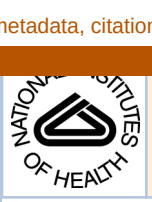

Published in final edited form as:

Osteoarthritis Cartilage. 2008 October ; 16(10): 1174–1177. doi:10.1016/j.joca.2008.03.007.

Absence of association of asporin polymorphisms and osteoarthritis susceptibility in US Caucasians

Uzma Atif[1], **Achamma Philip**[1], **Jennifer Aponte**[1], **Ernias M Woldu**[2], **Mandeep K Sekhon**[2], **Virginia B Kraus**[3], **Joanne M. Jordan**[4], **Michael Doherty**[5], **Anthony G Wilson**[6], **Roland W Moskowitz**[7], **Marc Hochberg**[8], **Richard Loeser**[9], **Jordan B. Renner**[4], and **Mathias Chiano**[10]

[1]GlaxoSmithKline, Research Triangle Park, NC, USA

[2]GlaxoSmithKline, Upper Providence, Philadelphia, USA

[3]Department of Medicine, Duke University Medical Center, Durham, NC, USA

[4]Thurston Arthritis Research Center, and Department of Medicine, Orthopedics and Radiology, University of North Carolina at Chapel Hill, Chapel Hill, NC

[5]Department of Medicine, University of Nottingham, UK

[6]Department of Medicine, University of Sheffield, UK

[7]Case Western Reserve University, OH, USA

[8]University of Maryland at Baltimore, MD, USA

[9]Wake Forest University School of Medicine, Winston-Salem, NC, USA

[10]GlaxoSmithKline, Harlow, UK

Abstract

Objective—An association between osteoarthritis (OA) and functional polymorphisms in the aspartic acid (D) repeat of the asporin (ASPN) gene was reported in Japanese and Han Chinese populations. The aim of this study was to assess the association of variants in the *ASPN* gene with the presence of radiographic hand and/or knee OA in a US Caucasian population.

Methods—Ten SNPs within the *ASPN* gene were genotyped in 775 affected siblings with radiographically confirmed hand or knee OA, and the allelic, genotypic and haplotypic association results were examined.

Results—One variant (SNP RS7033979) showed nominal evidence of association with both hand OA (P=0.042) and knee OA (P=0.032). Four additional SNPs showed nominal evidence of association with knee OA only. These associations were only observed with genotypic tests; the corresponding allelic and haplotype tests did not corroborate the single-point association results.

Conclusion—These data suggest that polymorphisms within *ASPN* are not a major influence in susceptibility to hand or knee OA in US Caucasians.

Introduction

Osteoarthritis (OA) is characterized by both degenerative and reactive changes in the articular cartilage and subchondral bone, thought to be driven by a spectrum of

Corresponding author: Uzma Atif Clinical Pharmacology & Discovery Medicine GlaxoSmithKline 5 Moore Drive Office location 5.5722.5A PO Box 13398 Research Triangle Park North Carolina 27709-3398, USA Tel: +1 919 483 5283 Fax: +1 919 315 0311 uzma_x_atif@gsk.com.

environmental and genetic factors operating via both biomechanical and biochemical mechanisms[1,2]. The changes in articular cartilage include the progressive loss of extracellular matrix (ECM) and collagen breakdown due to a combination of increased catabolism and decreased synthesis. Asporin (ASPN) is a member of the small leucine-rich proteoglycan subfamily of proteins[3], members of which bind to transforming growth factor-β (TGF-β), an important regulator of cartilage homeostasis, and to other ECM molecules of cartilage, including collagens[4]. ASPN is expressed at low levels in normal cartilage but is expressed abundantly in OA articular cartilage[3,5]. Variant ASPN proteins arise due to a microsatellite in the *ASPN* coding sequence that determines a variable number of aspartic acid (D) repeats in the amino-terminal end of the protein. A genetic association study conducted by Kizawa and colleagues identified a variant of ASPN as a susceptibility factor for OA[5]. Allele D14 was significantly more common in Japanese hip and knee OA patients compared with controls[5], suggesting that the D14 allele may lead to an increased OA liability in the Japanese population. An association of the D14 allele with knee OA susceptibility was also observed in a Han Chinese population[6,7]. However, subsequent replication studies in European Caucasians gave inconsistent results[8–10], suggesting that the association of *ASPN* polymorphisms with OA susceptibility varies in different ethnic populations. The D14 allele codes for a variant form of ASPN that appears to be more active than other forms at inhibiting TGF-β activity. A variant of ASPN coded by the D13 allele appears to have reduced binding to TGF-β and has been associated with a reduced susceptibility to OA[5]. The purpose of the present study was to assess the association of variants in the *ASPN* gene with OA in a US Caucasian population.

