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ORIGINAL RESEARCH

Association Between SPARC Polymorphisms and Ankylosing Spondylitis and Its mRNA and Protein Expression in a Chinese Han Population: A Case–Control Study

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Objective: We explore the association of polymorphisms in Secreted protein acidic and rich in cysteine (SPARC) with ankylosing spondylitis (AS) and detect SPARC mRNA and protein expression in a Chinese Han population.

Methods: Nine single-nucleotide polymorphisms (SNPs) of *SPARC* were genotyped in 768 AS patients and 768 controls by TaqMan genotyping assay. mRNA expression of SPARC was detected by real-time polymerase chain reaction (RT-PCR), and serum level of SPARC protein was detected by ELISA.

Results: The frequency of A allele of rs171121187 was significantly higher in AS patients than in controls (P_c=0.003, odds ratio [OR] =1.45, 95% confidence interval [95% CI] = 1.18–1.77), the AA and AC genotypes increased the risk of AS when compared with CC genotype (P_c=0.003, OR=3.96, 95% CI=1.80–8.75, and P_c=0.003, OR=1.27, 95% CI=1.01–1.61, respectively). The frequency of G allele of rs4958487 was significantly lower in AS than in controls (P_c=0.001, OR=0.60, 95% CI=0.47–0.68), the GG and GA genotypes reduced the risk of AS when compared with AA genotype (P_c=0.005, OR=0.46, 95% CI 0.18–1.14, and P_c=0.005, OR=0.60, 95% CI=0.45–0.79, respectively). The haplotype AA of rs17112187/rs4958487 significantly increased the risk of AS (P=2.31E-5, OR=1.60, 95% CI=1.28–1.98), while haplotype CG decreased the risk of AS (P=5.42E-5, OR=0.55, 95% CI=0.41–0.74). Expression levels of SPARC mRNA were significantly lower in both Peripheral blood mononuclear cells (PBMC) and granulocytes in AS patients than in controls (P=0.008 and P=0.005, respectively). SPARC protein levels were also reduced in AS patients versus the controls (P=0.002).

Conclusion: This study indicates that polymorphisms in *SPARC* are associated with AS susceptibility, and both mRNA and protein levels of SPARC are decreased in AS patients in a Chinese Han population.

Keywords: secreted protein acidic and rich in cysteine, SPARC, ankylosing spondylitis, single nucleotide polymorphism, SNP, case–control study

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease and the main subtype of spondyloarthritis. Clinical features of AS include sacroiliitis, spinal inflammation, asymmetrical peripheral oligoarthritis, and enthesitis.[1] The exact pathogenesis and etiology of AS are not fully elaborated till now, but it is well established that genetic factors play a key role in determining a patient's susceptibility to AS.[2] The gene encoding human leukocyte antigen B-27 (HLA-B27) is strongly associated with AS development.[3] However, only 1–5% of all HLA-B27 positive individuals will develop AS.[4,5] Previously, in addition to this well-known HLA-B27 gene, more than 14 susceptibility genes outside of the MHC region, such as *ERAP1, IL23R, ANTXR2, IL12B, CARD9, PTGER4, KIF21B,* and *STAT3*, have been identified as the risk factors for AS.[6–11]

Osteoporosis (OP) or osteopenia is common even in the early stages of AS.[12–14] Cortical bone loss starts early and is evident within the first 2 years of the disease.[15] It is reported that the prevalence of OP and osteopenia in AS patients

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ranged from 2.0% to 47.0% and 5.0%–78.3%, respectively.[16] Compared with patients with mechanical back pain, patients with AS showed significant bone loss assessed by Dual-energy x-ray absorptiometry (DXA) at the lumbar spine.[17] Osteoporosis of spine is much more common than that of femur, and presence of syndesmophytes did not affect estimation of osteoporosis of spine.[18] Inflammation is associated with trabecular bone loss leading to osteoporosis but also with cordal new bone formation.[17,19–21] The bone mineral density (BMD) at the femoral neck was reduced in AS patients and was correlated with increased risk of vertebral fractures, suggesting that bone mass loss is still the basic pathological change of this disease.[12]

