



ARTICLE

ASSOCIATION OF SMAD3 GENE RS12901499 VARIATION WITH KNEE OSTEOARTHRITIS IN INDONESIAN AGED WOMEN

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ABSTRACT

Small Mother Against Decapentaplegic 3 (SMAD3), an intracellular transducer protein in the TGF- β signaling pathway, has a role in maintaining joint cartilage. Many studies have been conducted to examine the effect of polymorphism in SMAD3 gene with the incidence of osteoarthritis (OA). The objective of this study is to identify the association of SMAD3 gene rs12901499 variation with the incidence of knee OA in Indonesian women. We conducted an analytic cross-sectional design involving 24 knee OA patients and 50 non-OA subjects. The DNA was taken from saliva and genotyped using a kit from Integrated DNA Technologies with the Real Time PCR method. In this study, we found that the GA genotype (heterozygous mutant) of the rs12901499 allele was the allele that most frequently (50%) appeared in knee OA patients as well as non-OA subjects (50%). The G allele frequency was higher than the A allele among all participants. The Chi-Square analysis showed that there was no statistically significant relationship between allele variations in the SMAD3 rs12901499 gene and knee OA ($p=1$). In conclusion, there was no statistically significant relationship between the rs12901499 genetic variation in the SMAD3 gene and the incidence of knee osteoarthritis in Indonesian aged women.

Keywords: Aged women; Indonesian; Knee Osteoarthritis; rs12901499; SMAD3.

АБСТРАКТ

Small Mother Against Decapentaplegic 3 (SMAD3), внутриклеточный трансдукторный белок в сигнальном пути TGF- β , играет роль в поддержании суставного хряща. Было проведено множество исследований по изучению влияния полиморфизма в гене SMAD3 на заболеваемость остеоартритом (ОА). Цель данного исследования - выявить ассоциацию вариации rs12901499 гена SMAD3 с заболеваемостью ОА коленного сустава у индонезийских женщин. Мы провели аналитическое перекрестное исследование с участием 24 пациенток с ОА коленного сустава и 50 человек без ОА. ДНК была взята из слюны и генотипирована с помощью набора от Integrated DNA Technologies методом ПЦР в реальном времени. В этом исследовании мы обнаружили, что генотип GA (гетерозиготный мутант) аллеля rs12901499 был аллелем, который наиболее часто (50%) встречался у пациентов с ОА коленного сустава, а также у лиц без ОА (50%). Частота аллеля G была выше, чем аллеля A среди всех участников. Анализ Хи-квадрат показал отсутствие статистически значимой связи между вариациями аллелей в гене SMAD3 rs12901499 и ОА коленного сустава ($p=1$). В заключение следует отметить отсутствие статистически значимой связи между генетической вариацией rs12901499 в гене SMAD3 и распространенностью остеоартрита коленного сустава у индонезийских пожилых женщин.

Ключевые слова: Пожилые женщины; индонезийцы; остеоартрит коленного сустава; rs12901499; SMAD3.

INTRODUCTION

OA is a joint disease caused by a process of biomechanical degeneration that triggers an inflammatory reaction. OA often occurs on the weight-bearing joints, like the hips, knees, spine, hands, and feet. Symptomatic OA will cause symptoms such as pain and stiffness in the joints, which can interfere with and hinder the activities of most patients, who are elderly.^{1,2}

OA is a complex disease that many factors could lead someone to experience OA.³ Genetic factor is one of the biggest risk factor that contributed 39-65% in the incidence of OA.⁴ The study, entitled "Genetic influence on the progression of radiographic knee osteoarthritis: a longitudinal twin study", found that there is genetic influence on the pathophysiology and progression of OA.⁵ Many loci are associated with OA pathogenesis and one of them is the SMAD3 gene⁶. SMAD3 gene encoded SMAD3 forming protein. SMAD3 is an intracellular transducer in the Transforming Growth Factor- β (TGF- β) signalling pathway that plays an important role in controlling chondrocyte differentiation.⁷ Several polymorphism studies showed the association between the SMAD3 gene and knee OA risk.⁸ Study from India showed the GA and GG genotype were found in SMAD3 rs12901499 and the G allele frequency was higher than the A allele.⁹ Zhang Li et al. reported the GG genotype of rs12901499 could decrease the risk of knee OA compared to AA genotype on a Chinese population.

However, there are still differences in results, and no studies have been conducted on the Indonesian population. The aim of this is to identify the association of SMAD3 rs12901499 genetic variation with the incidence of knee OA in the Indonesian women.

MATERIAL AND METHODS

Study Design and Ethical Approval

This study was conducted with an analytic cross-sectional design to compare the allele with the highest frequency in OA patients with

the other 2 alleles and compare them with controls/non-OA. This protocol was approved by The Ethics Committee of the Faculty of Medicine, UIN Syarif Hidayatullah Jakarta (B-077/F12/KEPK/TL.00/06/2022).

