Investigation of the Asporin Gene Polymorphism as a Risk Factor for Knee Osteoarthritis in Iran

Roshanak Jazayeri, MD, Mohammad Qoreishi, MD, Hamid Reza Seyyed Hoseinzadeh, MD, Mojgan Babanejad, MS, Enayatollah Bakhshi, PhD, Hossein Najmabadi, PhD, and Seyyed Mohammad Jazayeri, MD

Abstract

Osteoarthritis (OA) is a degenerative disease of the joints characterized by degradation of the hyaline articular cartilage and remodeling of the subchondral bone with sclerosis. The asporin (*ASPN*) gene encodes a cartilage extracellular protein belonging to the small leucine-rich proteoglycan family. Polymorphisms in the aspartic acid (D) repeat region in the second exon of this gene, which consist of *GAT* repeats, are associated with OA susceptibility. The D14 allele, which contains 14 D-repeats, is associated with increased OA susceptibility in the Japanese and the Han Chinese but is not an important factor in OA etiology among Caucasians, though the D15 allele is a risk allele for the Greek population.

To examine the possibility of this controversial association, we explored the effect of *ASPN* on Iranians with knee OA. The D-repeat polymorphism was genotyped in 100 knee OA patients and 100 controls, and the allelic association of the D-repeat was examined. Our data suggest that the D15 allele could be considered a risk allele significant only for women (P = .045, odds ratio = 1.73, 95% confidence interval [CI] = 1.01-2.94) in the Iranian population. This association is in part similar to that found for the Greek population.

Osteoarthritis (OA), the most prevalent form of arthritis, presents as focal areas of loss of the articular cartilage in synovial joints and is the most common late-onset disease limiting daily activities.[1] The knee is the most common outbreak joint, where OA causes pain, stiffness, swelling, and sometimes locking. Knee OA (KOA), in particular, is prevalent in Asia, including Iran. Incidence ranges from 1.4% to 20% (19.3% in rural areas, 15.3% in urban areas), and some reports have indicated an overall incidence of 17.3% in Iran and a higher incidence in women than in men.[2]

The role of genetic factors in the etiology of OA is considerable. Several predisposing genes have been found.[3-6] The asporin (*ASPN*) gene encodes a cartilage extracellular protein belonging to the small leucine-rich proteoglycan family. These proteins can regulate chondrogenesis by binding to the cartilage transforming growth factor β (TGFβ) and inhibiting expression of the TGFβ1-induced gene in cartilage.[7] TGFβ1 induces *ASPN* indirectly.[8] Asporin has tandem aspartic acid (D) residues (D-repeats) in the amino-terminal region of its mature protein directed by a microsatellite polymorphism.[6] Previous studies have indicated that *ASPN* can bind to TGFβ1 and block its interaction with the TGFβ type II receptor, and then sequentially inhibit TGFβ/Smad signaling and TGFβ1-induced chondrogenesis.[9,10] A D-repeat polymorphism of *ASPN* was first described in 2005 as an OA-associated polymorphism.[9] The D14 allele of *ASPN* was overrepresented in OA subjects, and more inhibition of TGFβ1 activity occurred in the D14 allele than in D13, the common allele. D14, an allele with 14 D-repeats, was associated with increased OA susceptibility, whereas the D13 allele encoded OA protection in the Japanese[9] and the Han Chinese[1] but is not an important factor in OA etiology among Caucasians,[5,7,9] though the D15 allele was considered a risk allele in the Greek population.[11]

Thus, the association between asporin and OA in different ethnic groups is controversial. We conducted a study with Iranian patients to examine the possibility of that association—to explore the effect of *ASPN* on KOA in Iran, where the incidence of KOA is high, and the population is heterogenous.