Human *SPARC* (Secreted protein acidic and rich in cysteine) is a single-copy gene localized on chromosome 5q31-33 with a high degree of evolutionary conservation. Termine et al primarily identified that SPARC was a pivotal noncollagenous constituent of bovine bone in 1981.[22] SPARC is also called osteonectin or BM-40, and it is a matricellular protein secreted by many cell types found in the extracellular (ECM) during a variety of processes such as bone formation, wound healing, adipogenesis, and angiogenesis.[23–25] It has been shown that matricellular SPARC regulates ECM tightness, dendritic cells migration, and T-cell priming with antigen-specific immune response.[26] When tissues undergo remodeling during normal development or in response to injury, the expression of SPARC mRNA and protein is markedly elevated.[27] While in some osteopenic states, such as osteogenesis imperfecta, osteonectin expression decreases,[28] as Delany et al reported that osteonectin-null mice had decreased bone formation, resulting in decreased bone remodeling with a negative bone balance and causing profound osteopenia. Mansergh et al have established a Sparc-deficient mouse model for human osteoporosis.[29] These data suggest that SPARC/osteonectin participated in bone remodeling and the maintenance of bone mass. Sharma et al first implicated SPARC as a component of the innate immune system in the pathogenesis of spondyloarthropathy (SpA).[30] In order to evaluate whether SPARC contributes to the susceptibility of AS, we studied SPARC polymorphisms and its mRNA and protein expression in a Chinese Han population.

Materials and Methods

Subjects

A total of 768 AS patients were recruited from the Department of Rheumatology and Immunology of Shandong Provincial Hospital affiliated to Shandong First Medical University from 2014 to 2016, 768 healthy controls were recruited from those who underwent physical examination in the Health Center of the same hospital, who had no previous medical history or family history diseases. All the participants underwent genotyping. For the following mRNA test, we recruited 68 patients from the AS group who were first diagnosed without any treatment, and collected 76 individuals randomly from the control group. For further protein analysis, we randomly chose 39 from 68 patients and 45 from 76 controls, respectively. All AS patients were diagnosed according to the modified 1984 New York criteria.[31] The exclusion criteria of this study included: those who were not of Chinese Han nationality, those with a family history of AS, those aged under 18 years; those with psoriasis, psoriatic arthritis, inflammatory bowel disease (IBD), diabetes, viral hepatitis, cirrhosis, severe hepatic or renal function insufficiency, thyroid disease, those who were diagnosed with cardiovascular or cerebrovascular diseases, those who underwent organ transplants, and patients with active tuberculosis or other infections. Blood samples were obtained after their informed consent. The study was approved by the Human Ethics Committee of Provincial Hospital affiliated with Shandong First Medical University (No. 2014–030). The main demographic and clinical characteristics of AS patients and controls are shown in Table 1. Questionnaires (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI)) were obtained before the initiation of treatments.

Tag SNP Selection and Genotyping

Tag SNPs were identified using International HapMap data with the Chinese Beijing Han population (CHB) of Phase I+II release, 24. The region of SNPs data was downloaded covering the SPARC gene and 2 kb upstream of the gene. We used Haploview software to figure out the linkage disequilibrium structure between SNPs, with minor allele frequency (MAF) >0.05 and r^2 >0.8. Finally, in order to contain the maximum information of the haplotype blocks, we chose the SNPs rs3776959, rs4958279, rs7733793, rs2881558, rs2347128, rs4958485, rs17112187, rs4958487and rs1545030 as tag SNPs. The basic information of the nine SNPs is shown in Table 2.

Table 1 The Main Demographic and Clinical Characteristics of the AS Patients and Controls

Characteristic	AS Patients	Controls
Number of subjects	768	768
Gender (Men, %)	61.7	57.8
Age (mean ± SD, years)	35.7±9.8	37.1±8.7
HLA-B27 positive, %	95	–
Disease duration (mean ± SD, years)	8.6±4.1	–
ESR (mean ± SD, mm/h)	30.03±12.84	–
CRP (mean ± SD, mg/L)	24.36±9.62	–
BASDAI (mean±SD)	4.67±1.62	–
BASFI (mean±SD)	3.86±2.14	–

Abbreviations: AS, ankylosing spondylitis; SD, standard deviation; HLA-B27, human leukocyte antigen B27; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath AS Functional Index.

