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Correlation of PON1 polymorphisms with ankylosing spondylitis susceptibility
A case–control study in Chinese Han population

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Abstract

Paraoxonase 1 (PON1) modulates the oxidative stress and inflammatory response, thus, it might relate to the risk of ankylosing spondylitis (AS). The aim of present study was to discover the correlation of *PON1* polymorphisms (rs662 and rs854560) with PON1 activity and AS risk.

Around 128 AS patients and 146 healthy controls were recruited in this case–control study. *PON1* polymorphisms were genotyped by direct sequencing. Serum PON1 activity was detected and compared by nonparametric test in different genotypes of *PON1* polymorphisms. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to present the relative risk for AS.

GG genotype and G allele of rs662 polymorphism were closely correlated with enhanced AS risk ($P = .034$, OR $= 2.318$, 95%CI $= 1.051–5.113$; $P = .032$, OR $= 1.485$, 95%CI $= 1.033–2.135$). PON1 activity was obviously higher in controls than that in AS patients. Significant difference of PON1 activity has been discovered in the different rs662 genotypes ($P < .01$). rs662 GG genotype carriers had the lowest PON1 activity, followed by AG carriers and the AA carriers. Besides, no significant relationship existed between rs854560 genotypes and AS risk.

PON1 rs662 polymorphism is significantly correlated with increased AS risk via inhibiting PON1 activity.

Abbreviations: AS = ankylosing spondylitis, BASDAI = bath ankylosing spondylitis activity index, BASFI = bath ankylosing spondylitis functional index, CI = confidence interval, CRP = C-reactive protein, CT = computed tomography, HDL = high-density lipoprotein, HLA = human leucocyte antigen, HWE = Hardy–Weinberg equilibrium, MRI = magnetic resonance imaging, mSASSS = modified stoke ankylosing spondylitis spine score, OR = odds ratio, PON1 = paraoxonase 1, RA = rheumatoid arthritis, SNP = single nucleotide polymorphism.

Keywords: ankylosing spondylitis, polymorphisms, PON1

1. Introduction

Ankylosing spondylitis (AS), belonging to rheumatism, is a chronic autoimmune disease.[1] Approximately 90% AS patients present positive human leucocyte antigen-B27 (HLA-B27). Axial skeleton, sacroiliac joints, and spine attachment points are the mainly predilection sites of AS.[2] Abnormal immune response presenting in these parts often leads to stiffness and fibrosis in the spine, finally results in the activity disability of spine.[3] AS is one of the seronegative spondyloarthropathies, and usually occurs in young adults especially young men.[4] The onset of AS is generally hidden, and has no clinical features. In the early stages, clinical features for AS are not specific, which contributing to challenges for early detection. Many cases develop advanced stages when initially diagnosed, missing the optimal opportunity for treatment. Despite no danger in live, restricted active ability decreases the life quality for AS patients and takes a heavy toll for family and society.[5,6] Thus, to explore AS pathogenesis and improve management of the disease are pivotal for the cases. Previous studies indicated that AS was a complex disease which was affected by the combination of various genetic and environmental factors.[7–13]

Paraoxonase 1 (PON1) is an arylesterase which is dependent on Ca^{2+}. PON1 is mainly synthesized in liver, and secreted to the bloodstream after binding to high-density lipoprotein (HDL). PON1 plays a pivotal role in regulating esterase activity and peroxidase activity.[14,15] The activity of PON1 was reported to be significantly correlated with the oxidation in mammals.[16] Given the close association between oxidative stress and inflammation,[17] we speculated that PON1 might play a functional role in inflammatory disorders. Recent study suggested that PON1 impairment was observed in rheumatoid arthritis (RA) which could verify our deduction.[18] Accumulating evidences have suggested that the polymorphisms in *PON1* gene might influence the enzymatic activity of PON1, thus participating in initiation and development of diseases,[19] however, very few studies were carried out to explore the correlation between *PON1* gene polymorphisms and AS in Chinese population.