Patients and Methods

Patients and controls

The design of the Genetics of Generalized Osteoarthritis (GOGO) study has been published previously[11]. The overall aim of the GOGO study is to identify regions in the human genome associated with increased susceptibility to generalized OA. For the current analysis, 786 siblings with multiple-joint OA from 533 nuclear families (330 families with one affected sibling and 203 families with more than one affected sibling) were sampled. Only affected siblings from the US sites (Case Western Reserve University, OH; Duke University Medical Center, NC; Rush Medical College, IL; University of Maryland, MD; and University of North Carolina, NC) were selected for inclusion in this study. In addition, a set of 513 unrelated matched controls were recruited from North Carolina. These were randomly selected from controls in the GO (Genetics of Osteoarthritis) study directed by Dr. Joanne Jordan at UNC. These controls underwent the same stringent radiographic phenotyping as cases and failed to meet the case definitions described below. After genotypic QC, 11 subjects from the case group and two from the control group were excluded from further analysis because of low subject genotyping efficiency (<70%). Therefore, the results presented here are based on 775 affected siblings from 528 nuclear families and 511 unrelated control subjects group-matched by age and sex. The phenotypes analyzed were generalized OA of the hand joints and of at least one knee (740 and 572 affected siblings had hand OA and knee OA phenotypes, respectively). All family-based cases and unrelated controls were Caucasian, ≥18 years and provided written informed consent. The ascertainment and phenotypic analysis of participating individuals and families were performed as previously described[11]. Demographic characteristics of individuals included in this study are summarized in Table 1.

Case definitions

Hand OA was characterized radiographically using the Kellgren-Lawrence (KL) grading scheme[12]. Radiographs were also assessed for individual features, including joint space

narrowing (JSN), osteophytes and other joint-specific features using a photographic standard atlas[13]. Individuals were classified as having hand OA if they met the following criteria: (1) involvement of ≥3 joints (KL grade ≥2), including at least one distal interphalangeal (DIP) joint of digits 2–5; (2) two of the three involved joints within the same joint group (DIP, proximal interphalangeal [PIP] or carpal metacarpal [CMC]); and (3) evidence of OA observed in both hands (bilateral hand involvement).

Knee OA (limited to X-ray information from the medial and lateral tibio-femoral views of the knee) was defined as KL grade ≥2 in at least one knee or a verified history of joint replacement for OA.

Genotyping

A marker selection algorithm was used to select SNPs that were likely to contribute independent genetic information from the HapMap data[14]. The average spacing between markers was 1 SNP per 3.5 kb for production genotyping. The critical aspartic acid (D) repeat polymorphism, nonsynonomous coding SNPs and SNPs in intron lariat regions were added to create the final marker set for the *ASPN* gene.

The D repeat microsatellite was genotyped by PCR amplification using 20 ng of genomic DNA with the primers 5'-6-FAM-ATTCCTGGCTTTGTGCTCTG-3' and 5'-CTCGTGAATAGCACTGACATCC-3'. PCR was performed using AmpliTaq Gold (Applied Biosystems, Foster City, CA) as follows: 7 cycles at 98°C for 10 seconds, 60°C for 1 minute and 70°C for 1 minute, followed by 31 cycles at 96°C for 10 seconds and 68°C for 1 minute. Amplified PCR products were diluted 1:30 with water, from which 1.0 μl of each sample was transferred into a 19.0 μl master mix containing 18.5 μl HiDi Formamide (Applied Biosystems), and 0.5 μl GeneScan 500 ROX Size Standard (Applied Biosystems). Samples were heated to 95°C for 5 minutes and immediately placed on ice before being analyzed on the 3730×l DNA Analyzer (Applied Biosystems). Peak profiles were analyzed using GeneMapper v3.0 software (Applied Biosystems). The number of aspartic acid repeats was determined by PCR fragment size.

Genotyping for the other nine SNPs was performed by a modification of the single base chain extension (SBCE) assay[15]. Following genotyping, the data were scored using a modification of Spotfire Decision Site (version 8.1).

Genotypes for all assays that passed our internal quality control assessment were then exported to an analysis database for statistical analysis.

Statistical analysis

Each SNP was tested for association with hand and knee OA. In addition, haplotypic tests were also performed for each phenotype. In order to perform case-control analysis using related cases and/or controls, it was necessary to account for correlations between related individuals due to identity-by-descent. The CCREL method[16] accounts for these correlations, employing a composite likelihood approach with weights based on kinship coefficients. An EM-style algorithm is used to resolve missing genotypes and phase, and *P* values for the case-control tests are based on asymptotic approximation to a chi-square distribution.