Table 2 The Basic Information of the Nine SNPs

SNPs	Genotyping Assay Part Number	Region
SPARC-rs3776959	C___2086343_10	Intron8
SPARC-rs4958279	C__28037505_10	Intron6
SPARC-rs7733793	C___2086331_20	Intron2
SPARC-rs2881558	C__16083139_10	Intron1
SPARC-rs2347128	C__15779576_10	Intron1
SPARC-rs4958485	C__27927153_10	Intron1
SPARC-rs17112187	C__34334451_10	Intron1
SPARC-rs4958487	C___2086329_10	Intron1
SPARC-rs1545030	C___8949874_10	5'flanking

Abbreviations: SNP, single nucleotide polymorphism; SPARC, Secreted protein acidic and rich in cysteine.

DNA samples from the 768 AS patients and 768 controls were extracted from peripheral blood leukocytes using TIANGEN Genomic DNA Purification Kit according to the manufacturer's instructions and stored at −80°C before genotyping. The nine SNPs were genotyped using TaqMan chemistry of the Applied Biosystems real-time Prism 7900HT Sequence Detection System (ABI Inc. CA, USA). Primers and the TaqMan fluorogenic probes were designed using the Primer Express software V2.0 (ABI Inc., Foster City, CA, USA) and procured from Applied Biosystems (ABI, Warrington, UK). One probe (for allele 1) was labeled with VIC dye and the other (for allele 2) with FAM dye at the 5'-end, and serial dilutions were run to determine the optimal working concentrations. For each reaction, a 25 μL mixture was prepared by mixing 5 μL containing 50 ng DNA, 12.5 μL of 2x Universal mix (Eurogentec, Liege Science Park, Seraing Belgium), 1.25 μL of 20x probe assay mix, and 6.25 μL DNase-free distilled water. Three no-template controls were included in each plate for normalization of emission signal. The thermal profile for amplification for the first cycle occurred at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 94°C for 15 sec and 60°C for 30 sec. The plates were then scanned for FRET signal using the 7900HT sequence detection system and data analyzed using SDS 2.0 software (ABI, Foster City, CA, USA).

Isolation of PBMC and Granulocytes

Blood samples from 68 patients and 76 healthy controls were deposited in tubes with sodium citrate. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by Ficoll gradient centrifugation (Lymphoprep, Nycomed Pharma AS). Cells from the interphase were collected and washed twice with HBSS (HyClone). Granulocytes were collected from heparinized blood samples after being processed by lymphocyte separation liquid and red cell lysing reagent, washed twice with Hanks liquid. The PBMC and granulocytes are stored in 1mL of Trizol (Invitrogen) at –80°C, respectively, until total RNA extraction.

Total RNA Extraction and cDNA Synthesis

Total RNA from PBMC was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. RNA quantitation and quality were determined using an Agilent 2100 Bioanalyzer (Agilent) and the RNA 6000 Nano Kit (Agilent), according to the manufacturer's manuals. The isolated RNA samples were quantified by a spectrophotometer (Titertek-Berthold Colibri, Germany). cDNA was synthesized using the UEIris reverse transcription real-time polymerase chain reaction system. Total RNA (500 ng/mL in a final volume of 2 μL) was added to a mixture (10 μL) containing RNase-free water, 10 × DNase I buffer, and DNase I. This mixture was placed into a thermal cycler (Bioer Life Express, China) at 37°C for 10 min, before 1 μL of 50 mM EDTA was added to the mix. This mixture was heated to 65°C for 10 min to deactivate DNase I; then, UEIris RT MasterMix (5×) RNase-free water was added to the mix (20μL). This mix was placed into a thermal cycler, and a reverse transcription reaction was facilitated to produce cDNA at 55°C for 30 min, with a final step conducted for 10 sec at 85°C.

