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Exploring Aspartic Acid D-repeat Polymorphism as a Potential Risk Factor for Primary Hip Osteoarthritis in the Iranian Population

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Abstract:
Background: The *ASPN* gene encodes a cartilage extracellular protein (Asporin) that is known to be involved in the pathological paths of osteoarthritis (OA). Many research efforts have explored the link between aspartic acid D-repeat polymorphism in the asporin (*ASPN*) gene and the risk of OA susceptibility, yet the findings are inconsistent. Our study involved a case-control analysis to examine the relationship between D allele polymorphism in asporin and primary hip osteoarthritis (HOA) among the Iranian population.
Methods: The asporin D repeat polymorphism was genotyped in primary HOA patients (N=70) and healthy controls (N=70). Each group consisted of 28 women and 42 men. Patients were classified into three subgroups based on the radiographic severity of osteoarthritis. Statistical analysis was performed on gender, severity, and primary HOA position.
Odds ratios (ORs) along with 95% confidence intervals (95% CIs) were utilized to assess the association between D-repeats in the ASPN gene and primary hip osteoarthritis.
Results: Three common D-repeat variants (D13, D14, and D15) of the *ASPN* gene were obtained. The most frequent allele in the patient group was observed at D13, while it was D15 among controls. In both cohorts, the least frequent allele was D14. Our findings indicate no statistically significant association between any D-repeats with primary HOA according to the sex of patients or the severity of the disease.
Conclusion: Our findings indicate that polymorphisms in the *ASPN* D-repeat are not linked to a higher risk of primary HOA in the Iranian population. However, future large studies are needed to validate these findings.

INTRODUCTION

HOA is an age-related form of OA and a leading cause of musculoskeletal disability. HOA is a degenerative joint disease that affects the articular surface of the joint. It is characterized as either primary (or idiopathic) or secondary in the elderly population (1). Incidence rate estimations by the World Health Organization (WHO) indicate that 10% of men and 18% of women aged over 60 years suffer from HOA (2). Furthermore, this disease's prevalence is expected to increase significantly shortly.

Symptoms related to HOA significantly affect patients' social and physical well-being and also represent a considerable economic burden on society. HOA is influenced by multiple risk factors such as body mass index (BMI), age, sex, and genetic background (3). The exact mechanism underlying the pathogenesis of OA remains unclear. However, numerous studies have investigated the link between aspartic acid (D) repeat polymorphism in the ASPN gene and the risk of developing knee osteoarthritis (KOA) and HOA across various

populations (4) The *ASPN* gene produces Asporin, a cartilage extracellular protein that belongs to the small leucine-rich proteoglycan (LRR) family.

The *ASPN* gene is cytogenetically located on human chromosome 9 within a specific gene cluster between 9q21.3 and 9q22 Asporin is a critical regulator of transforming growth factor beta 1 (*TGFb1*), and through an inhibitory mechanism, it affects *TGFb1*-induced gene expression, which negatively regulates chondrogenesis in cartilage (5). The N-terminal region of asporin consists of aspartic acid residues (D-repeat). The poly-Asp region is involved in binding calcium (3). Heterogeneity in the number of D-repeats in this region has been reported to be associated with susceptibility to osteoarthritis, although the results are inconsistent. The contradictions led us to investigate the validity of this association among Iranians. In addition, we will answer the question of whether this association with osteoarthritis is worth considering.

According to the principle of multifactorial disease, genetic background and environmental differences influence genetic diversity and phenotype between different ethnicities, which may lead to controversial results across studies in different populations. To our knowledge, no study has investigated the connection between *ASPN* gene Asp repeat polymorphism and primary Hip OA penetrance in the Iranian population. Therefore, we performed a case-control study based on a robust stratified analysis of gender and primary HOA severity rates with a reliable sample size to further elucidate the role of asporin D allele polymorphism in primary HOA susceptibility among the Iranian population. Multi-haplotype analysis (D13, D14, and D15 alleles) enabled us to increase the evidence for hip association.

