Brunei International Medical Journal

OFFICIAL PUBLICATION OF THE MINISTRY OF HEALTH, BRUNEI DARUSSALAM

Volume 14 29 May 2018 (13 Ramadhan 1439H)

ASSOCIATION BETWEEN SMAD3 GENE rs6494629 C/T POLYMORPHISM AND KNEE OSTEOARTHRITIS IN INDONESIAN POPULATION.

B MARPAUNG, OM SJAH
Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

ABSTRACT

Background. Osteoarthritis (OA) risk factors include older age, obesity, physical activity, trauma, female gender, and genetic factors. One of the genetic factors suspected to be involved in OA development is the Sma and Mad homologue 3 (SMAD3) polymorphism. Previous studies had examined the association between SMAD3 rs6494629 C/T polymorphism and risk of knee OA, unfortunately the results were controversial. The aim of this study was to evaluate the association between SMAD3 rs6494629 C/T polymorphism and knee OA in Medan, Indonesia. **Methods.** This study was a case control study on 80 consecutive OA patients and 100 healthy controls recruited from Adam Malik General Hospital, Medan, Indonesia. The diagnosis of knee OA was confirmed by clinical examination and knee radiographs, which fulfilled the American College of Rheumatology criteria. Only patients with Kellgren–Lawrence (K-L) score of ≥2 were included in the study. *SMAD3* gene rs6494629 C/T polymorphism was determined using PCR-RFLP method. Data were analyzed using SPSS version 22. **Results.** Case and control groups were homogeneous by age and sex. There was a significant association between SMAD3 gene rs6494629 C/T polymorphism and knee OA in the ≤50 years age group. Patients with TT genotype and T allele were at risk for developing knee OA ($p < 0.05$), but this association did not appear within the >50 years age group. **Conclusion.** Our findings suggested that knee OA in the younger age is predominantly affected by genetic factors, whereas knee OA in older age group is influenced by other factors.

Keywords: Knee osteoarthritis, SMAD3 protein, polymorphism, TGF-β, molecular epidemiology

Brunei Int Med J. *2018;14:67-72*

ISSN 1560 5876 Print
ISSN 2079 3146 Online

Online version of the journal is available at www.bimjonline.com

Brunei International Medical Journal (BIMJ)
Official Publication of the Ministry of Health, Brunei Darussalam

ISSN 1560-5876 Print
ISSN 2079-3146 Online

Aim and Scope of Brunei International Medical Journal

The Brunei International Medical Journal (BIMJ) is a six monthly peer reviewed official publication of the Ministry of Health under the auspices of the Clinical Research Unit, Ministry of Health, Brunei Darussalam.

The BIMJ publishes articles ranging from original research papers, review articles, medical practice papers, special reports, audits, case reports, images of interest, education and technical/innovation papers, editorials, commentaries and letters to the Editor. Topics of interest include all subjects that relate to clinical practice and research in all branches of medicine, basic and clinical including topics related to allied health care fields. The BIMJ welcomes manuscripts from contributors, but usually solicits reviews articles and special reports. Proposals for review papers can be sent to the Managing Editor directly. Please refer to the contact information of the Editorial Office.

Instruction to authors

Manuscript submissions

All manuscripts should be sent to the Managing Editor, BIMJ, Ministry of Health, Brunei Darussalam; e-mail: editor-in-chief@bimjonline.com. Subsequent correspondence between the BIMJ and authors will, as far as possible via should be conducted via email quoting the reference number.

Conditions

Submission of an article for consideration for publication implies the transfer of the copyright from the authors to the BIMJ upon acceptance. The final decision of acceptance rests with the Editor-in-Chief. All accepted papers become the permanent property of the BIMJ and may not be published elsewhere without written permission from the BIMJ.

Ethics

Ethical considerations will be taken into account in the assessment of papers that have experimental investigations of human or animal subjects. Authors should state clearly in the Materials and Methods section of the manuscript that institutional review board has approved the project. Those investigators without such review boards should ensure that the principles outlined in the Declaration of Helsinki have been followed.

Manuscript categories

Original articles

These include controlled trials, interventional studies, studies of screening and diagnostic tests, outcome studies, cost-effectiveness analyses, and large-scale epidemiological studies. Manuscript should include the following; introduction, materials and methods, results and conclusion. The objective should be stated clearly in the introduction. The text should not exceed 2500 words and references not more than 30.