Materials and Methods

One hundred KOA patients (72 women, 28 men) were consecutively enrolled in the clinic of the Akhtar Orthopedic Educational Hospital, which is affiliated with the Shahid Beheshti University of Medical Science in Tehran. Patients and controls were Iranians (Fars ethnicity) living in or around Tehran. Inclusion criteria were clinical and radiologic KOA

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Table I. Allele Frequencies of Aspartic Acid (D) Repeats in Iranian Knee Osteoarthritis Patients and Controls

Group	Alleles, n (%)									
	D11	D12	D13	D14	D15	D16	D17	D18	D19	Total
Patients										
Total (N=100)	0	4	82	32	52	22	7	0	1	200
	(0%)	(2%)	(41%)	(16%)	(26%)	(11%)	(3.5%)	(0%)	(0.5%)	(100%)
Female (n = 72)	0	1	59	21	41	16	5	0	1	144
	(0%)	(0.5%)	(29.5%)	(10.5%)	(20.5%)	(8%)	(2.5%)	(0%)	(0.5%)	(72%)
Male (n = 28)	0	1	25	11	11	6	2	0	0	56
	(0%)	(0.5%)	(12.5%)	(5.5%)	(5.5%)	(3%)	(1%)	(0%)	(0%)	(28%)
Controls										
Total (N = 100)	1	4	91	40	45	12	6	1	0	200
	(0.5%)	(2%)	(45.5%)	(20%)	(22.5%)	(6%)	(3%)	(0.5%)	(0%)	(100%)
Female (n = 72)	1	3	63	35	27	8	6	1	0	144
	(0.5%)	(1.5%)	(31.5%)	(17.5%)	(13.5%)	(4%)	(3%)	(0.5%)	(0%)	(72%)
Male (n = 28)	0	1	28	5	18	4	0	0	0	56
	(0%)	(0.5%)	(14%)	(2.5%)	(9%)	(2%)	(0%)	(0%)	(0%)	(28%)

Table II. Association Between the Aspartic Acid (D) Repeat Polymorphism and Knee Osteoarthritis in Iranians

Groups Compared	D14 vs Others			D13 vs Others			D14 vs D13		
	OR	P	95% CI	OR	P	95% CI	OR	P	95% CI
All patients (N = 100) vs All controls (N = 100)	0.76	.3	0.46-1.27	0.83	.37	0.56-1.24	0.89	.68	0.51-1.55
Female patients (n = 72) vs Female controls (n = 72)	0.53	.032*	0.3-0.95	0.84	.47	0.53-1.35	0.64	.17	0.34-1.22
Male patients (n = 28) vs Male controls (n = 28)	2.50	.11	0.8-7.75	0.81	.58	0.38-1.72	2.62	.12	0.78-8.80
	D15 vs Others			D13 vs D15			D14 vs D15		
	OR	P	95% CI	OR	P	95% CI	OR	P	95% CI
All patients (N = 100) vs All controls (N = 100)	1.21	.41	0.77-1.90	0.79	.34	0.48-1.29	0.70	.24	0.38-1.27
Female patients (n = 72) vs Female controls (n = 72)	1.73	.045*	1.01-2.94	0.60	.09	0.33-1.08	0.39	.007*	0.20-0.77
Male patients (n = 28) vs Male controls (n = 28)	0.51	.13	0.22-1.23	1.51	.38	0.61-3.74	3.63	.05	0.998-13.18

Abbreviations: CI, confidence interval; OR, odds ratio.
* P<0.05.

symptoms (chronic pain, limited mobility) and age 50 to 75 years. Exclusion criteria were history of inflammatory articular disease, postinfection arthritis, joint dysplasia or congenital disorder, crystalopathy, posttraumatic arthritis, malalignment, avascular necrosis, and obesity (body mass index [BMI], >30). Clinical data (eg, age, sex, BMI, and family history) were collected, and patient pedigrees, including 3 generations, were drawn by a genetic counselor physician. One hundred healthy age-matched controls (72 women, 28 men) were enrolled in the Center of Physical Examination. Mean age of controls at time of recruitment was 63 years (range 50-75 years). Controls had never experienced any signs or symptoms of arthritis or joint disease (ie, pain, swelling, tenderness, restriction of movement). No patients or controls dropped out of the study. The study protocol was approved by ethics committees of the Medical School of Shahid Beheshti University and the Medical Genetics Research Center at the University of Social Welfare and Rehabilitation Sciences, also in Tehran.