MATERIALS AND METHODS

Patients

A case-control study was conducted with a sample size (N =70) for each group, of which 28 women and 42 men were regarded as control cases, as well as OA patients according to exclusion/inclusion criteria. The standard deviation for sample size calculation is obtained from the previous study of our team on knee osteoarthritis in Iran (6). All subjects in the study were of Iranian nationality and came from the same geographical area of the country (Tehran).

All procedures and clinical research were carried out in accordance with the ethical standards and subsequent amendments outlined in the declaration of Helsinki. All participants signed the written informed consent. Cases were defined as patients based on inclusion criteria including chronic pelvic pain, radiological verification, limitations in mobility, those without any history of inflammatory joint disease, inflammatory arthritis, joint dysplasia or congenital disorder, etc. Clinical symptoms and radiographic evidence of hip osteophytes and narrowing of the joint space were used to diagnose hip OA. All patients with hip OA were categorized into three grades according to the Tönnis classification. Tönnis grading scale of HOA (7, 8):

-Grade 1: This classification involves mild narrowing of the joint space, minor lipping at the joint margin, and slight sclerosis of either the femoral head or the acetabulum.

- Grade 2: This grade is characterized by the presence of small cysts in the femoral head or acetabulum, increased narrowing of the joint space, and moderate loss of femoral head sphericity.

-Grade 3: This classification includes large cysts, severe narrowing or complete obliteration of the joint space, severe deformity of the femoral head, and avascular necrosis (7). The controls were healthy people with normal radiographs of the hip joint space. (Table S1 gives information about the demographics of both groups). Samples were collected from Akhtar Hospital-Orthopaedic Surgery Center at Shahid Beheshti University of Medical Sciences. The Institutional Review Board Committee at Shahid Beheshti University of Medical Sciences in Tehran, Iran, approved the study.

DNA extraction

DNA was extracted from 10 ^{cc} of collected peripheral blood into an EDTA tube according to standard protocols (9). DNA degradation was assessed using 1% agarose gels. Protein contamination in all DNA samples was evaluated by the A260/A280 ratio, and reagent contamination was determined by the A260/A230 ratio using a NanoDrop ND 1000 spectrophotometer (NanoDrop, Wilmington, DE, USA).

Primer design and PCR

The polymorphic Asp repeat region was identified by sanger sequencing for exon 2 of the *ASPN* gene (described by Mustafa and colleagues in 2005) (10). Primers flanking the regions, including a minimum of 60 bp from the exon-intron boundary of exon 2 of the *ASPN* gene (NM_017680.5), were designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The forward primer sequence is 5'- GCTTTGTGCTCTGCCAAACCC -3', and the reverse primer sequence is 5'-CACTGACATCCAAATGGACAC-3'.

These primers were synthesized by Macrogen in Korea. The amplification of target sequences was performed in an ABI thermal cycler (Applied Biosystem Veriti™ Thermal Cycler, Thermo Fisher, Waltham, MA, USA) using reaction volumes of 25 µL. Each reaction contained 50 ng of DNA, 1× PCR buffer, 2.5 mM MgSO4, 200 µM of each dNTP, 10

pmol of each forward and reverse primer, and 5 U of Taq DNA polymerase (Macrogen, Korea). The PCR cycle conditions were set as follows: an initial denaturation at 95°C for 5 minutes; 30 cycles of 30 seconds at 94°C, 1 minute at 60°C, and 30 seconds at 72°C; and a final extension at 72°C for 3 minutes.

Genotyping

All the PCR products were directly sequenced using the BigDye v3.1 Terminator Cycle Sequencing Kit and the 3130 Genetic Analyzer (Applied Biosystems, Foster City, California). Sequencing results were identified by CodonCode Aligner software (CodonCode Corp, Centerville, Massachusetts).

