Association of an asporin repeat polymorphism with ankylosing spondylitis in Han Chinese Population: A case-control study

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\textsuperscript{Manuscript submitted 9th December, 2009
Manuscript accepted 14th January, 2010


Abstract

Objective: To investigate the role of a functional polymorphism consisting of an aspartic acid (D) repeat located in the asporin gene (\textit{ASPN}) gene in the susceptibility to and clinical outcome of ankylosing spondylitis (AS).

Methods: A total of 374 Chinese patients with ankylosing spondylitis and 421 controls of the same ethnic origin matched for age and sex were included in the study. The asporin D repeat polymorphism was genotyped by polymerase chain reaction with a fluorescent primer.

Results: Significant differences between AS patients and controls were detected in the distribution of the 7 alleles found in our population. D14 and D16 alleles were significantly over-represented in AS patients (D14, \textit{P}=0.001, odds ratio (OR)=1.857, 95\% confidence interval (CI) 1.27-2.715; and D16, \textit{P}< 0.0001, OR=2.605, 95\% CI 1.75-3.879). D16 over-representation was more common in early-onset patients than in late-onset patients, although the difference did not reach significance (\textit{P}= 0.071).

Conclusion: The results support a role for an asporin D repeat polymorphism in the susceptibility to AS and an influence of this gene on the outcome of the disease. D14 and D16 allele variants of \textit{ASPN} might be the susceptibility alleles for AS in the Han Chinese population, whereas the D13 allele variant may have a protective effect on the onset of AS.

Ankylosing spondylitis (AS) is the second most common form of inflammatory arthritis worldwide with a prevalence ranging from 0.2\% to 1.1\%.\textsuperscript{1, 2} Disease susceptibility is clearly attributable to genetic factors, because the sibling recurrence risk ratio is 82,\textsuperscript{3} and twin- based studies estimate that disease heritability exceeds 90\%.\textsuperscript{4} HLA–B27, and its association with AS discovered more than 20 years ago, was a breakthrough in our understanding of this inflammatory condition. However, there is considerable evidence that susceptibility to AS is more complex than was initially thought. Many genetic and environmental factors have been suggested to play roles in the development of AS.\textsuperscript{5} Among these is the asporin, a novel member of the leucine-rich repeat protein family closely related to decorin and biglycan.\textsuperscript{6, 7} Asporin contains a putative propeptide, 4 amino-terminal cysteines, 10 leucine-rich repeats, and 2 C-terminal cysteines. However, asporin is different from decorin and biglycan in that it is not a proteoglycan and it contains a unique stretch of aspartic acid residues in its amino-terminal region encoded by a microsatellite polymor-
phism. Kizawa et al. first reported a strong association of the asporin gene (ASPN) with knee and hip osteoarthritis (OA) in the Japanese population. Together with convincing functional evidence, they showed that the D14 allele (an allele containing 14 D-repeats) was over-represented, and the D13 allele was under-represented in OA. Torres et al. were the first to investigate the potential genetic role of an asporin D repeat polymorphism in rheumatoid arthritis (RA). Their work did not support a major role for the asporin repeat polymorphism in the susceptibility to RA, but they supported an influence of D13 and D14 on the outcome of the disease. The purpose of the present study was to assess the association of the D-repeat polymorphism in asporin with AS in the Han Chinese population.

Patients and Methods

Subjects and protocol

A total of 374 patients (36 women and 338 men) meeting the American College of Rheumatology modified New York Criteria for AS were recruited from Department of Rheumatology and Immunology, Shandong Provincial Hospital. The diagnosis of AS was established according to the clinical information collected at the same time. The control group was made up of 421 normal individuals who underwent a physical examination in Health Center of Shandong Provincial Hospital. They had no previous medical history and no abnormal laboratory results. All subjects (cases and controls) were of Chinese origin matched for age and sex. Blood samples were obtained after the subjects provided written informed consent. This study was approved by the ethics review committee of Shandong Provincial Hospital.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using TIANGEN Genomic DNA Purification Kit (Beijing, China) according to the manufacturer’s instructions. The D repeat region was amplified using the forward primer 5’-FAM- CCCTTCTTT AGCCCTTCACAC-3’(forward) and 5’-CACTGACA TCCAAATGGACAC-3’(reverse). The polymerase chain reaction (PCR) was carried out with a mixture consisting of 50 ng of genomic DNA and 10×PCR buffer (Mg2+) 2 µl, 1.6 µl of each dNTP, 2.5 µl of each primer, 0.1 µl of Taq DNA polymerase and double distilled water up to a final volume of 20 µl. PCR was performed in a Gene Amp PCR System 9700 (ABI, Foster City, CA, USA) as follows: 30 cycles consisting of 1 min of denaturation at 94°C, 1 min of annealing at 60°C and 1 min of extension at 72°C with an initial denaturation step of 5 min at 94°C and a final extension of 30 min at 72°C. We separated 5’-FAM-labeled PCR products containing the D-repeat polymorphisms of asporin by size on an ABI Prism 3100-Avant Genetic Analyzer.