Quantitative Real-Time PCR (qRT-PCR)

Expression levels of SPARC mRNA were detected by qRT-PCR using QuantiFast SYBR Green PCR Kit (Qiagen) following the manufacturer's instructions. Primers for SPARC gene were designed and obtained from the GenBank Database, which were as follows: forward 5'-AGAAGGTGTGCAGCAATGAC-3', reverse: 5'-TGTAGTCCAGGTGGAGCTTG-3'. PCR amplification conditions were as follows: one cycle at 95°C for 2 min, 40 cycles at 95°C for 5 sec, one cycle at 60°C for 15 sec, and elongation at 72°C for 25 sec. Each cDNA sample was analyzed in triplicate, and β-actin was used as the endogenous control. The relative expression of SPARC mRNA was calculated using the $2^{-\Delta\Delta Ct}$ comparative method, where −ΔCt refers to the difference between the numbers of cycles (Ct) of the target gene and the endogenous control.

Enzyme-Linked Immunosorbent Assay (ELISA)

The blood samples of 39 AS patients and 45 healthy controls were centrifuged, and the serum specimens were stored at −80°C. Serum SPARC protein levels were detected by Human SPARC ELISA kit from R&D systems (Minneapolis, MN55413, USA) according to the manufacturer's instructions. In this ELISA system, human SPARC ELISA kit was used to generate the standard curve to quantify the SPARC protein based on absorbance data. The intra- and inter-assay CVs were 5.3% and 9.2%, respectively. All samples were tested in duplicates in a blinded manner.

Statistical Analysis

The data were analyzed using GraphPad Prism software (version 5.0). Mann–Whitney *U*-tests were used to compare mRNA expression and serum protein levels of SPARC in patients with AS and healthy controls. *P* value of less than 0.05 was considered significant. The Hardy–Weinberg equilibrium (HWE) of the nine SNPs was tested by chi-square test. Genotypic and allelic frequencies between patients and controls, the odds ratio (OR) with 95% confidence interval (CI), *P* value were calculated with respect to the minor allele compared with the major allele, and haplotype frequencies analyzed by SHEsis and SNPStats program. The linkage disequilibrium (LD) was calculated using Haploview 4.2. P_c values were corrected by the Bonferroni method, and the statistical significance was defined as $P_c < 0.05$.

Results

Overall, 768 AS patients (474 men, mean age 35.7±9.8 years) and 768 controls (444 men, mean age 37.1±8.7 years) were enrolled in our study. The age of all participants ranged from 18 to 60. There were no statistical differences in gender and age between AS patients and controls (for gender, P=0.119; for age, P=0.537). In the AS group, positivity of HLA-B27 was 95%, mean BASDAI was 4.67±1.62, and mean BASFI was 3.86±2.14.

Genetic Association Between *SPARC* SNPs and AS

Nine SNPs of *SPARC* were genotyped in all subjects. The distribution of the nine *SPARC* SNPs was in accordance with the Hardy-Weinberg equilibrium (HWE) (P>0.05, Supplementary Table S1). In this study, Minor Allele Frequency (MAF) was consistent with the International HapMap project data of CHB.

The allele and genotype frequencies of the tested *SPARC* SNPs are illustrated in Table 3. The results revealed that rs17112187 and rs4958487 of the tested SNPs correlated with the development of AS. For rs17112187, the major A allele was associated with the increased risk of AS (P_c=0.003, OR=1.45), the AA and AC genotypes appeared to increase the risk of AS when compared with CC genotype (P_c=0.003, OR=0.25 and P_c=0.003, OR=0.79, respectively). For rs4958487, the minor G allele frequency in AS patients was significantly lower than that in controls (P_c=0.001, OR=1.67), the GG and GA genotypes appeared to reduce the risk of AS when compared with AA genotype (P_c=0.005, OR=2.18 and P_c=0.005, OR=1.68, respectively). The haplotype AA of rs17112187/rs4958487 was strongly associated with AS and significantly increased the risk of AS (*P*=2.31E-5, OR=1.60), while the haplotype CG reduced the risk (*P*=5.42E-5, OR=0.55), we further got a global *P*=1.90E-7, which suggests the haplotypes of both SNPs were related to AS susceptibility (Table 4). The linkage analysis was performed by Haploview software 4.2, all the nine SNPs haplotypes were compared between AS patients and healthy controls, but all of them were in weak linkage disequilibrium (Supplementary Figure S1).