STATISTICAL ANALYSIS

All case-control qualitative data were presented by number and percentages. We performed a case-control study for the ASPN D13, D14, and D15 alleles and hip OA susceptibility to assess the strength of the association respectively by calculating odds ratios (ORs) and 95% confidence intervals (CIs). We used the Chi-square test or Fisher exact test to investigate the association between genotype and the presence of increased levels of hip osteoarthritis (Severity of radiopathological symptoms) (11). Statistical analyses were conducted using the SPSS version 17.0 statistical package (SPSS, Chicago, IL, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA). A p-value of less than 0.05 was deemed statistically significant, and all tests were two-sided. The variables, such as age and BMI, were compared between the case and control groups using the t-test and chi-square test.

RESULTS

Three different alleles (D13, D14, and D15) were identified in the study groups.

Table 1 displays the distribution of allele frequencies for each case-control study, categorized by sex both before and after stratification. According to the Tönnis grading scale of primary hip osteoarthritis, the patient group was classified into three groups: grade 1, grade

Table 1. Allele frequencies of the asporin D repeat in hip OA patients and controls from the Iran.

Alleles	D13	D14	D15	Total
All patients (n=70)	62 (44.3%)	20 (14.3%)	58 (41.4%)	140 (100%)
Female patients (n=28)	25 (17.8%)	8 (5.7%)	23 (16.4%)	56 (40%)
Male patients (n=42)	37 (26.4%)	12 (8.5%)	35 (25%)	84 (60%)
All controls (n=70)	53 (37.9%)	18 (12.9%)	69 (49.3%)	140 (100%)
Female controls (n=28)	22 (15.7%)	6 (4.2%)	28 (20%)	56 (40%)
Male controls (n=42)	31 (22.1%)	12 (8.5%)	41 (29.2%)	84 (60%)

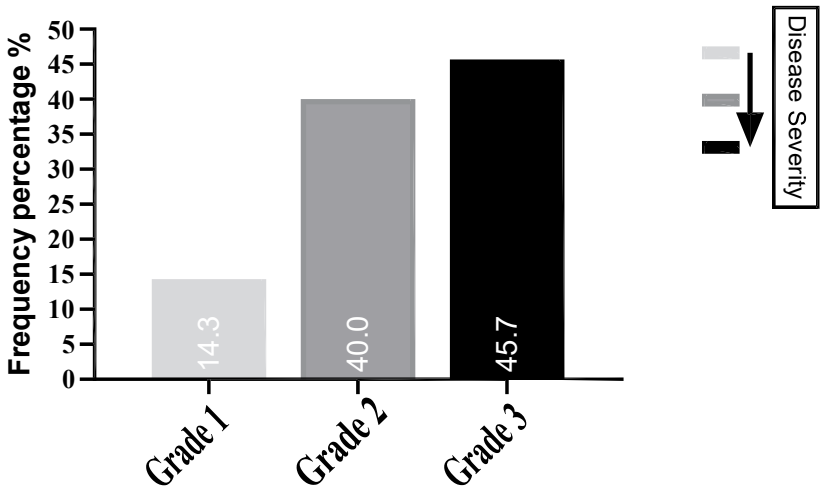

Fig 1. The bar plot demonstrates the value frequencies of the disease grades for hip OA group. The severity of the disease represented by color intensity. Most of the participants belongs to sever form of disease (45.7%)

Table 2. correlation between Asp allele repeats and Hip OA Tönnis grades in patients' group.