Statistical analysis

Genotypic and allelic frequencies were obtained by direct counting. Statistical analysis to compare distributions was performed by the $\chi^2$ test. Stratification analysis by sex and the age at onset was performed using SPSS 15.0 system software. Odds ratio (OR), P value and 95% confidence interval (CI) were calculated with respect to the minor allele compared with the major allele. We tested the difference of the age at onset of AS in the D genotypes using Mann–Whitney and Kruskal–Wallis tests. We also applied a survival analysis using the Kaplan–Meier method and log-rank test for the date.

Results

The ages of the patients and the controls (mean ± SD) were 23.98±15.2 (range 16–55) years and 26.23±16.7 (range 17–69) years, respectively. There was no statistical difference between the two groups. Seven different alleles were identified, containing 12–18 D repeats. The most common allele was D13 in both patients and controls. D17 was detected only in the
control group not in the AS group while D18 was detected only in the AS group (Table 1). There were 16 genotypes. Distributions of genotypes in the AS and control groups were conformed to Hardy–Weinberg equilibrium (P=0.241 and P=0.473, respectively). A significant difference in the allelic frequency was observed in comparison of D14 and D16 versus other alleles combined (P=0.001 and P<0.0001; Table 2). The association was also found in male group after stratification by sex (P=0.004 and P=0.001). No significant association was detected for D13 versus all the remaining alleles combined.

A significant difference was observed in genotype D13D16 and genotypes including D14 versus others (P=0.003 and P=0.010). There was a significant correlation between D13D16, D14D14 and hip involvement (P<0.0001 and P<0.0001). D16 was more frequent in early-onset patients than in late-onset patients. The age at onset in patients with D16 allele (15.24±5.24 years) was younger than that in those

TABLE 1. Allelic frequency of the aspartic acid (D)-repeat polymorphism of asporin in the AS in a Han Chinese population

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>No. of alleles (%)</th>
<th>D12</th>
<th>D13</th>
<th>D14</th>
<th>D15</th>
<th>D16</th>
<th>D17</th>
<th>D18</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td></td>
<td></td>
<td>374</td>
<td>90(12.03)</td>
<td>467(62.43)</td>
<td>74(9.89)</td>
<td>31(4.14)</td>
<td>82(10.82)</td>
<td>0(0)</td>
<td>4(0.53)</td>
</tr>
<tr>
<td>Male</td>
<td>338</td>
<td></td>
<td>82(12.13)</td>
<td>418(61.83)</td>
<td>63(9.32)</td>
<td>31(4.59)</td>
<td>7911.6900</td>
<td>0(0)</td>
<td>3(0.44)</td>
<td>676</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td></td>
<td>8(11.11)</td>
<td>49(68.06)</td>
<td>11(15.28)</td>
<td>0(0)</td>
<td>3(4.17)</td>
<td>0(0)</td>
<td>1(1.39)</td>
<td>72</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td>421</td>
<td>143(16.98)</td>
<td>558(66.27)</td>
<td>47(5.58)</td>
<td>36(4.28)</td>
<td>38(4.51)</td>
<td>20(2.38)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Male</td>
<td>376</td>
<td></td>
<td>129(17.15)</td>
<td>497(66.09)</td>
<td>40(5.32)</td>
<td>34(4.52)</td>
<td>33(4.39)</td>
<td>19(2.53)</td>
<td>0(0)</td>
<td>752</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td></td>
<td>16(16.67)</td>
<td>61(67.78)</td>
<td>7(7.77)</td>
<td>2(2.22)</td>
<td>5(5.56)</td>
<td>1(1.11)</td>
<td>0(0)</td>
<td>90</td>
</tr>
</tbody>
</table>

TABLE 2. Association of the D-repeat of asporin in patients with AS in the Han Chinese population

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>D14 vs. others</th>
<th>D16 vs. others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P</td>
</tr>
<tr>
<td>All patients</td>
<td>1.857</td>
<td>0.001</td>
</tr>
<tr>
<td>Male patients</td>
<td>1.829</td>
<td>0.004</td>
</tr>
<tr>
<td>Female patients</td>
<td>2.138</td>
<td>0.131</td>
</tr>
<tr>
<td>All patients</td>
<td>1.024</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male patients</td>
<td>1.026</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female patients</td>
<td>1.011</td>
<td>0.372</td>
</tr>
</tbody>
</table>

FIGURE 1. Survival function of patients with the D16 allele and without the D16 allele. Average onset age of AS in patients with D16 allele (n = 82) tended to be earlier (P=0.071) than without D16 patients (n = 292).
without D16 (19.66±7.12 years) although the difference did not reach significance (P=0.071, Fig 1). A survival analysis using the Kaplan–Meier method showed no significant difference in a dominant mode of D16 (P=0.1).

