.300	0.370	0.490	0.140	0.376	0.482	0.141	0.986	1.00
rs312024	G	0.422	0.447	0.450	0.902	0.92	0.848–1.450	0.330	0.497	0.173	0.288	0.529	0.182	0.644	0.95
rs41494349	G	0.090	0.082	0.689	1.102	0.92	0.584–1.502	0.823	0.173	0.003	0.841	0.153	0.006	0.787	0.97
rs638051	G	0.336	0.300	0.256	1.181	0.82	0.835–1.328	0.408	0.512	0.080	0.476	0.447	0.076	0.346	0.87
rs4930573	C	0.177	0.195	0.494	0.888	0.97	0.631–1.249	0.030	0.294	0.676	0.041	0.308	0.651	0.753	0.97
rs686921	C	0.254	0.256	0.954	0.991	1.00	0.743–1.369	0.545	0.401	0.054	0.571	0.347	0.082	0.305	0.89
rs471966	G	0.273	0.262	0.719	1.057	0.95	0.700–1.279	0.528	0.398	0.074	0.547	0.382	0.071	0.927	1.00
rs531163	G	0.271	0.268	0.914	1.017	1.00	0.728–1.328	0.528	0.401	0.070	0.535	0.394	0.071	0.988	1.00
LRP5SNP2	A	0.085	0.097	0.534	0.864	0.92	0.546–1.368	0.007	0.157	0.837	0.000	0.194	0.806	0.341	0.90
LRP5SNP3	A	0.219	0.131	0.0018	1.859	0.0087	1.255–2.755	0.121	0.195	0.684	0.034	0.195	0.772	0.0098	0.0316

P_c: p value after multiple testing correction.

Table 3. Haplotype analysis of LRP5 in cases and controls. All haplotype frequencies < 0.03 were left out of the analysis.

Haplotype	Frequency (case)	Frequency (control)	Fisher's p	OR	P_c	95% CI
AGA	0.1577	0.0691	0.0002	2.5359	0.0051	(1.5297, 4.2039)
AGG	0.5440	0.6423	0.0035	0.6329	0.0482	(0.4653, 0.8607)
GAG	0.0539	0.0588	0.7635	0.9105	0.9999	(0.4941, 1.6776)
GGG	0.1844	0.1681	0.5590	1.1185	0.9935	(0.7681, 1.6288)

P_c: p value after mutiple testing correction.

tion (G171V) in the same gene was associated with a high bone mass[12]. Mice overexpressing the human G171V mutation had a phenotype with increased bone mass. G171V inhibits the ability of DKK-1 and potentially other proteins to bind to LRP5 and inhibit Wnt signaling[12]. Therefore, if LRP5SNP3 were a gain-of-function variant, LRP5 would serve as a switch of inflammation/ossification, and play a pathologic role independent of DKK-1 and tumor necrosis factor in AS. LRP5 would not only cause increased OPG, inhibiting bone resorbing, but would also facilitate osteoblast proliferation and differentiation.

Multivariate logistic regression analysis showed that the LRP5SNP3 was probably a functionally relevant SNP not due to linkage disequilibrium. The LRP5SNP3 is located in intron 21, just 73 bp downstream of exon 21 and 20 bp downstream of rs2291466. We hypothesized that LRP5SNP3 may play a pathological role through interference with splicing the exon-intron junction. We found no possible effect of the LRP5SNP3 on RNA splicing. LRP5SNP3 may be involved in the pathogenesis through other mechanisms, such as the effect of gene expression.

Osteoarthritis (OA) is characterized by cartilage degradation, formation of osteophytes, and subchondral sclerosis. In AS the formation of osteophytes and subchondral sclerosis is similar to that occurring in OA. OA susceptibility locus is chromosome 11q12-13[13], a region harboring the LRP5 gene. A common haplotype (C-G-C-C-A) in LRP5 provided a 1.6-fold increased risk of OA[14]. Therefore, through researching LRP5 in the pathogenesis of OA, we could find some clues in the LRP5 playing a role in the pathogenesis of AS.

Because the sample size in each clinical phenotype is too small, we cannot rule out the association between LRP5 and kyphosis, bamboo spine, and radiology 3-4 grade, as well as age of onset in AS. The question remains of how the LRP5 gene led to the etiology of AS. Our study would be more persuasive using a disease that manifests only inflammation and no ossification (e.g., rheumatoid arthritis) as controls, and a larger sample size.

Cheung, *et al*[15] found associations of rs686921 with BMD in young southern Chinese Han women. LRP5 is a susceptibility gene of osteoporosis, which is predominant in women. One reason for these phenomena could be the gain-of-function or loss-of-function variants in LRP5.

Our study supports the importance of LRP5 in the etiology of AS. Further association studies involving more cases and different ethnic groups will be required.

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