ALLELES	GRADE 1	GRADE 2	GRADE 3	PEARSON CHI-SQUARE	DF	OR
D13	40.00%	42.90%	25.00%	0.992	2	0.016
D14	20%	21.40%	21.90%	0.319	2	2.283
D15	40.00%	35.70%	53.10%	0.383	2	1.92

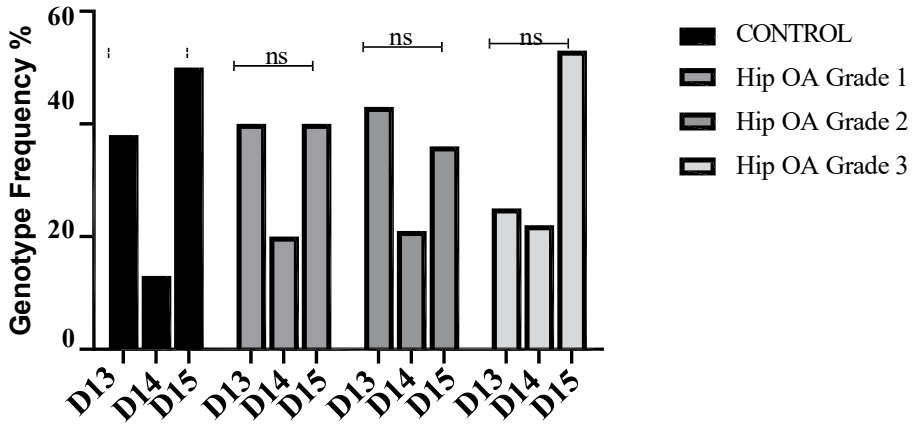

Fig 2. Bar plot represents association between Asp repeats genotype and Hip OA severity.no significant differences were observed between three alleles (D13, D14 and D15) and mild, moderate and severe form of Hip OA in patients group.

2, and grade 3. The frequencies of the disease grade classification for the patient group were 14.3% for grade 1, 40.0% for grade 2, and 45.7% for grade 3 (Fig 1).

The association between genotype and primary hip osteoarthritis severity was tested, and the following values were obtained p=0.319 for D13, p=0.992 for D14, and p=0.383 for D15 (Pearson Chi-Square test). The results demonstrated that there is no association between D allele repeats and primary Hip OA severity (Table 2 and Fig 2).

Targeted comparison analyses were performed for each allele frequency compared to all other frequencies combined, followed by a comparison of the most frequent alleles (D13, D15, and D14) in the patient group versus the control group before and after stratification according to sex and disease severity.

All alleles.

The overall allele frequency percentages in the study groups were (patient groups: D13:44.3%, D14: 14.3%, and D15: 41.4%) and (control group: D13: 37.9%, D14: 12.9%, and D15: 49.3%). Stratification was performed according to the sex criteria, and the results were; patient group: (D13:17.8% in women and 26.4% in men), (D14: 5.7% in women and 8.5% in men), and (D15: 16.4% in women, and 25% in men) and control group: (D13: 15.7% in women and 22.1% in men), (D14: 4.2% in women and 8.5% in men), and (D15: 20% in women and 29.2% in men). The findings showed that there were no significant differences ($P \geq 0.05$) in allele frequencies between primary HOA patients and controls, both with and without stratification.

D13 vs Other Alleles Combined

No significant differences were observed between the two groups either before or after stratification. (P = 0.27; OR=1.3; 95% CI, 0.81-2.1).

D14 vs Other Alleles Combined

There were no significant differences noted between the two groups, both before and after stratification. (P =0.72; OR=1.13; 95% CI, 0.57-2.24).

D15 vs Other Alleles Combined

Significant differences were not identified between the two groups, both before and after stratification. (P = 0.18; OR=0.72; 95% CI, 0.45-1.16).

D13 vs D14

No significant differences were found, regardless of whether stratification was applied.

D13 vs D15

No significant differences were found, regardless of whether stratification was applied.

D14 vs D15

No significant differences were found, regardless of whether stratification was applied.