Research Subjects

This study involved 22 patients with knee OA and 50 non-OA of aged women in Banten area. Participants were selected by consecutive sampling. All of them were Indonesian women who met the research criteria. After participants were fully explained the rights, risks, and benefits of our research and signed written informed consent, 10 ml of saliva was collected for simple, inexpensive, and harmless DNA collection.¹⁰ Saliva samples that have been collected but not directly extracted was stored at -20°C.¹¹ This research was conducted between July-October 2022.

Inclusion and Exclusion Criteria

Subjects were divided into 2 groups: OA and non-OA as a control. OA subjects were elderly women diagnosed with OA by Kellgren-Lawrence radiographic classification at FK UIN Research Teaching Clinical Unit (RCTU) in Banten, Indonesia.¹² Non-OA subjects were women in Banten area who were aged ≥ 45 years and did not meet OA criteria according to The American College of Rheumatology.⁷

Subjects who did not agree to join and had a history of knee trauma or deformity were excluded.

DNA Extraction and Genotyping

Saliva samples were centrifuged to separate the supernatants and the pellets. Then the pellets were washed using 5 mL of 0.9% NaCl. After that, 1 mL of 0.9% NaCl was added to each washed pellet to produce ready-to-extract samples. The DNA extraction was performed using the Quick-DNA™ Miniprep Plus Kit from ZYMO Research.¹³ The Quick-DNA Miniprep Plus Kit was the most efficient and cost-effective to extract genomic

DNA.¹⁴ Then the extracted DNA was measured for purity and concentration using the Nanodrop DeNovix DS-11+Spectrophotometer.¹⁵

Genotyping rs12901499 was performed using The rhAmp-SNP Genotyping Integrated DNA Technologies Assay Kit for rs12901499 in Real-Time PCR (LightCycler® 480 II Instrument from Roche), which connected with LightCycler® 480 Software for further genotype data analysis.¹⁶ We used rhAmp SNP genotyping for its reliability and cost-effective option for targeted genotyping.¹⁷ The rs12901499 polymorphism includes the GG (homozygous mutant), GA (heterozygous mutant), and AA (wildtype) genotypes.⁸

Data Analysis and Management

The data analysis used an endpoint genotyping experiment with filter combinations for X and Y axes on LightCycler® 480 Software. The results of the genotyping of rs12901499 were managed using Microsoft Office Excel and IBM SPSS Statistic 24. We compared the allele with the highest frequency in OA patients with the other 2 alleles and compared them with

controls/non-OA. The results were analysed with descriptive statistic and continuity correction Chi-square hypothesis testing. Statistically significance is determined at p -value <0.05 .¹⁸

RESULT

A total of 72 aged women from Banten area were participated to this study. According to The American College of Rheumatology, being >50 years old is one of the criteria for diagnosing someone with OA.¹⁹ Based on that, we divided the participants age category into ≤ 50 years old and >50 years old. As shown in Table 1, all the OA patients and 24 the non-OA participants were older than 50 years. While 26 the non-OA participants were younger than 50 years.

As shown in Table 2, the GA allele (heterozygous mutant) was found with the highest frequency of all allele variations in OA patients, followed by the GG (homozygous mutant) and AA (wildtype) alleles. Consistent with the results of OA patients, we also found the GA allele to be the allele with the highest frequency in non-OA participants.

Table 1. The characteristic of participants (n=72)

Variable	OA		Non-OA	
	n	%	n	%
Age				
≤50 yo	0	0	26	52
>50 yo	22	100	24	48
Total	22	100	50	100

Table 2. The genotyping of rs12901499

SNP	Allele	OA		Non-OA	
		n	%	n	%
rs12901499	GG	7	31.9	17	34
	GA	11	50	25	50
	AA	4	18.1	8	16
	Total	22	100	50	100