Expression Levels of SPARC mRNA and Protein

The subjects included 68 AS patients (59 male and 9 female, mean age 33.2±9.1 years) and 76 healthy controls (64 males and 12 females, mean age 36.7±8.3 years). There were no statistical differences in gender and age between the AS patients and healthy controls (for gender, P=0.814; for age, P=0.726). The SPARC mRNA expression levels were

Table 3 Frequencies of Alleles and Genotypes of *SPARC* Polymorphisms in AS Patients and Controls

SNPs	Genotypes	AS n (%)	Controls n (%)	*P* values	OR (95% CI)	P_c values
rs3776959	G	870(56.6)	854(55.6)		1	
	C	666(43.4)	682(44.4)	0.561	0.96(0.83–1.11)	
	GG	234(30.5)	226(29.4)		1	
	CC	132(17.2)	140(18.2)	0.829	1.10(0.81–1.48)	
	CG	402(52.3)	402(52.3)		1.04(0.82–1.30)	
rs4958279	G	925(60.2)	938(61.1)		1	
	A	611(39.8)	598(38.9)	0.631	1.04(0.90–1.20)	
	GG	278(36.2)	278(36.2)		1	
	AA	110(14.3)	108(14.1)	0.841	0.94(0.69–1.29)	
	AG	391(50.9)	382(49.7)		0.94(0.75–1.17)	
rs7733793	T	888(57.8)	882(57.4)		1	
	C	648(42.2)	654(42.6)	0.827	0.98(0.85–1.14)	
	TT	250(32.6)	241(31.4)		1	
	CC	130(16.9)	127(16.5)	0.826	1.07(0.85–1.34)	
	CT	388(50.5)	400(52.1)		1.01(0.75–1.37)	
rs2881558	G	890(61.7)	948(61.7)		1	
	A	646(42.1)	588(38.3)	0.033	1.17(1.01–1.35)	0.299
	GG	270(35.2)	286(37.2)		1	
	AA	148(19.3)	106(13.8)	0.016	0.68(0.50–0.91)	0.140
	AG	350(45.6)	376(49.0)		1.01(0.81–1.27)	
rs2347128	C	872(56.8)	834(54.3)		1	
	G	664(43.2)	702(45.7)	0.168	1.11(0.96–1.27)	
	CC	258(33.6)	228(29.7)		1	
	GG	154(20.1)	162(21.1)	0.258	1.19(0.90–1.58)	
	GC	356(46.4)	378(49.2)		1.20(0.96–1.51)	
rs4958485	C	1120(72.9)	1057(68.8)		1	
	T	416(27.1)	479(31.2)	0.012	1.22(1.04–1.43)	0.112
	CC	404(52.6)	359(46.7)		1	
	TT	52(6.8)	70(9.1)	0.040	1.51(1.03–2.23)	0.362
	TC	312(40.6)	339(44.1)		1.22(0.99–1.51)	

(*Continued*)

Table 3 (Continued).

