Association of the aspartic acid-repeat polymorphism in the asporin gene with age at onset of knee osteoarthritis in Han Chinese Population

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Abstract Recent genetic studies for osteoarthritis (OA) have been focused on *ASPN*, the gene encoding asporin, where aspartic acid (D)-repeat polymorphisms are associated with OA in several ethnic groups. The purpose of the present study is to further examine the association of the D-repeat polymorphism in a Han Chinese population. The D-repeat polymorphism was genotyped in 354 knee OA patients (257 women and 97 men) who suffered from primary symptomatic knee OA with radiographic confirmation, and the association of the repeat with various clinical parameters was examined. The age at onset (years) in patients with the D14 allele (mean: 51.9, SD: 8.5) was younger than that those without the D14 allele (mean: 54.9, SD: 10.9) ($P = 0.023$). Survival analysis showed D14 was significantly associated with age at onset of OA ($P = 0.004$ in the dominant model). The average age at onset of patients with

the D13/D13 genotype (mean: 56.1, SD: 11.1) was older than those without the D13/D13 genotype (mean: 53.0, SD: 9.9) ($P = 0.013$). Our study further highlighted the significance of asporin in OA.

Keywords Asporin · Association · Knee osteoarthritis · Age at onset · Han Chinese

Introduction

Osteoarthritis (OA, OMIM 165720) is the most prevalent form of arthritis. A recent study in China show that the prevalence of radiographic knee OA increased in proportion to age: 13.5, 41.9, 64.1, and 71.4% for the 40-, 50-, 60-, and 70-year-old age groups, respectively (Du et al. 2005). The risk of disability in mobility in knee OA is greater than any other diseases in the people age ≥ 65 years (Hunter et al. 2006). Genetic factors have been implicated in its etiology (Ikegawa et al. 2003; Loughlin 2005) and several susceptibility genes with reasonable functional significance have been reported (Loughlin et al. 2004; Kizawa et al. 2005; Mototani et al. 2005; Miyamoto et al. 2007).

Recent genetic studies for OA have been focused on *ASPN*, the gene encoding asporin (Kizawa et al. 2005; Mustafa et al. 2005; Kaliakatsos et al. 2006; Rodriguez-Lopez et al. 2006; Ikegawa et al. 2006; Jiang et al. 2006). Asporin is a new member of the small leucine-rich proteoglycan family (Lorenzo et al. 2001; Henry et al. 2001). Asporin has consecutive aspartic-acid residues (D-repeat) in the amino-terminal region of its mature protein encoded by a microsatellite polymorphism. Kizawa et al. (2005) first reported a strong association of *ASPN* with knee and hip OA in Japanese. Together with convincing functional evidence, they show that the D14 allele (an allele

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containing 14 D-repeats) is over-represented, and the D13 allele is under-represented in OA. The replication of association was not clear in the subsequent studies in European Caucasians (Mustafa et al. 2005; Kaliakatsos et al. 2006; Rodriguez-Lopez et al. 2006), but was definitely positive in the Han Chinese population (Jiang et al. 2006).

The purpose of the present study is to further examine the association of the D-repeat polymorphism in asporin for various clinical parameters in the Han Chinese population. We found the significant association of D-repeat with age at onset of OA, which has not been reported in the previous studies.

Patients and methods

Subjects, DNA samples and genotyping

A total of 354 knee OA patients (257 women and 97 men) were enrolled consecutively at Center of Diagnosis and Treatment for Joint Disease, Drum Tower Hospital, affiliated to Medical School of Nanjing University. All subjects included in the study were Han Chinese who are living in and around Nanjing. The inclusion and exclusion criteria were as described previously (Jiang et al. 2006). Clinical information including age, sex, body mass index (BMI), and age at onset were collected by participating doctors from the subjects at their visits to the hospital. Age at onset is defined as the year when patients began to have not only definite signs and symptoms of OA, but also radiographic evidence. No subjects dropped out during the process of the study. The study protocol was approved by an ethical committee of Medical School of Nanjing University. A written informed consent was obtained from patients. Extraction of genomic DNA and genotyping of the *ASPN* D-repeat polymorphisms were as described previously (Jiang et al. 2006).

Statistical analysis

Association of the *ASPN* D-repeat polymorphisms was tested for asporin genotypes by stratification with a variety of clinical information. χ^2 test was used to assess the

Hardy-Weinberg equilibrium. Mann-Whitney and Kruskal-Wallis tests were used in this study. We also applied survival analysis using Kaplan-Meier method and log-rank test for the data.

Results

The age of the knee OA patients (mean $\pm$ SD) was 60.6 ± 10.9 (range 40–89) years and the age at onset (mean $\pm$ SD) was 54.4 ± 10.6 (range 18–77) BMI of the patients was 26.6 ± 6.8 kg/m². Over 60% of patients had a K/L score of three or four. Seven different alleles containing from 11 to 17 D-repeats were identified, which composed 17 genotypes. Frequencies of alleles were shown in Table 1. Frequencies of genotypes were similar to the previous study (Jiang et al. 2006). Distributions of genotypes were conformed to Hardy-Weinberg equilibrium ($P = 0.98$).