Overall, there were no significant differences between the two groups, neither before and after the stratification nor in the status of radiological severity in

Table 3. Association of the D-repeat asporin polymorphism with Hip OA in the Iranian population

GROUPS COMPARED	D14 VS. D15			D15 VS. D13			D15 VS. OTHERS		
	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI	*P* value	OR
All PATIENTS(N=70) *VERSUS* ALL CONTROLS(70)	0.63-2.73	0.45	1.32	0.43-1.19	0.2	0.72	0.45-1.16	0.18	0.72
FEMALE PATIENTS(N=28) *VERSUS* FEMALE CONTROLS(N=28)	0.26-4.91	0.85	1.14	0.22-1.42	0.22	0.057	0.26-1.46	0.27	0.62
MALE PATIENTS(N=42) *VERSUS* MALE CONTROLS(N=42)	0.76-4.63	0.17	1.87	0.42-1.78	0.7	0.87	0.39-1.43	0.38	0.75
GROUPS COMPARED	D14 vs. D13			D13 vs. others			D14 vs. others		
	95%CI	*P* value	OR	95%CI	*P* value	OR	95%CI	*P* value	OR
ALL PATIENTS(N=70) *VERSUS* ALL CONTROLS(N=70)	0.45-1.98	0.89	0.95	0.81-2.1	0.27	1.3	0.57-2.24	0.72	1.13
FEMALE PATIENTS(N=28) *VERSUS* FEMALE CONTROLS(N=28)	0.14-2.90	0.57	0.65	0.7-4.15	0.23	1.71	0.21-3.56	0.85	0.87
MALE PATIENTS(N=42) *VERSUS* MALE CONTROLS(N=42)	0.65-4.11	0.29	1.63	0.49-1.86	0.91	0.96	0.76-4.07	0.18	1.76

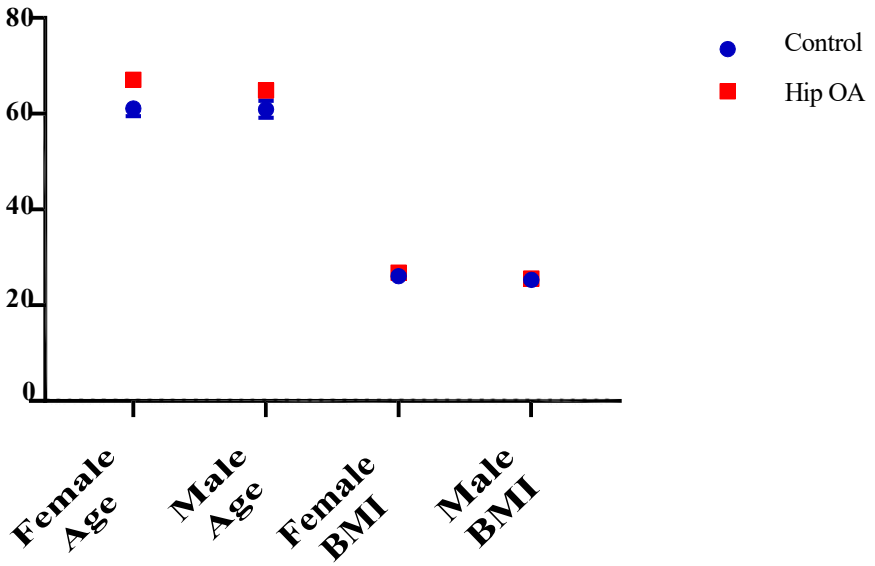

Fig 3. Dot plot demonstrates independent samples t-Test results for age and BMI by mean and variances between sexes in two cohorts. both populations (case and control) for each group located in close area and no significant differences were observed.

the patient group. (Table 3 demonstrates the statistical values). Using a *t*-test demonstrated that there was no difference in the outcome variables (age and BMI) between the primary hip OA patients and healthy control groups (Fig 3).

DISCUSSION

The present study demonstrates that there is no association between D13, D14, and D15 alleles and increased primary Hip OA risk in the Iranian population.

In recent years, several studies have paid particular attention to the correlation between asporin D- repeat polymorphisms and OA susceptibility in different populations. D-repeat is the unique aspartate-rich N terminus region that expands in exon 2 of the *ASPN* gene and encodes a cartilage extracellular protein.

Previously, it has been reported that *ASPN* gene polymorphism has an impact on OA disease and that asporin expression levels differ between various populations such as Caucasians vs. Japanese (12).