For each patient and each control, a peripheral blood sample (10 mL) was drawn, and genomic DNA was extracted.[12] Direct

sequencing of the exon 2 *ASPN* gene, described by Mustafa and colleagues[7] in 2005, was used to detect the exact number of D-repeat microsatellites. D-repeat was amplified with forward primer 5′GCTTTGTGCTCTGCCAAACCC3′ and reverse primer 5′CACTGACATCCAAATGGACAC3′. Polymerase chain reaction products were direct-sequenced with the BigDye v3.1 Terminator Cycle Sequencing Kit and the 3130 Genetic Analyzer (Applied Biosystems, Foster City, California). Alleles were discriminated with CodonCode Aligner software (CodonCode Corp, Centerville, Massachusetts).

Generalized regression models were used to compare allele frequencies in patients and controls. Odds ratios (ORs) with 95% confidence intervals (CIs) and Ps for the association of the D-repeat were estimated for the minor and major alleles. For each analysis, patients were compared with sex-matched controls. All calculations were performed with SPSS Version 16 software (IBM, Chicago, Illinois).

Results

Table I lists the allele frequencies of the D-repeat in our patients and controls. Nine different alleles (11 to 19 D-repeats) were identified. Patients and controls did not differ in allele frequencies (P = .45). Results were similar for each sex (female patients vs female controls, P = .13; male patients vs male controls, P = .26). The D13 allele was the most common (control frequency, 45.5%), and the D15 allele was second most common (control frequency, 22.5%).

Table II lists the Ps adjusted by age and sex for the association of the D-repeat. The following comparisons were made between patients and controls, without stratification and after stratification according to sex:

- **All Alleles.** No significant differences (P≤.05) in allele frequencies between patients and controls, with or without stratification.
- **D15 vs Other Alleles Combined.** No significant differences in unstratified analysis. After stratification, however, a significant difference (P = .045; OR, 1.73; 95% CI, 1.01-2.94) was found in D15 allele frequencies between female patients (20.5%) and female controls (13.5%).
- **D14 vs Other Alleles Combined.** No significant differences in unstratified analysis. After stratification, however, a significant difference (P = .032; OR, 0.53; 95% CI, 0.3-0.95) was found in D14 allele frequencies between female patients (10.5%) and female controls (17.5%).
- **D13 vs Other Alleles Combined.** No significant differences, with or without stratification.
- **D13 vs D15.** No significant differences, with or without stratification.
- **D14 vs D15.** A significant difference (P = .007; OR, 0.39; 95% CI, 0.2-0.77) was found between female patients and female controls. D14 versus D15 was lower in female patients.
- **D13 vs D14.** No significant differences, with or without stratification.

Overall, there were minor differences in D14 allele frequency and D15 allele frequency between patients and controls. These differences, however, were significant only for women. In Iranian females, the D15 allele was significantly (P = .045) associated with KOA, and the D14 allele was significantly (P = .032) protective against KOA.

Discussion

It has been shown that asporin has a basic role in the molecular pathogenesis of OA.[10] Asporin protein, which includes a unique large expanse of D-residues in its amino-terminal region, was partly purified from human articular cartilage and meniscus.[13] It was reported that a D-repeat polymorphism in *ASPN* was significantly associated with OA of knee and hip joints in Japanese people; the D14 allele of the *ASPN* polymorphism was overrepresented, and the D13 allele underrepresented.[9] These findings were not duplicated in a study performed in Caucasians in the United Kingdom.[7] Genotyping Greek KOA patients for the D-repeats showed that the D15 allele can be considered a risk allele for the Greek population.[11] Subsequent reports indicated that the asporin polymorphism is not an important factor in OA susceptibility among European Caucasians.[5] The Han Chinese replication association study for *ASPN* repeat polymorphism and KOA susceptibility had findings similar to those for the Japanese but different from those for the European Caucasians. Therefore, the OA susceptibility gene association differed between these ethnic groups,[1] and the implication is that KOA genetic polymorphisms differ between Japanese and Caucasians.[14] Nakamura and colleagues[15] performed a meta-analysis of 7 association studies to assess incompatibility between studies and to evaluate the common genetic effect of the D-repeat polymorphism on OA. They determined that there are ethnic differences for the universal relatedness association of the *ASPN* D14 allele and KOA. The Korean population study found no significant difference in frequency of D13 (or D14) and other alleles between patients and controls (P = .1082) but did find a significant difference between female patients and female controls in the frequency of D13 versus other alleles (P = .0245).[16] These findings imply that polymorphisms within *ASPN* do not have a major effect on KOA susceptibility in US Caucasians.[17]