SNPs	Genotypes	AS n (%)	Controls n (%)	*P* values	OR (95% CI)	P_c values
rs17112187	C	1273(82.9)	1344(87.5)		1	
	A	263(17.1)	192(12.5)	3.14E-4	1.45(1.18–1.77)	0.003
	CC	534(69.5)	584(76.0)		1	
	AA	29(3.8)	8(1.0)	2.84E-4	0.25(0.11–0.56)	0.003
	AC	205(26.7)	176(22.9)		0.79(0.62–0.99)	
rs4958487	A	1431(93.2)	1368(89.1)		1	
	G	105(6.8)	168(10.9)	6.57E-5	1.67(1.30–2.16)	0.001
	AA	670(87.2)	614(79.9)		1	
	GG	7(0.9)	14(1.8)	0.001	2.18(0.88–5.44)	0.005
	GA	91(11.8)	140(18.2)		1.68(1.26–2.23)	
rs1545030	T	1171(76.2)	1220(79.4)		1	
	C	365(23.8)	316(20.6)	0.033	1.20(1.01–1.43)	0.300
	TT	456(59.4)	482(62.8)		1	
	CC	53(6.9)	30(3.9)	0.029	0.54(0.34–0.85)	0.258
	CT	259(33.7)	256(33.3)		0.94(0.75–1.16)	

Abbreviations: AS, ankylosing spondylitis; SNP, single nucleotide polymorphism; OR, odds ratio; CI, 95% confidence interval; p_c was corrected by Bonferroni method; significance level p_c <0.05.

Table 4 The Haplotypes Between rs17112187 and rs4958487 Related to AS

Haplotype	Case (Freq)	Control (Freq)	p value	Global p	OR (95% CI)
AA*	232.66 (0.151)	153.92 (0.100)	2.31E-5		1.60 (1.28–1.98)
CA*	1198.34 (0.780)	1214.08 (0.790)	0.314		0.91 (0.76–1.09)
CG*	74.66 (0.049)	129.92 (0.085)	5.42E-5	1.90E-7	0.55 (0.41–0.74)
AG	30.34 (0.020)	38.08 (0.025)	0.344		0.793(0.49–1.28)

Note: *Haplotype with a frequency >3%. Significance level p <0.05.
Abbreviations: AS, ankylosing spondylitis; OR, odds ratio; CI, 95% confidence interval.

examined using qRT-PCR. The results showed that SPARC mRNA expression levels in PBMC of the 68 AS patients were significantly lower than that of the 76 healthy controls (*P*=0.008, Figure 1A). Further, the granulocyte mRNA expression levels were also significantly lower in AS patients than in healthy controls (*P*=0.005, Figure 1B). Next,

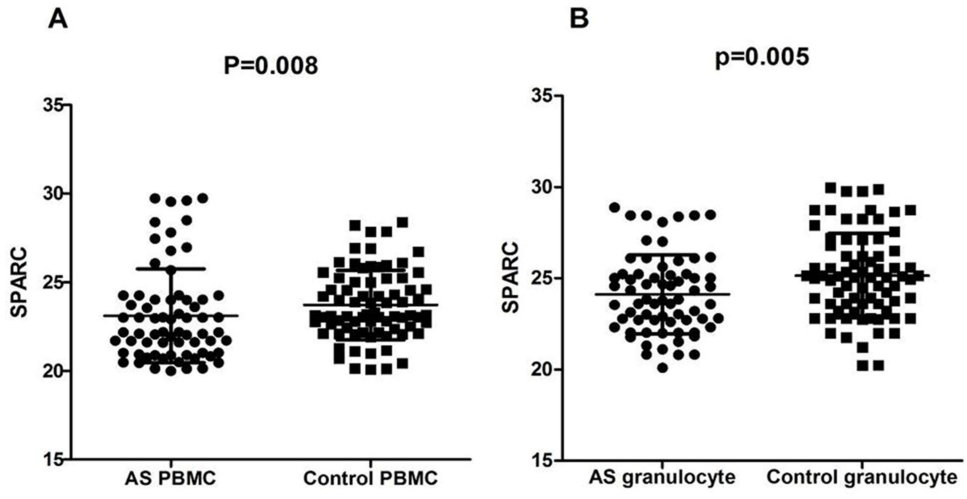

Figure 1 Comparison of SPARC mRNA expression levels in both PBMC (**A**) and granulocyte (**B**) between AS patients and healthy controls. The scatter-plot showed that the quantification levels of normalized mRNA. The p value refers to unpaired nonparametric comparison of the two groups (Mann–Whitney *U*-test).
Abbreviations: AS, ankylosing spondylitis; SPARC, secreted protein acidic and rich in cysteine; PBMC, peripheral blood mononuclear cell.