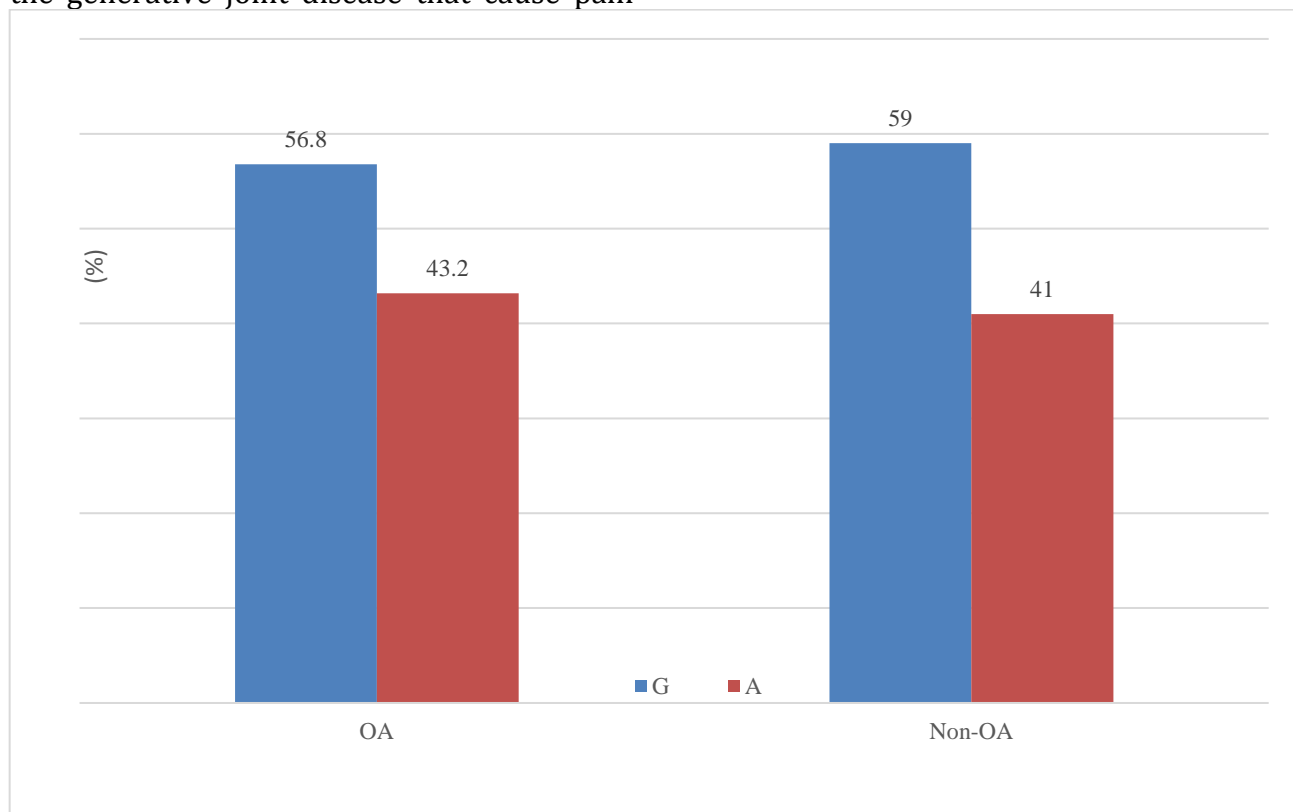
Table 3. The comparison of the highest frequent allele with the other alleles in OA and non-OA

Allele	GA	OA		Non-OA		<i>p</i>
		n	%	n	%	
	Non-GA	11	50	25	50	1.00
Total		22	100	50	100	

DISCUSSION

Single Nucleotide Polymorphisms (SNPs) are genetic variations that occur when a single nucleotide in the DNA sequence changes.²⁰ The SNP rs12901499 is associated with OA, the generative joint disease that cause pain

and disability millions people worldwide. The genotype of this SNP can be used to predict or identify people who are at a high risk for developing OA and enable physicians to be more intense in monitoring the patients' disease.

**Figure 1.** Allele G and A frequency in OA and non-OA

We found the GA allele (heterozygous mutant) was the highest frequency of all allele variations in OA patients. This result is in line with a study in a Chinese Han population.²¹ Moreover, in this study we compared the allele with the highest frequency in OA patients with the other 2 alleles and compared them with controls/non-OA. As shown in Table 3, the result was in both OA patients and non-OA participants: the GA (heterozygous

mutant) allele frequency was 50%, as was the other alleles frequency (accumulation of GA and GG alleles).

We performed the continuity correction value of the Chi-Square test to our results to analyse the relationship between genetic variation in SMAD3 gene rs12901499 with the incidence of knee OA. We found a *p*-value >0.05, which means the null hypothesis is accepted. Based on the analysis of the research results, we found that there was no

statistically significant relationship between the rs12901499 genetic variation in the SMAD3 gene and the incidence of knee osteoarthritis.

Furthermore, the calculation of allele G and allele A frequencies among the participants, as shown in Figure 1, the G allele frequency was higher than the A allele. We indicate that the G allele could increase the risk of knee OA compared with the A allele. This result is in line with Ana M Valdes et al., who found that the G allele was found to be increased in OA patients.²² This result is also reinforced by research conducted by Amar Chandra Sharma et al. and Fuhua Zhong et al., whose research concluded that the G may increase the risk of developing OA.^{9,21}

In a contrast, study conducted by Zhang Li et al. found the GG genotype of rs12901499 could decrease the risk of knee OA compared to AA genotype.¹ This matter can occur because the distribution of allele variations is different in each region. Quoted from asia.ensembl.org, the distribution of the rs12901499 allele in the Asian continent grouped into two. First, in East Asia (EAS), the allele G found as much as 52% and the allele A as much as 48%. Second, in Asia South (SAS), the G allele was found in 32% and the A allele in 68%. The results of this study appear to be more in line with the proportions of alleles present in East Asia.²³ Further study with more number and diverse ethnic populations are needed to confirm these results. Not only about genetic, the incidence of knee OA also corelated of other environmental factors like IMT, diet, history of trauma, and many more.³

CONCLUSION

In conclusion, there was no statistically significant relationship between the rs12901499 genetic variation in the SMAD3 gene. Thus, the incidence of knee osteoarthritis in Indonesian aged women might due to the influence of other genetic and environmental factors.

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DECLARATIONS

The authors declare no conflict of interest.

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