The D14 allele is the least common in the entire cohort (14.3% for patients and 12.9% for controls) However, the control and patient groups differ in that D13 is the most prevalent allele in patients, followed by D15, whereas D15 is the most frequent, followed by D13, in controls. The data regarding D14 allele frequency are consistent with our previous report on KOA among the Iranian population (6).

Reports vary regarding the most prevalent allele; D13 has been previously identified as more common among controls, while D14 is often found more frequently in patients within the UK Caucasian population (10). This demonstrates the different genetic diversity between Iranians and Europeans. However, meta-analyses by Sobhan et al. in eleven case-control studies in ten publications implied that D13 is more prevalent in case and control groups, whereas D14 is less common in both groups(3), similar to our results about the D14 allele.

Previously, D13 was reported to be associated with low OA risk and, thus, was considered a protective allele of Greek origin (13). This finding, however, was duplicated in a Japanese study (14). Also, gender-wise stratification indicated a high risk for HOA among women, and it was believed that D13 was a protective marker in men in the Romanian population (15). A meta-analysis by Wang. H et al. performed using twelve qualified studies from various ethnicities confirmed the critical role of the D13 allele as a protective marker in OA (4). Subsequently, the under-representation of D13 in OA patients was observed in the Han Chinese population (16). Hence, several studies from different populations have consented that the D13 allele is associated with a decreased risk for OA. However, our study detected no significant difference in allele frequencies between the primary HOA cohort and controls. Other meta-analyses in Asians and Europeans (3, 17) are consistent with our results. Moreover, functional studies demonstrated no significant difference in the inhibitory effects on TGF-beta between D13 and other alleles (16), which strongly supports our findings. Taken together, we assumed that the D13 allele does not have a major impact on primary HOA susceptibility in the Iranian population.

Regarding the D14 allele, the high frequency has been observed in developmental dysplasia of the hip (DDH) in the Han Chinese population and is claimed to be associated with early-onset OA (16). Furthermore, the over-representation of the D14 allele was reported as being associated with HOA and increased alongside disease severity, which is an outcome of the greater inhibition of TGF-beta for D14 (18). A high expression of the D14 allele was reported in OA cartilage tissue, which could mediate the suppression of the TGF-beta signaling pathway (13). Also, a meta-analysis revealed that D14 is a prominent risk factor for KOH in Caucasians (17). This result was duplicated in Caucasian and Japanese populations (19). Similarly, the D14 allele was associated with the risk of KOA development in Japanese and Chinese populations. In support of the above-mentioned reports, D14 is a common risk allele for KOA among Asians and male Caucasians (10, 20). In contrast with these data, our research team previously implied a protective role of the D14 allele among female KOA patients in the Iranian population (6). Moreover, D14 was associated with a high risk for HOA in men but was a protective marker in women. In a previous study, D14 had a significant association with increased radiological severity in both sexes in the Romanian population. (15) These data are inconsistent with our results, as we detected no significant association between D14 and primary HOA in the Iranian population. Previous meta-analyses of Europeans and Asians support our findings (3, 17). Considering functional studies and controversial results in various populations, we assume these findings could imply the ethnic dependence of the D14 allele's role in OA susceptibility.

The D15 allele was reported either to have an association with OA risk via a different mechanism, as a risk factor or to have a protective role in the Mexican and Iranian populations (3, 5). Our research team demonstrated that the D15 allele is a risk factor for KOA in Iranian female patients (6). However, this association is not confirmed for primary HOA in the same population in the present study.