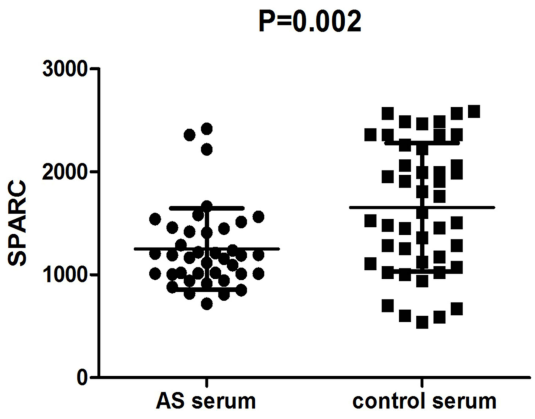

Figure 2 Comparison of serum SPARC protein levels between AS patients and healthy controls by ELISA analysis. The scatter-plot showed that the quantification levels of normalized serum levels of SPARC. The p value refers to unpaired nonparametric comparison of the two groups (Mann–Whitney *U*-test).
Abbreviations: AS, ankylosing spondylitis; SPARC, secreted protein acidic and rich in cysteine.

SPARC protein levels in the serum were investigated by ELISA, the result indicated that SPARC protein levels in 39 AS patients were significantly reduced compared with 45 healthy controls (P=0.002, Figure 2). All the results showed that the mRNA and protein levels of SPARC were significantly decreased in patients with AS.

Discussion

In this case–control study based on a Chinese Han population, we evaluated the association between SPARC genetic polymorphisms and AS susceptibility, we then detected mRNA and protein expression levels of SPARC in AS patients and healthy controls. We found the allele frequencies and genotype of rs17112187 and rs4958487 correlated with the development of AS. Both mRNA and protein levels of SPARC were significantly decreased in AS patients compared with controls.

SPARC is a 34,632 Dalton extracellular matrix-associated glycoprotein in the extracellular matrix (ECM), also called osteonectin or BM-40.[32] Termine et al first described that SPARC was a bone-specific protein enriched in mineralized bone trabeculae, connecting hydroxyapatite and collagen to compose a bone matrix.[22,33] SPARC could influence pathways involved in extracellular matrix assembly such as procollagen processing and collagen fibril formation.[34] In many studies of animal models, SPARC was found to participate in the regulation mechanism of growth factors and matrix protease in the microenvironment. SPARC could link with TGFβ/TGFβRII complex combination to enhance its downstream signals.[35] In HMVEC and BAEC cells, SPARC inhibits the β-FGF receptor phosphorylation, combines with VEGF and its receptor against angiogenesis.[36] In fibroblasts, SPARC inhibits the binding of PDGF-B to its cell-surface receptors.[37] SPARC mediates peripheral blood mononuclear cells to express MMP-1 and MMP-9, and mediates fibroblasts to express MMP-1, MMP-3, and MMP-9.[38] Moreover, SPARC participates in a variety of signal transduction pathways, such as PI3K/AKT, MAPK, and Wnt signal transduction pathway,[39,40] relative to the occurrence of glioma, colorectal cancer, and ovarian cancer.[41,42] SPARC interacts with actin in skeletal muscle in vitro and in vivo, and appears to be an important modulator of the actin cytoskeleton, implicating maintenance of muscular function, which suggests a new role of SPARC during tissue remodeling.[43] A recent animal study demonstrated that SPARC played a role in maintaining the stiffness of skeletal muscle by regulating collagen accumulation, showing that accumulation of fibrillar collagen was significantly reduced in SPARC-null mice compared to WT mice after 5 months of age.[44] SPARC can also adjust the osteoblasts and osteoclasts to promote the growth of bone.[34] In osteoblasts of patients with osteogenesis imperfecta, expression of SPARC was significantly lower than in that of healthy controls. In SPARC-null mice, the numbers of osteoclasts and osteoblasts were significantly decreased.[45–47] It indicated that SPARC was one of the necessary factors in the phases of osteoblast formation, maturity, and survival. A newly published paper demonstrated that SPARC regulated the extracellular matrix mineralization of osteoblasts through P38 signaling pathway.[48] Jundt et al reported that in bone cells, SPARC was a marker of osteoblastic functional differentiation.[49]