The effective numbers of cases and controls represent the equivalent number of unrelated individuals yielding the same amount of information. Because correlations between related individuals result in reduced information, a group of related individuals has an effective sample size less than the total number of individuals in the group. The CCREL method does not allow for cases to be related to controls. Therefore, a set of independent controls

(unrelated to cases) were used to test for association in this experiment. While the tests are based on asymptotic approximation, the results are fairly robust to small sample sizes and low allele/genotype/haplotype frequencies.

The D repeat polymorphism has 10 alleles. Only 3 of the 10 alleles occur at more than 10% frequency in these data. We tested for association with this marker by testing each of the common alleles, in turn, against the rest, pooled.

For each marker that showed evidence of association with OA, genotypic odd ratios (ORs, with 95% CIs) were calculated with respect to carriers of the minor allele compared with the major allele

Results

Ten markers, including the critical aspartic acid (D) repeat polymorphism, were analyzed in the *ASPN* gene. The single-point allelic and genotypic association results are presented in Table 2. The D repeat polymorphism that was originally found to be associated with OA in a Japanese cohort was not associated with OA in this population. However, one variant (SNP RS7033979) did show nominal evidence of association with both hand OA (P=0.042; OR=0.59, 95% CI 0.37–0.95) and knee OA (P=0.032; OR=0.62, 95% CI 0.39–0.98). This SNP was not typed in the original Japanese study. Four additional SNPs showed nominal evidence of association with knee OA only; SNP RS8067 (P=0.021; OR=1.7, 95% CI 1.16–2.49), SNP RS1924243 (P=0.013; OR=0.53, 95% CI 0.35–0.80), SNP RS3739606 (P=0.016; OR=1.84, 95% CI 1.22–2.78) and SNP RS7860786 (P=0.012; OR=0.51, 95% CI 0.34–0.77). For SNPs RS7033979, RS1924243 and RS7860786, ORs for genotypes homozygous for the minor allele were similar to ORs for the heterozygous genotypes, suggesting that variation at these sites might be associated with OA in a dominant fashion. For SNPs RS8067 and RS3739606, the association with OA was limited to genotypes homozygous for the minor allele. None of these associations remained statistically significant after adjusting for multiple testing. Furthermore, these nominal genotype associations were not observed with allelic tests or combinations of alleles (haplotype tests) across the gene.

Discussion

The purpose of the present study was to assess the association of variants in the *ASPN* gene with OA in a US Caucasian population. An association between OA and functional polymorphisms in the *ASPN* gene was reported in Japanese and Han Chinese populations[5–7]. Subsequent replication studies in European Caucasian populations were unable to demonstrate an association with the D14 allele, the risk allele in the Japanese and Chinese studies[8–10]. However, knee OA studies in all three European populations did show a decreased allelic frequency of the D13 allele (protective allele) and increased D14 frequency in the case group, paralleling the trend in the Japanese study. Recently, Valdes *et al* performed an analysis of combined data from all three European Caucasian studies and provided some evidence of an association between the D14 allele and an increased risk of OA[17]. Furthermore, results from these combined data were suggestive of a decreased risk of knee OA in carriers of the D13 allele. This raises the possibility of an association between *ASPN* and knee OA in Caucasian, as well as Japanese and Chinese, populations. A similar trend was not observed in the present study; carriers of the D13 allele variant did show an association with a reduced risk of knee OA but this trend was not statistically significant (P=0.06). In addition, variants in the D repeat polymorphism and SNPs in linkage disequilibrium with this repeat (haplotypes) were not found to be associated with knee OA

in this study, although it is possible that the study did not capture all the genetic variation necessary to fully mark this allele.

The Japanese study that identified association with the D repeat polymorphism and OA was conducted in patients with knee OA (n=530) and hip OA (n=593) compared with 374 controls[5]. More recent studies in Caucasian populations[8,17] have investigated similar numbers of patient samples. Power estimates performed by Mustafa *et al*[8] suggested that with approximately 300 patients with knee OA, there was a greater than 80% power to detect genetic association when the odds ratio was 1.5; an odds ratio of 1.5 was smaller than the smallest odds ratio observed in the Japanese study. The equivalent number of independent cases analyzed in the present study, on average, was 570 patients, allowing for familial correlation in siblings, and 511 matched controls. This study was therefore comparable in size with previous studies investigating the impact of the D repeat variant in Caucasian populations, and was powered to detect modest genetic effects, if they existed.