Review articles

These are, in general, invited papers, but unsolicited reviews, if of good quality, may be considered. Reviews are systematic critical assessments of literature and data sources pertaining to clinical topics, emphasising factors such as cause, diagnosis, prognosis, therapy, or prevention. Reviews should be made relevant to our local setting and preferably supported by local data. The text should not exceed 3000 words and references not more than 40.

Special Reports

This section usually consist of invited reports that have significant impact on healthcare practice and usually cover disease outbreaks, management guidelines or policy statement paper.

Audits

Audits of relevant topics generally follow the same format as original article and the text should not exceed 1,500 words and references not more than 20.

Case reports

Case reports should highlight interesting rare cases or provide good learning points. The text should not exceed 1000 words; the number of tables, figures, or both should not be more than two, and references should not be more than 15.

Education section

This section includes papers (i.e. how to interpret ECG or chest radiography) with particular aim of broadening knowledge or serve as revision materials. Papers will usually be invited but well written paper on relevant topics may be accepted. The text should not exceed 1500 words and should include not more than 15 figures illustration and references should not be more than 15.

Images of interest

These are papers presenting unique clinical encounters that are illustrated by photographs, radiographs, or other figures. Image of interest should include a brief description of the case and discussion with educational aspects. Alternatively, a mini quiz can be presented and answers will be posted in a different section of the publication. A maximum of

three relevant references should be included. Only images of high quality (at least 300dpi) will be acceptable.

Technical innovations
This section include papers looking at novel or new techniques that have been developed or introduced to the local setting. The text should not exceed 1000 words and should include not more than 10 figures illustration and references should not be more than 10.

Letters to the Editor
Letters discussing a recent article published in the BIMJ are welcome and should be sent to the Editorial Office by e-mail. The text should not exceed 250 words; have no more than one figure or table, and five references.

Criteria for manuscripts
Manuscripts submitted to the BIMJ should meet the following criteria: the content is original; the writing is clear; the study methods are appropriate; the data are valid; the conclusions are reasonable and supported by the data; the information is important; and the topic has general medical interest. Manuscripts will be accepted only if both their contents and style meet the standards required by the BIMJ.

Authorship information
Designate one corresponding author and provide a complete address, telephone and fax numbers, and e-mail address. The number of authors of each paper should not be more than twelve; a greater number requires justification. Authors may add a publishable footnote explaining order of authorship.

Group authorship
If authorship is attributed to a group (either solely or in addition to one or more individual authors), all members of the group must meet the full criteria and requirements for authorship described in the following paragraphs. One or more authors may take responsibility 'for' a group, in which case the other group members are not authors, but may be listed in an acknowledgement.

Authorship requirement
When the BIMJ accepts a paper for publication, authors will be asked to sign statements on (1) financial disclosure, (2) conflict of interest and (3) copyright transfer. The correspondence author may sign on behalf of co-authors.

Authorship criteria and responsibility
All authors must meet the following criteria: to have participated sufficiently in the work to take public responsibility for the content; to have made substantial contributions to the conception and design, and the analysis and interpretation of the data (where applicable); to have made substantial contributions to the writing or revision of the manuscript; and to have reviewed the final version of the submitted manuscript and approved it for publication. Authors will be asked to certify that their contribution represents valid work and that neither the manuscript nor one with substantially similar content under their authorship has been published or is being considered for publication elsewhere, except as described in an attachment. If requested, authors shall provide the data on which the manuscript is based for examination by the editors or their assignees.

Financial disclosure or conflict of interest
Any affiliation with or involvement in any organisation or entity with a direct financial interest in the subject matter or materials discussed in the manuscript should be disclosed in an attachment. Any financial or material support should be identified in the manuscript.

Copyright transfer
In consideration of the action of the BIMJ in reviewing and editing a submission, the author/s will transfer, assign, or otherwise convey all copyright ownership to the Clinical Research Unit, RIPAS Hospital, Ministry of Health in the event that such work is published by the BIMJ.

Acknowledgements
Only persons who have made substantial contributions but who do not fulfill the authorship criteria should be acknowledged.

Accepted manuscripts
Authors will be informed of acceptances and accepted manuscripts will be sent for copyediting. During copyediting, there may be some changes made to accommodate the style of journal format. Attempts will be made to ensure that the overall meaning of the texts are not altered. Authors will be informed by email of the estimated time of publication. Authors may be requested to provide raw data, especially those presented in graph such as bar charts or figures so that presentations can be constructed following the format and style of the journal. Proofs will be sent to authors to check for any mistakes made during copyediting. Authors are usually given 72 hours to return the proof. No response will be taken as no further corrections required. Corrections should be kept to a minimum. Otherwise, it may cause delay in publication.