Ultimately, our findings are different from previous investigations. Nevertheless, some studies support our results by indicating a negative association between OA susceptibility and D-repeat alleles

in different populations (21-23). Meta-analyses performed by Sobhan, Nakamura, Wang, H et al. and AA Brisola, et al. similarly reported no association for D-repeat in asporin and OA among Europeans and Asians (3, 17, 24, 25). Additionally, meta-analyses by Xing et al. suggest that the *ASPN* D-repeat polymorphism may not be a significant marker for KOA susceptibility in both Caucasian and Asian populations (21). As mentioned above, there are some discrepancies between reports about the link between the D-repeat allele and OA. Our results do not support the connection between susceptibility to primary HOA and D allele polymorphism. Generally, the KOA is more prevalent than the HOA (26), and so most research is focused on KOA. Thus, the problem of making valid comparisons across available documents is one of the pivotal limitations of studying HOA. Moreover, OA is a multifactorial disorder that is influenced by genetic background, age, sex, and environmental factors. These facts could explain the discrepancies between the results of research work conducted in different populations. The advantages of the present study, which due to the high level of consanguinity in Iran, could be more prominent in the genetic role compared to other nongenetic factors in this study.

Our study addresses a significant gap by investigating the *ASPN* gene's D-repeat polymorphism in the Iranian population, previously underrepresented in osteoarthritis genetics research. Despite the smaller sample size, our findings contribute preliminary insights into the unique genetic landscape of this group, highlighting the importance of including diverse populations in genetic research. This approach not only helps validate or challenge existing data but also enhances understanding of osteoarthritis's multifactorial nature, paving the way for future research and potential precision medicine advancements.

CONCLUSION

The findings suggest a lack of an association between aspirin D allele polymorphism and primary HOA susceptibility among the Iranian population. This is the first assessment of the association between primary HOA and D polymorphism in the *ASPN* gene in Iran. However, further investigations of larger populations and involving diverse ethnic cohorts are required to confirm the role of asporin genetic variation on primary HOA risk.

Abbreviations

ASPN = asporin, CI = confidence interval, LRR = leucine-rich, KOA = knee osteoarthritis, primary HOA = primary Hip osteoarthritis, OA =osteoarthritis, OR = odds ratio, TGF-b =transforming growth factor-b, D = aspartic acid, DDH = developmental dysplasia of the hip.

Statements and Declarations

Consent to participate and Publication

All participants read and signed the consent form.

Availability of data and materials

The data supporting the findings of this study can be obtained upon request from the corresponding author, R Jazayeri. These data are not publicly accessible due to restrictions such as containing information that could compromise research participant privacy.

Ethics approval and consent to participate

Written informed consent has been obtained from all participants in the study.

Conflicts of Interest: M Qoreishy, A Sajedi, M Qoreishi, M Makvand, and R Jazayeri declare that they have no conflict of interest.

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Authors' contributions

M Qoreishi, A Sajedi, M Qorbani, M Makvand, and R Jazayeri were instrumental in designing and implementing the research, analyzing the results, and contributing to the writing of the manuscript.

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REFERENCES

1.Lespasio MJ, Sultan AA, Piuzzi NS, Khlopas A, Husni ME, Muschler GF, et al. Hip osteoarthritis: a primer. The Permanente Journal. 2018;22.

2.Organization WH, Ageing WHO, Unit LC. WHO global report on falls prevention in older age: World Health Organization; 2008.

3.Sobhan MR, Mehdinejad M, Jamaladini MH, Mazaheri M, Zare-Shehneh M, Neamatzadeh H. Association between aspartic acid repeat polymorphism of the asporin gene and risk of knee osteoarthritis: A systematic review and meta-analysis. Acta Orthopaedica et Traumatologica Turcica. 2017;51(5):409-15.

4.Wang H, Zhang X, Wu W, Zhang M, Sam NB, Niu L. Association between the aspartic acid D-repeat polymorphisms and osteoarthritis susceptibility: an updated systematic review and meta-analyses. Medicine. 2018;97(45).

5.González-Huerta NC, Borgonio-Cuadra VM, Zenteno JC, Cortés-González S, Duarte-Salazar C, Miranda-Duarte A. D14 repeat polymorphism of the asporin gene is associated with primary osteoarthritis of the knee in a Mexican Mestizo population. International Journal of Rheumatic Diseases. 2017;20(12):1935-41.