In summary, evidence to support the hypothesis that *ASPN* is involved in OA susceptibility is not substantiated by the data from the Caucasian cohort analyzed in this study. However, as previously shown, it would be useful to explore the possibility of performing a combined analysis of several Caucasian samples with similar inclusion criteria and disease classification to further elucidate the role of *ASPN* as a susceptibility gene in osteoarthritis. Interestingly, an influence of *ASPN* polymorphism on the outcome of rheumatoid arthritis has been reported[18], although it had no association with susceptibility to the disease. This raises the possibility that the influence of *ASPN* extends beyond osteoarthritis to a broader spectrum of bone and joint diseases.

Acknowledgments

We acknowledge the following individuals from Glaxo SmithKline for their contributions and insights: Ian D. Latham and Santhi Subramanian (data management), Meg Ehm, Karen F. Lewis (experimental design & management), Anita Nelsen, Morlisa Dixon, Jill Ratchford and Ray Thomas (sample management and plate presentation), Donna Brashear and Elizabeth Harris (genotyping). We also thank sample donors for their participation in this study. This study was supported by a grant from GlaxoSmithKline.

At Duke University Medical Center this study was performed on the Duke General Clinical Research Unit funded by NIH MO1-RR-30, National Center for Research Resources. At Case Western Reserve University this study was supported by the Northeast Ohio Multipurpose Arthritis and Musculoskeletal Disease Center-AR-20618-23.

References

1. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med. 2000; 133:635–646. [PubMed: 11033593]
2. Peach CA, Carr AJ, Loughlin J. Recent advances in the genetic investigation of osteoarthritis. Trends Mol Med. 2005; 11:186–191. [PubMed: 15823757]
3. Henry SP, Takanosu M, Boyd TC, Mayne PM, Eberspaecher H, Zhou W, et al. Expression pattern and gene characterization of asporin. a newly discovered member of the leucine-rich repeat protein family. J Biol Chem. 2001; 276:12212–12221. [PubMed: 11152695]
4. Ameye L, Young MF. Mice deficient in small leucine-rich proteoglycans: novel in vivo models for osteoporosis, osteoarthritis, Ehlers-Danlos syndrome, muscular dystrophy, and corneal diseases. Glycobiology. 2002; 12:107R–116R.
5. Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, et al. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nat Genet. 2005; 37:138–144. [PubMed: 15640800]

6. Jiang Q, Shi D, Yi L, Ikegawa S, Wang Y, Nakamura T, et al. Replication of the association of the aspartic acid repeat polymorphism in the asporin gene with knee-osteoarthritis susceptibility in Han Chinese. J Hum Genet. 2006; 51:1068–1072. [PubMed: 17024313]
7. Shi D, Nakamura T, Dai J, Yi L, Qin J, Chen D, et al. Association of the aspartic acid-repeat polymorphism in the asporin gene with age at onset of knee osteoarthritis in Han Chinese population. J Hum Genet. 2007; 52:664–667. [PubMed: 17603749]
8. Mustafa Z, Dowling B, Chapman K, Sinsheimer JS, Carr A, Loughlin J. Investigating the aspartic acid (D) repeat of asporin as a risk factor for osteoarthritis in a UK Caucasian population. Arthritis Rheum. 2005; 52:3502–3506. [PubMed: 16255042]
9. Kaliakatsos M, Tzetis M, Kanavakis E, Fytili P, Chouliaras G, Karachalios T, et al. Asporin and knee osteoarthritis in patients of Greek origin. Osteoarthritis Cartilage. 2006; 14:609–611. [PubMed: 16377215]
10. Rodriguez-Lopez J, Pombo-Suarez M, Liz M, Gomez-Reino JJ, Gonzalez A. Lack of association of a variable number of aspartic acid residues in the asporin gene with osteoarthritis susceptibility: case-control studies in Spanish Caucasians. Arthritis Res Ther. 2006; 8:R55. [PubMed: 16542493]
11. Kraus VB, Jordan JM, Doherty M, Wilson AG, Moskowitz R, Hochberg M, et al. The Genetics of Generalized Osteoarthritis (GOGO) study: study design and evaluation of osteoarthritis phenotypes. Osteoarthritis Cartilage. 2006; 15:120–127. [PubMed: 17113325]
12. Kellgren JH, Lawrence JS. Radiological assessment of rheumatoid arthritis. Ann Rheum Dis. 1957; 16:485–493. [PubMed: 13498603]
13. Burnett, S.; Hart, DJ.; Cooper, C.; Spector, TD. A Radiographic Atlas of Osteoarthritis. Springer Verlag; London: 1994.
14. Meng Z, Zaykin DV, Xu CF, Wagner M, Ehm MG. Selection of genetic markers for association analyses, using linkage disequilibrium and haplotypes. Am J Hum Genet. 2003; 73:115–130. [PubMed: 12796855]
15. Taylor JD, Briley D, Nguyen Q, Long K, Iannone MA, Li MS, et al. Flow cytometric platform for high-throughput single nucleotide polymorphism analysis. Biotechniques. 2001; 30:661–669. [PubMed: 11252801]
16. Browning SR, Briley JD, Briley LP, Chandra G, Charnecki JH, Ehm MG, et al. Case-control single-marker and haplotypic association analysis of pedigree data. Genet Epidemiol. 2005; 28:110–122. [PubMed: 15578751]
17. Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M, et al. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum. 2007; 56:137–146. [PubMed: 17195216]
18. Torres B, Orozco G, Garcia-Lozano JR, Oliver J, Fernandez O, Gonzalez-Gay MA, et al. Asporin repeat polymorphism in rheumatoid arthritis. Ann Rheum Dis. 2007; 66:118–120. [PubMed: 16707531]