Offprint
Contributors will not be given any offprint of their published articles. Contributors can obtain an electronic reprint from the journal website.

ASSOCIATION BETWEEN SMAD3 GENE rs6494629 C/T POLYMORPHISM AND KNEE OSTEOARTHRITIS IN INDONESIAN POPULATION.

B MARPAUNG, OM SJAH
Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

ABSTRACT

Background. Osteoarthritis (OA) risk factors include older age, obesity, physical activity, trauma, female gender, and genetic factors. One of the genetic factors suspected to be involved in OA development is the Sma and Mad homologue 3 (SMAD3) polymorphism. Previous studies had examined the association between SMAD3 rs6494629 C/T polymorphism and risk of knee OA, unfortunately the results were controversial. The aim of this study was to evaluate the association between SMAD3 rs6494629 C/T polymorphism and knee OA in Medan, Indonesia. **Methods.** This study was a case control study on 80 consecutive OA patients and 100 healthy controls recruited from Adam Malik General Hospital, Medan, Indonesia. The diagnosis of knee OA was confirmed by clinical examination and knee radiographs, which fulfilled the American College of Rheumatology criteria. Only patients with Kellgren–Lawrence (K-L) score of ≥2 were included in the study. *SMAD3* gene rs6494629 C/T polymorphism was determined using PCR-RFLP method. Data were analyzed using SPSS version 22. **Results.** Case and control groups were homogeneous by age and sex. There was a significant association between SMAD3 gene rs6494629 C/T polymorphism and knee OA in the ≤50 years age group. Patients with TT genotype and T allele were at risk for developing knee OA ($p < 0.05$), but this association did not appear within the >50 years age group. **Conclusion.** Our findings suggested that knee OA in the younger age is predominantly affected by genetic factors, whereas knee OA in older age group is influenced by other factors.

Keywords: Knee osteoarthritis, SMAD3 protein, polymorphism, TGF-β, molecular epidemiology

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease and the main cause of chronic pain and disability.[1] OA occurs most commonly in knee, hands, feet, and spine joints, and is relatively common in shoulder and hip joints. The prevalence of OA continues to increase due to population ageing. Risk factors for OA include older age, obesity, physical activity, trauma, female gender, and genetic factors.[2-7]

One of the genetic factors suspected to be involved in OA development is the Sma and Mad homologue 3 (SMAD3) polymorphism.[8] SMAD3 protein is a member of SMAD family and it is encoded by the SMAD3 gene located on chro-mosome 15q21-22, including 9 exons and 8 introns.[8,9] SMAD3 protein is the most important medium in transforming growth factor-β (TGF-β)/Smad signal transduction.[10,11] The protein encoded

Correspondence: Blondina Marpaung, MD., Ph.D. Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Dr. Mansyur 5 Medan, Indonesia. Tel: +6281262186275. Email: blondina@usu.ac.id

by this gene forms the family of protein SMAD which is an intracellular molecule or protein associated with TGF-β signalling pathways. SMAD3 expression has been postulated to play a role in OA development.[12-14] SMAD3 protein acts as a mediator that has the effect of inhibiting TGF-β on chondrocyte maturation.[15]TGF-β is thought to play a role in protecting against cartilage damage. The low levels of TGF-β are associated with cartilage damage and OA progression.[16]

Previous studies had examined the relationship between SMAD3 rs6494629 C/T polymorphism and risk of knee OA, but with controversial results, which may be due to racial/ geographical differences in susceptibility of SMAD3 polymoprhism to knee OA.[17-22] Hence, the aim of this study is to investigate the association between SMAD3 rs6494629 C/T polymorphism and knee OA in the Indonesian population.