Among the 374 AS patients, the frequencies of D13D16 and D14/- genotypes were significantly higher than those in the control group (17.8% vs 7.62%, P=0.000 and 59.8% vs 20.74%, P<0.0001). Anterior uveitis was the most common extra-articular manifestation in D14/- genotypes (60.5% vs 7.38%, P=0.000). Severe sacroiliac erosion was more frequently seen in D13D16 genotypes (78.68% vs 48.75%, P<0.0001), whereas mild sacroiliitis was more common in D13D13 versus other genotypes (56.03% vs 49.32%, P=0.042).

Discussion

Asporin, a new member of the class I small leucine-rich repeat proteoglycan (SLRP) family, was first described by Lorenzo in 2001. Asporin contains a putative propeptide, 4 amino-terminal cysteines, 10 leucine-rich repeats, and 2 C-terminal cysteines, but it is distinct from the other two members of class I SLRPs, decorin and biglycan, by possessing a unique stretch of aspartate residues at its N terminus. Some studies have confirmed that since its N terminus aspartate repetitive sequence is close to a Zinc finger protein binding site, polymorphisms might lead to protein architectonic arrangement, and an increase in the frequency of such polymorphisms might be associated with progressive cartilage degeneration.

Studies on the association of asporin and inflammatory arthritis began only 4 years ago, when in 2005 Kizawa et al. first identified a variant of ASPN as a susceptibility factor for knee and hip OA in the Japanese population. Subsequently, there have been various studies on its association with OA in different ethnic groups but the results have been inconsistent. In Japanese and Han Chinese populations, D14 allele and D13 allele were reported to have a susceptibility and protective effect for OA, respectively, whereas in European and US Caucasians such results were not replicated. The Greek study found D15 instead of D14 as a risk allele for OA, and in the study of UK Caucasians, D14 was only found to be over-represented in male patients who underwent hip replacement. In 2007, Torres et al. first investigated the association of the D-repeat polymorphism in asporin with RA, supporting a role for D14 and D13 in the outcome of RA. These data suggested that ASPN was involved in the immuno-pathogenesis of RA, which might influence the prognosis of this disease.

In this study, we first investigated the genetic association of the aspartic acid (D) repeat of asporin with AS in the Han Chinese population. Our data showed the frequencies of D14 and D16 were significantly higher in AS patients than in controls, suggesting that D14 and D16 might be susceptibility alleles for AS. However, when the data was stratified by gender, the significance was only observed in male comparison groups, indicating the roles of D14 and D16 as risk alleles for AS might be different in males and females. Genotype analysis showed the frequencies of D13D16 and genotypes including D14 (D14homozygote, D14D15 and D14D16) were significantly higher in AS patients than in controls, and also higher in those with hip joints involvement than in those without. Our data also suggest that the genotypes D13D16 and D14 might be predictors of a worse prognosis for AS. For the study of ASPN and age at onset of AS, patients homozygous for D13 had a later onset of disease and less destruction of cacroiliac joint compared with patients harboring other genotypes, suggesting that D13 had a protective effect for AS. Our study is in agreement with the studies on OA and RA. Moreover, we detected four patients carrying the D18 allele in the AS group, but due to the limited
number, it is not clear whether D18 is a risk allele as it was found to be in the study on knee OA in the Greek population. Further studies should be conducted with larger sample size in different ethnic groups to address this issue.

In vitro assays have shown that asporin binds specifically to transforming growth factor-beta (TGF-β), leading to the negative regulation of chondrogenesis. TGF-β is a cytokine that can promote protein synthesis and cell proliferation in the articular cartilage, thus is of great relevance to cartilage regeneration. TGF-β can also inhibit the activity of matrix metalloproteinase which plays an important role in the digestion of the extracellular matrix (ECM) in both normal and degenerative articular cartilage. In a mouse model, injection of TGF-β into knee joints resulted in increased synthesis of articular cartilage and accumulation of proteoglycan. In another murine OA model, treatment with soluble TGF-β Type II receptor, which acts as a TGF-β antagonist, led to a decrease in articular cartilage proteoglycan and cartilage thinning. Moreover, interference with the TGF-beta/Smad signaling pathway might induce progressive loss of cartilage and a decrease in proteoglycan. Further evidence from Nakajima et al has shown that asporin binds to TGF-β, and inhibits the TGF-β/Smad signaling pathway and chondrogenesis forming a functional feedback loop with TGF-β1 and regulating its chondrogenic potential. Jaakkola et al. reported in 2004 a weak association between the TGF-β1 +1632 polymorphism and AS in the Finnish population. Taken together, these data demonstrate asporin and TGF−β1 play a critical role in joint disease associated with AS.

In conclusion, our study suggests that ASPN, which is involved in the joint degeneration process, might be one of the predisposing genes for AS. D14 and D16 allele variants of ASPN are associated with AS progression and prognosis, whereas D13 allele variant may have a protective effect on AS.

Acknowledgments

This work was supported by the Shandong Provincial Nature Science Foundation of China (QZ2007C09 and 2008GG10002009) and Shandong Provincial Medical Foundation of China(2007QZ014).

References


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