Table 1

Demographic characteristics of the case and control samples

	Controls (n=511)		Cases (n=775)	
	Female (n=341)	**Male (n=170)**	**Female (n=630)**	**Male (n=145)**
Mean age (yrs)[SD]	67.5 [7.1]	69.6 [7.0]	70.8 [8.6]	70.9 [7.6]
Mean BMI (kg/m^2) [SD]	26.5 [4.5]	28.1 [4.1]	29.6 [6.5]	29.1 [5.5]

Table 2

Association of the D repeat of ASPN in patients with hand or knee osteoarthritis (significant associations shown in bold)

	Hand OA				Knee OA			
RefSeq Number	**Allelic *P* value**	**Genotypic *P* value**	**Haplotypic Win2 *P* value**	**Haplotypic Win3 *P* value**	**Allelic *P* value**	**Genotypic *P* value**	**Haplotypic Win2 *P* value**	**Haplotypic Win3 *P* value**
RS8067	0.20825	0.08242	0.38729	0.55833	0.13659	**0.02062**	0.2721	0.42854
RS3174352	0.68177	0.18551	0.91549	0.42644	0.53847	0.10218	0.79982	0.58827
RS17591776	0.80182	0.97578	0.27394	0.36844	0.6706	0.91976	0.45573	0.50171
RS4744132	0.10972	0.10508	0.21633	0.30305	0.23487	0.23198	0.35105	0.37404
RS7033979	0.40091	**0.04283**	0.50359	0.67503	0.3573	**0.03193**	0.36919	0.57446
RS1924243	0.26518	0.10127	0.47972	0.4203	0.15664	**0.0133**	0.37429	0.33736
RS10992350	0.60734	0.61809	0.87366	0.34726	0.34178	0.56173	0.63027	0.26256
Aspartic Acid D repeat*								
D13 vs others	0.98351	0.11158	0.23542	0.26907	0.90338	0.05921	0.14552	0.17729
D14 vs others	0.42511	0.51518	0.35717	0.38451	0.29767	0.43079	0.25925	0.29851
D15 vs others	0.57046	0.53326	0.60626	0.68233	0.3944	0.48494	0.43791	0.51028
RS3739606	0.18939	0.07853	0.2212	0.2212	0.10949	**0.01578**	0.13556	0.13556
RS7860786	0.25846	0.07853	0.2212	0.40164	0.13016	**0.01232**	0.13556	0.27841

*The D repeat polymorphism has 10–19 D residues (10 alleles) in our data (cases and controls combined; number of repeats [freq]: D10 [0.0007], D11 [0.0007], D12 [0.0588], D13 [0.4840], D14 [0.1344], D15 [0.2146], D16 [0.0702], D17 [0.0167], D18 [0.0134] and D19 [0.0067]). Only three of the ten alleles occured at more than 10% frequency in these data. The frequencies of the common alleles in controls were: D13 (0.485), D14 (0.139), D15 (0.207); the frequences of the corresponding alleles in cases were: D13 (0.483), D14 (0.133), D15 (0.218). We tested for association with this marker by testing each of the three common alleles, in turn, against the rest, pooled. In the above table, only results for the allele showing the most evidence for association are listed. Note that the variant with13 D residues (D13) is the most common variant in both Japanese and Caucasian samples (D13 freq, 0.48 in Caucasians vs 0.67 in Japanese). However, the variant with 14 D residues (D14), for which association was reported in Japanese samples, is fairly common in Caucasians (D14 freq, 0.13 in Caucasians vs 0.05 in Japanese).