Table I lists allele frequencies of the asporin D-repeat in Iranian KOA patients and controls. As is true in the Japanese, Greek, Chinese, Caucasian, and Korean populations, the most common variant in the Iranian population was the D13 allele in both patients (41%) and controls (45.5%). The second most common variant was the D15 allele (26% in patients, 22.5% in controls), and the third was the D14 allele (16% in patients, 20% in controls). Our study detected no significant difference in allele frequencies between KOA patients and controls (P = .045). However, it detected significant differences in D14 and D15 allele frequencies between female patients and female controls. The D15 allele was significantly (P = .045) more common in female patients with KOA than in female controls. Previously, genotyping Greek KOA patients for the D-repeats showed that the D15 allele can be a risk allele for this population.[11] Therefore, our finding regarding an association between the D15 allele and KOA is similar to that for the Greek population. The D14 allele was significantly (P = .032) less common in female patients with KOA than in female controls and had a protective role in

Iranian women. Other studies have reported a different finding—an association between the D14 allele and KOA.[1,9]

Table II shows the association between the D-repeat polymorphism and Iranian patients with KOA. No differences were detected in D13 allele frequencies between KOA patients and controls (P = .37). The same pattern was found when the analysis was repeated after stratification by sex. Likewise, D15 and D14 allele frequencies were similar for patients and controls (**Table II**), but a significant difference was found after stratification by sex. The D15 allele was significantly more common (P = .045) and the D14 allele significantly less common (P = .032) in female patients with KOA than in female controls. We then used more sensitive tests to compare patients' and controls' D13, D14, and D15 allele frequencies versus other alleles, versus one another, and then according to sex (**Table II**). The significant findings were that the D14 allele was less common than the other alleles (P = .032; OR, 0.53; 95% CI, 0.3-0.95, approaching 1), and the D15 allele was more common than the others (P = .045; OR, 1.73; 95% CI, 1.01-2.94, again very near 1). The D14 allele was significantly less common than the D15 allele, because of the strongest D14-versus-D15 comparison (P = .007; OR, 0.39; 95% CI, 0.2-0.77).

These findings suggest the possibility of a weak association between asporin polymorphism and KOA susceptibility among Iranian females. This was the first investigation of the association between the asporin gene polymorphism and KOA in Iran. Findings may become clearer as studies are conducted with larger sample sizes and more power. Failure to detect a compelling association in our cohort does not call into question the veracity of the Japanese and Han Chinese reports. Instead, D-repeat may influence disease risk only with environmental factors that are present in the other Asian population but absent in the Iranian population. Alternatively, there may be interaction between the D-repeat polymorphism and alleles at other loci that are more common in the other Asian populations, such as Japanese and Chinese. In addition, OA frequency differs between the Iranian and Japanese populations,[2] which implies differences in frequency of risk factors—genetic or nongenetic—between these populations. Different allele distributions of the D-repeat among the different studies could have several causes, including ethnicity, habits, and other genetic and nongenetic factors that influence disease development. It is imperative that additional cohorts from the same population and from different ethnic groups be genotyped to test the veracity and global relevance of each association.

Dr. R. Jazayeri is Resident, Medical Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. Dr. Qoreishi is Orthopaedic Surgery Resident, and Dr. Hoseinzadeh is Orthopaedic Surgeon Specialist, Akhtar Orthopedic Educational Hospital, Medical School of Shahid Beheshti University, Tehran, Iran. Ms. Babanejad is Resident, Medical Genetics Research Center, Dr. Bakhshi is Associate Professor, Department of Biostatistics, and Dr. Najmabadi is Professor, Medical Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. Dr. S. Jazayeri is Associate Professor and Orthopedic Surgeon Specialist, Akhtar Orthopedic Educational Hospital, Medical School of Shahid Beheshti University, Tehran, Iran.

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Address correspondence to: Seyyed Mohammad Jazayeri, MD, Akhtar Orthopedic Educational Hospital, Medical School of Shahid Beheshti University, Tehran, Iran (tel, 98-212-200-1072; fax, 98-212-260-1967; e-mail, smjazayeri110@yahoo.com).

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