In recent years, the complex interactions between the skeletal and immune systems have become more and more clear, and some investigators have verified that SPARC is one of the critical factors in both systems.[50] Several studies

indicated that SPARC was associated with some autoimmune diseases such as rheumatoid arthritis (RA), primary Sjogren's syndrome (PSS), and scleroderma. SPARC synthesis was increased in the synovial cells of RA and OA joints, and the levels of SPARC in synovial fluids of patients with RA were significantly elevated.[51] In addition, another study conducted by our team suggested that two SNPs (rs3210714 and rs1950384) of *SPARC* gene were associated with the pathogenesis of RA.[52] Strong expression of SPARC promoted calcinosis cutis in PSS and scleroderma.[53,54] Zhou et al first reported that polymorphisms of the *SPARC* gene were associated with susceptibility to systemic sclerosis (SSc) and its clinical manifestations.[55] Evidence showed that serum SPARC expression levels in AS patients accompanied with OP were significantly lower than that in healthy controls, and the correlation analysis showed that serum SPARC levels were negatively correlated with TNF-α, while positively correlated with L2-4BMD, TGF-β1 and BSAP.[56] In the present study, we first investigated the association between SPARC and the susceptibility to AS in a Chinese Han population by functional and genetic association studies.

Our study showed that two SNPs of *SPARC* gene were associated with susceptibility to AS. The major A allele, AA and AC genotype of rs17112187 was associated with an increased risk of AS. The minor G allele, GG and GA genotype of rs4958487 appeared to reduce the risk of AS. The haplotypes for both rs17112187 and rs4958487 were related to AS susceptibility. We also found that the expression of SPARC mRNA levels in PBMC and granulocytes of AS patients was significantly lower than in those of the healthy controls. Moreover, the serum level of SPARC protein was also significantly reduced in AS patients. Thus, we suppose that SPARC might be a protective factor in the pathogenesis of AS. To our knowledge, our study is the first to demonstrate that both SPARC gene and its protein are significantly associated with AS. Bone metabolism disorder is one of the pathological changes in AS, SPARC may be related to the bone metabolic disorder of AS.

There are some limitations in our study, the first is that our study just enrolled a small size of AS patients of Han population in a single center of local hospital, the result could not represent the whole Chinese population, more RCTs studies with larger sample size are needed to elaborate the association between SPARC and AS. Secondly, the present conclusion is just based on the initial SNP study of SPARC in AS patients, we need to do further research to determine the effect of SPACR on disease manifestations such as X-ray progression. Thirdly, when recruiting AS patients, we set the inclusion criteria as that who were first diagnosed without any treatment, we did not account for effect of treatments on our measurements. Therefore, whether the treatment of AS would affect the expression level of SPARC protein and the relationship between SPARC level and inflammatory markers still needs to be further elaborated.

Conclusions

Genetic polymorphisms and mRNA and protein expression of SPARC are associated with AS in a Chinese Han population. How the specific physiological process of SPARC influences AS is still not fully elucidated. Further studies are needed to figure out the specific pathway of SPARC in the pathogenesis of AS, so as to provide a better therapeutic strategy for AS.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Statement

The studies titled "Association between SPARC Polymorphisms and Expression and Ankylosing Spondylitis in a Chinese Han Population: A Case-Control Study" involving human participants were reviewed and approved by Shandong Provincial Hospital Affiliated to Shandong First Medical University. Patients/participants provided their written informed consent to participate in this study. Participants were assured of confidentiality and anonymity of information relating to the survey. This study was conducted according to the guidelines of the Declaration of Helsinki.

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Disclosure

The authors declare that there is no conflict of interest for this work.

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