In the present study, we explored the effects of rs662 (c.575A>G, p.Gln192Arg) and rs854560 (c.163T>A, p.Leu55Met) single

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nucleotide polymorphisms (SNPs) in *PON1* gene coding region on serum PON-1 activity and AS susceptibility in Chinese Han population.

2. Materials and methods

2.1. Study subjects

This study was authorized by the ethnic committee of The Yongchuan Hospital of Chongqing Medical University. All of the subjects were Chinese Han population and signed the written informed consent.

Between January 2014 and June 2016, AS patients who were diagnosed by x-ray, magnetic resonance imaging (MRI), computed tomography (CT), and laboratory inspections in The Yongchuan Hospital of Chongqing Medical University were recruited as cases. Healthy individuals who received a medical examination in the same hospital were recruited as controls. Controls were matched with the cases in age and gender. All of the participants had no other bone diseases, systemic diseases, and immune or inflammatory diseases in recent 3 months.

2.2. Sample collection and genotyping method

About 10 ml blood sample was collected from elbow vein of every participant. Blood samples were anticoagulated by EDTA-Na$_2$, centrifuged, and collected white blood cells and serum, then stored in $-80\,°C$ until to use.

Genomic DNA was extracted from blood cells using a DNA extraction kit (Tiangen, Beijing). *PON1* gene rs662 and rs854560 polymorphisms were amplified by PCR and genotyped by direct sequencing.

2.3. Serum PON1 activity

Serum PON1 activity was examined according to previous study.[20]

2.4. Statistical analysis

Hardy–Weinberg equilibrium (HWE) test of the *PON1* polymorphisms was performed by PLINK. Quantitative data were presented by mean$\pm$SD. Quantitative and qualitative data between cases and controls were, respectively, compared by t test (or nonparametric test) and χ^2 test (or Fisher's exact test). Differences of genotype and allele frequencies between case and control groups were assessed by χ^2 test. Relative risk of *PON1* polymorphisms with AS risk was revealed by odds ratios (ORs) with 95% confidence intervals (CI). Independent-samples t test was utilized to explore the association of *PON1* polymorphisms with plasma PON1 activity. All of the calculations were performed by SPSS 18.0 software. Significant level was set to 0.05.

3. Results

3.1. Basic and clinic features

The case group included 97 males and 31 females with the mean age of 29.90 ± 5.68 years old. Healthy controls contain 111 males and 35 females, and their mean age was 30.49 ± 6.73 years. The HLA-B27, C-reactive protein (CRP) level, and PON1 activity were significantly different between cases and controls (Table 1, $P<.001$ for all). While other features had no significant difference between case and control groups ($P>.05$ for all).

Table 1

Basic and clinic features for subjects.

Features	Case n = 128 (%)	Control n = 146 (%)	*P*
Age (mean ± SD)	29.90 ± 5.68	30.49 ± 6.73	.707
Gender			.962
Male	97 (75.78)	111 (76.03)	
Female	31 (24.22)	35 (23.97)	
Smoking			.185
Yes	36 (28.13)	31 (21.23)	
No	92 (71.88)	115 (78.77)	
Alcohol abuse			.147
Yes	13 (10.16)	8 (5.48)	
No	115 (89.84)	138 (94.52)	
Exercise habit			.244
Yes	43 (33.59)	59 (40.41)	
No	85 (66.41)	87 (59.59)	
Family history			.053
Yes	6 (4.69)	1 (0.68)	
No	122 (95.31)	145 (99.32)	
HLA-B27			<.001
Positive	106 (82.81)	17 (11.64)	
Negative	22 (17.19)	129 (88.36)	
CRP, mg/L	19.34 ± 5.08	4.74 ± 1.85	<.001
PON1 activity, U/L	211.65 ± 37.89	234.23 ± 42.50	<.001
Kyphosis			
Positive	55 (42.97)	—	
Negative	73 (57.03)	—	
Bamboo spine			
Positive	39 (30.47)	—	
Negative	89 (69.53)	—	

CRP = C-reactive protein, HLA = human leucocyte antigen, PON1 = paraoxonase 1.