To evaluate the effect of *ASPN* for the age at onset of knee OA, we examined the difference of the age at onset of knee OA between the genotypes using the Mann-Whitney and Kruskal-Wallis tests. The age at onset in patients with the D14 allele (mean: 51.9 years, SD: 8.5) was younger than that those without the D14 allele (mean: 54.9 years, SD: 10.9) ($P = 0.023$). We also applied survival analysis using the Kaplan-Meier method and the log-rank test for the date (Table 2 and Fig. 1). The log-rank test showed significant difference between the D-repeat genotypes (in the dominant mode: $P = 0.004$, in the genotype mode: $P = 0.016$). When stratified by gender, similar results were found in female patients (dominant mode: $P = 0.007$, genotype mode: $P = 0.026$). The age at onset years in female patients with the D14 allele (mean: 51.8, SD: 7.7) was younger than that those without the D14 allele (mean: 54.8, SD: 10.6). No significant results were detected in male samples ($P > 0.05$) and in stratification by age, K/L grade, and BMI in all subjects (all $P > 0.05$). When we focused on survival functions of patients with and without D13/D13 genotype, significant difference of onset age was detected by log-rank test ($P = 0.001$). The average age at onset of patients with D13/D13 genotype (mean: 56.1, SD: 11.1) was older than those without D13/D13 genotype (mean: 53.0, SD: 9.9), and a statistically significant difference

Table 1 Allelic frequency of the aspartic acid (D)-repeat polymorphism of asporin in knee osteoarthritis (KOA) in a Han Chinese population

Group	No. of subject	No. of allele (%)							
		D11	D12	D13	D14	D15	D16	D17	Total
All	354	1 (0.1)	116 (16.4)	477 (67.3)	62 (8.8)	16 (2.3)	28 (4.0)	8 (1.1)	708
Female	257	1 (0.2)	87 (16.9)	341 (66.4)	46 (8.9)	12 (2.3)	21 (4.1)	6 (1.2)	514
Male	97	0	29 (14.9)	136 (70.1)	16 (8.3)	4 (2.1)	7 (3.6)	2 (1.0)	194

Table 2 The results of analysis for the age at onset

Allele	Mode of inheritance	Allele 1 ^a		Both ^a		Allele 2 ^a		P-value	
		Mean	SD	Mean	SD	Mean	SD	Non-parametric test	Log-rank test
D13	Dominant	54.8	10.8	–	–	51.7	8.4	0.105	0.018
D13	Recessive	56.1	11.1	–	–	53.0	9.9	0.013	0.001
D13	Genotype	56.1	11.1	52.3	10.2	51.7	8.4	0.031	0.001
D14	Dominant	51.9	8.5	–	–	54.9	10.9	0.023	0.004
D14	Recessive	53.5	2.1	–	–	54.4	10.6	0.959	0.593
D14	Genotype	53.5	2.1	51.9	8.6	54.9	10.9	0.072	0.016
D12	Dominant	52.6	10.5	–	–	55.2	10.5	0.085	0.040

^a If the mode of inheritance is dominant, allele 1 means genotype with target allele and allele 2 means genotype without target allele. If the mode of inheritance is recessive, allele 1 means homozygote of target allele and allele 2 means genotypes except for homozygote of target allele. If the mode of inheritance is genotype, allele 1, both, allele 2 mean homozygote of target allele, heterozygote of target allele, genotype without target allele, respectively

Fig. 1 Survival functions of patients with the D14 allele and without the D14 allele (**a**), and of those with D13/D13 genotype and without D13/D13 genotype (**b**). **a** Average onset age of patients with D14 allele ($n = 60$) was significantly earlier ($P = 0.004$) than without D14

patients ($n = 294$). **b** Average onset age of patients with D13/D13 genotype ($n = 160$) was earlier ($P = 0.001$) than patients without D13/D13 genotype ($n = 194$)

was found ($P = 0.013$). No significant differences were detected when we focused on other alleles of *ASPN* (all $P > 0.05$, shown in the Table 2).

Discussion

Not only susceptibility to disease occurrence of OA, but also various aspects of OA, including its progression, severity, and response to certain treatment can be associated with genetic determinant(s). In the present study, we found that the D-repeat polymorphism in *ASPN* is associated with the onset of knee OA in Han Chinese population. Patients with D14 allele, the susceptibility allele had earlier onset, which is consistent with its suspected pro-OA function in our previous studies (Kizawa et al. 2005; Nakajima et al. manuscript in submission). Our data also suggest the protective effect of D13 allele for onset of OA.

However, functional study does not support it, as the inhibitory effect of D13 allele on TGF- β function is not lower than other alleles except for the D14 allele. Later onset of OA in D13 homozygotes may be just the reflection of their not having the D14 allele. Valdes et al. (2007) detected several important candidate gene associations with OA and the fact that many of these associations were strongly dependent on sex and ethnicity and were often probably site specific. We also detected different results in stratification by sex when age at onset in patients with or without the D14 allele was compared. Just as in the susceptibility to the disease, the present result also needs replication in other populations, preferably of different ethnic origins. Well-designed, prospective studies will answer the question.

ASPN is the second gene that associated with age at onset of knee OA. Williams et al. (1993) found that the type II pro-collagen gene (*COL2A1*) is associated with

severe, early-onset knee OA. We found no association of *ASPN* with severity of OA, neither in stratification by age, K/L grade, BMI. *ASPN* is also reported to be associated with clinical outcome of rheumatoid arthritis (Torres et al. 2007). Individuals with D14 produced rheumatoid factor more often than the rest. The age of onset is higher in the group of individuals harboring D13 allele compared with the rest ($P = 0.001$). TGF- β is a key growth factor for bone and joint metabolism. The supposed *ASPN* function to the pathogenesis of OA, excessive inhibition of TGF- β can be applicable to other bone and joint diseases. *ASPN* may influence outcome of a broad spectrum of bone and joint diseases.

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