6.Jazayeri R, Qoreishi M, Hoseinzadeh H, Babanejad M, Bakhshi E, Najmabadi H, et al. Investigation of the asporin gene polymorphism as a risk factor for knee osteoarthritis in Iran. Am J Orthop. 2013;42(7):313-6.
7.Kovalenko B, Bremjit P, Fernando N. Classifications in brief: Tönnis classification of hip osteoarthritis. Clinical orthopaedics and related research. 2018;476(8):1680.
8.Busse J, Gasteiger W, Tonnis D. [A new method for roentgenologic evaluation of the hip joint--the hip factor]. Arch Orthop Unfallchir. 1972;72(1):1-9.
9.MWer S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids res. 1988;16(3):1215.
10.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 & Rheumatism. 2005;52(11):3502-6.
11.Bolboacă SD, Jäntschi L, Sestraş AF, Sestraş RE, Pamfil DC. Pearson-Fisher chi-square statistic revisited. Information. 2011;2(3):528-45.
12.Mishra A, Awasthi S, Raj S, Mishra P, Srivastava RN. Identifying the role of ASPN and COMP genes in knee osteoarthritis development. Journal of Orthopaedic Surgery and Research. 2019;14(1):1-9.
13.Kaliakatsos M, Tzetis M, Kanavakis E, Fytili P, Chouliaras G, Karachalios T, et al. Asporin and knee osteoarthritis in patients of Greek origin. Osteoarthritis and cartilage. 2006;14(6):609-11.
14.Xu L, Li Z, Liu S-Y, Xu S-Y, Ni G-X. Asporin and osteoarthritis. Osteoarthritis and Cartilage. 2015;23(6):933-9.
15.Ilea C, Lupan I, Leucuta D-C, Lucaciu D. Investigation of asporin gene polymorphism (rs387906276) as a risk factor for primary hip osteoarthritis in Romanian population. Med Con Oct. 2015;10(3):13-8.
16.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. Journal of human genetics. 2007;52(8):664-7.
17.Song GG, Kim J-H, Lee YH. A meta-analysis of the relationship between aspartic acid (D)-repeat polymorphisms in asporin and osteoarthritis susceptibility. Rheumatology international. 2014;34(6):785-92.
18.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. Nature genetics. 2005;37(2):138-44.
19.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 & Rheumatism. 2007;56(1):137-46.
20.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. Journal of human genetics. 2006;51(12):1068-72.
21.Xing D, Ma X-L, Ma J-X, Xu W-G, Wang J, Yang Y, et al. Association between aspartic acid repeat polymorphism of the asporin gene and susceptibility to knee osteoarthritis: a genetic meta-analysis. Osteoarthritis and Cartilage. 2013;21(11):1700-6.
22.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 research & therapy. 2006;8(3):1-4.
23.Wang J, Yang A, Zhang J, Sun N, Li X, Li X, et al. Genetic polymorphism in the asporin gene is not a key risk factor for osteoarthritis: Evidence based on an updated cumulative meta-analysis. Experimental and Therapeutic Medicine. 2018;15(4):3952-66.
24.Nakamura T, Shi D, Tzetis M, Rodriguez-Lopez J, Miyamoto Y, Tsezou A, et al. Meta-analysis of association between the ASPN D-repeat and osteoarthritis. Human molecular genetics. 2007;16(14):1676-81.
25.Brisola AA, Colovati MES, Cernach MCSP, Riera R, Pacheco RL, Crizol GR, et al. Association between genetic polymorphisms and osteoarthritis development. Overview of systematic reviews. International Journal of Rheumatic Diseases. 2022.
26.Cooper DJ, Scammell BE, Batt ME, Palmer D. Factors associated with pain and osteoarthritis at the hip and knee in Great Britain's Olympians: a cross-sectional study. British Journal of Sports Medicine. 2018;52(17):1101-8.