3.2. Association of PON1 polymorphisms with AS risk

Genotype and allele distributions of *PON1* rs662 and rs854560 polymorphisms in control group did not deviate from the HWE test (Table 2, $P>.05$). This result indicated that subjects in present study could represent the general population.

Frequencies of rs662 AA, AG, GG genotype were 8.59%, 38.28%, 53.13% in AS patients and 16.44%, 39.73%, 43.84% in healthy controls, respectively. GG genotype of rs662 SNP had higher frequency in AS patients, and might be significantly

Table 2

Association of PON1 polymorphisms with AS risk.

Genotype/ allele	Case n = 128 (%)	Control n = 146 (%)	*P*	OR (95%CI)
rs662				
AA	11 (8.59)	24 (16.44)	—	—
AG	49 (38.28)	58 (39.73)	.135	1.843 (0.821–4.138)
GG	68 (53.13)	64 (43.84)	.034	2.318 (1.051–5.113)
A	71 (27.73)	106 (36.30)	—	—
G	185 (73.27)	186 (63.70)	.032	1.485 (1.033–2.135)
P_{HWE}	0.611	0.088		
rs854560				
AA	120 (93.75)	137 (93.84)	—	—
AT	8 (6.25)	9 (6.16)	.977	1.015 (0.380–2.713)
TT	0	0	\	\
A	248 (96.88)	283 (96.92)	—	—
T	8 (3.12)	9 (308)	.977	1.014 (0.385–2.669)
P_{HWE}	0.715	0.701		

HWE = Hardy–Weinberg equilibrium.

Genotype	Case n = 128 (%)	Control n = 146 (%)
rs662		
AA	247.45 ± 23.71	261.58 ± 36.66
AG	218.47 ± 32.63*	231.53 ± 41.61*
GG	200.94 ± 38.91*,†	226.42 ± 41.80*,†
rs854560		
AA	211.67 ± 37.80	233.58 ± 42.12
AT	211.37 ± 41.94	244.11 ± 49.69

* Compared with AA genotype $P < .01$.
† Compared with AG genotype, $P < .01$. Significant level was adjusted by the Bonferroni method.

associated with increased AS risk ($P = .034$, OR = 2.318, 95% CI = $1.051 - 5.113$). A and G allele of rs662 SNP were 27.73% and 73.27% in cases, 36.30% and 63.70% in controls. Significantly difference of rs662 allele frequencies was observed between case and control groups, indicating G allele might distinctly increase the AS risk ($P = .032$, OR = 1.485, 95% CI = 1.033–2.135). For rs854560 SNP, no TT genotype was observed in AS patients and healthy controls. Only 8 (6.25%) and 9 (3.08%) AT genotypes were respectively discovered in case and control groups. The AT genotype and T allele had no significant association with AS risk.

3.3. Correlation of PON1 polymorphisms with PON1 activity in AS patients

PON1 activity was significantly higher in controls than that in cases (Table 1, $P < .001$). PON1 gene rs662 AA, AG, GG genotypes showed obvious association with PON1 activity (Table 3, $P < .01$). PON1 activity was highest in rs662 A allele homozygote, the least in G allele homozygote. However, the PON1 activity had no significant difference between rs854560 genotypes. These results suggested that rs662 G allele was distinctly correlated with decreased PON1 activity.

3.4. Relationship between rs662 genotypes and AS activity

In AS patients, GG genotype carriers exhibited the highest BASDAI, BASFI, and mSASSS activity scores than AA genotype carriers (Table 4). But the association did not exist between rs662 AA and AG genotypes ($P > .05$ for all).

4. Discussion

Smoking status, alcohol abuse, as well as exercise habit are confirmed as risk factors for AS. However, not all of the individual exposed in risk environment will suffer from AS. As an autoimmune disease, AS is attributed by the interactions of various genetic and environmental factors especially the ingredients of inflammatory system (such as immune cells and

Activity score	AA	AG	GG
BASDAI	4.19 ± 1.56	4.59 ± 1.41	5.14 ± 1.65*
BASFI	3.61 ± 1.59	4.24 ± 1.49	4.95 ± 1.66*
mSASSS	11.49 ± 12.01	12.67 ± 11.87	13.87 ± 12.06*

BASDAI = bath ankylosing spondylitis activity index, BASFI = bath ankylosing spondylitis functional index, mSASSS = modified stoke ankylosing spondylitis spine score.

cytokines). For example, HLA-B27 is more frequently detected in AS patients, and the level of CRP exhibits high in AS cases. Besides, family history is also considered as a risk factor for AS development, revealing the strong association between genetic factors and AS risk.

PON1, as the first member of PON family, could modulate the oxidative stress and inflammatory responses.[21] In addition, PON1 has been confirmed to be correlated with several immune and inflammatory diseases, including AS.[22–24]PON1 gene is located in chromosome 7q21.3, including 9 exons. Polymorphisms in the coding region of the gene might alter the structure and function of the protein. Two widely studied SNPs in the exons of PON1 gene, rs662 (c.575A>G, p.Gln192Arg) and rs854560 (c.163T>A, p.Leu55Met) were associated with the PON1 activity and diseases susceptibility in several populations.[25,26] Previous study showed that AS patients exhibited significantly decreased PON1 activity, and its decreased enzymatic activity was negatively associated with the disease duration.[27] Thus we speculated that these two SNPs might influence the development of AS.

In the present study, rs662 GG genotype was significantly associated with 2.318 times increased AS risk. Rs662 G allele approximately increased 1.485 times AS risk. This result was accorded with the previous studies. PON1 rs662 SNP was significantly related to RA risk.[28] This SNP showed close association with the RA risk, especially the oxidative stress and some systemic inflammatory markers.[29] In the current study, PON1 rs854560 polymorphism showed no association with AS susceptibility.

A microarray analysis based on whole blood samples collected from 18 AS cases and 18 healthy individuals (GEO: GSE25101) demonstrated that the expression pattern of PON1 was obviously different between AS and healthy controls.[30] Previous studies showed that PON1 activity in serum was significantly decreased in RA and AS patients.[27,31] In the current study, we found that serum PON1 activity was significantly different between AS cases and healthy individuals. Moreover, decreased PON1 activity was observed in serum samples collected from AS patients It was reported that the concentration and activity of PON1 were significantly determined by rs662 SNP in diabetes patients.[20] In AS patients, we found that GG genotype of PON1 rs662 SNP significantly elevated the PON1 activity. Whereas G heterozygote also increased the PON1 activity both in cases and controls. It is indicated that rs662 SNP promote the AS susceptibility might via regulating the PON1 activity. A recent study indicated showed that rs662 SNP was correlated with PON1 activity in rheumatoid arthritis cases.[18] In addition, we also analyzed the effects of PON1 rs662 polymorphism on AS activity. The results demonstrated that AS cases carrying GG genotype were more likely to undergo worse scores. Based on the above researches, we deduced that GG genotype of PON1 rs662 polymorphism could suppress the enzymatic activity of PON1, thus contributing to initiation and development of AS. However, the correlation between PON1 activity and clinical manifestations were not investigated in this study. Further researches will be required.

To sum up, rs662 G allele is positively associated with AS risk via regulating PON1 activity. Although we obtained a meaningful result, several limitations should not be omitted. First, small sample size of this study might lead to a lower test power. Second, this study was not verified by other ethnicity. Third, all of the results were not adjusted by confounding factors. Fourth, interactions of gene–gene and gene–environment were not

researched in current study. Finally, all of the pathogenesis of PON1 for AS risk was not explored in this study. Therefore, further studies with large sample size and well designed contents should be performed to address the issues.

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