METHOD

Patient Selection

This study was a prospective case control study on 80 consecutive knee OA patients recruited from the Rheumatology clinic at Adam Malik General Hospital, Medan, Indonesia between October 2017 and March 2018. One hundred healthy controls attending other clinics at the hospital, matched to age and gender were also recruited. Demogrphic data on age, gender, height and weight were collected, the latter two for calculation of body mass index (BMI). The diagnosis of knee OA was confirmed by clinical examination and Knee radiographs, which also fulfilled the criteria of the American College of Rheumatology.[23] The radiographic film evaluation was performed on anteroposterior and lateral radiographic views of the knee. Severity on the knee OA population was assessed using the Kellgren–Lawrence (K–L) grading scale.[24] OA was classified into mild (K-L grade 2), moderate (K-L grade 3), and severe (K-L grade 4). Only patients with radiographic evidence of OA, defined as K-L score of ≥2, were included in the study. Subjects with other etiologies of knee joint disorders such as rheumatoid arthritis, gouty arthritis, septic arthritis, post traumatic, or dysplasias were excluded. This study also included 100 age and sex-matched healthy control participants with no symptoms of joint disease (pain, swelling, tenderness, or restriction of movement) in whom standard radiograph of knee joints confirmed the absence of radiographical knee OA. Subjects in both groups with chronic diseases like cardiopulmonary disease, diabetes, hypertension, as well as tumor or autoimmune disease also were excluded. Written informed consent were obtained from all participants. This study was approved by the Institutional Review Board of Universitas Sumatera Utara.

SMAD3 rs6494629 C/T

Genomic DNA was extracted from whole blood collected in ethylenediamine tetraacetic acid (EDTA) tubes from knee OA and control groups using the standard isothiocyanate guanidine (GTC) extraction method and/or the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Single nucleotide polymorphism (SNP) in the *SMAD3* gene, rs6494629 C/T was determined using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method.[25] Amplification reaction was performed with 200 ng of genomic DNA in a 50 -µl PCR mixture using 10 pmol of each primer, 0.25 mM each deoxyribonucleoside triphosphate (dNTP) (Qiagen), 1 U HotStar Taq polymerase (Qiagen) and ×1 PCR buffer (containing 1.5 µM magnesium chloride; Qiagen). The PCR primers used for SMAD3 rs6494629 C/T polymorphisms were 5′-CATCTTTCCTCCTGGCCATA-3′ (forward) and (5′-CTTAGCGAAGGAAACCAGCA-3′ (reverse). The PCR cycle conditions consist of an initial denaturation step at 95°C for 15 min, followed by 35 cycles at 94°C for 30 s, 55.6°C for 60 s

and 72°C for 60 s, with an extension at 72°C for 10 min. Ten µl of PCR product [430 base pairs (bp) in length] was digested with 1 µl HpaII (Fermentas/Fisher Scientific, UK) at 37°C for 15 min, separated on a 2.5% agarose gel and visualized with ethidium bromide staining under ultraviolet light. HpaII digestion of the PCR product yielded 155 and 275 bp for allele C and 399 bp for the undigested allele T. Randomly selected patients were analysed by direct sequencing, using an ABI PRISM Sequencer (Applied Biosystems) to confirm the accuracy of the method.

Statistical Analysis

Total knee OA subjects had met minimum sample size of the estimation sample calculation. Case and control groups were divided into two groups based on age $\leq$ 50 years and > 50 years to determine association between SMAD3 gene polymorphism and knee OA in younger and older age group. Data analysis was performed through simple logistic regresion using SPSS 22nd version (SPSS Inc., Chicago). A value of $p < 0.05$ with 95% confidence interval was considered statistically significant.

RESULTS

Case and control groups were homogeneous by age and sex. BMI was significantly higher in cases than control group. Majority of cases (93.75%) were patients with mild to moderate (K-L grade 2-3) OA (Table 1).

There was no significant association between SMAD3 gene rs6494629 C/T polymorphism and knee OA in cases as compared to controls (Table 2). However, when the cases and controls were divided into two groups based on age $\leq$ 50 years and > 50 years, there was a significant association between SMAD3 gene rs6494629 C/T polymorphism and knee OA in the younger age groups ($\leq$50 years), particularly cases with TT genotype and T allele were at higher risk for developing knee OA (TT genotype, OR 2.54; T allele, OR 1.71). However this association did not appear in the > 50 years age group (Table 3).

DISCUSSION

SMAD protein is a family of intracellular protein that acts as a signal transduction for TGF-β, from cell membran to nucleus.[14] Low SMAD3 increased bone morphogenetic protein signaling and accelerated chondrocytes differentiation.[26] TGF-β can regulate MMP through SMAD3 protein levels during the OA progression process.[27] Van der Kraan et al reported that TGF-β might regulate the differentiation of chondrocytes that were important for OA. TGF- β has an anabolic effect on chondrocytes through SMAD3 gene signals that play a role for synovial joint homeostasis.[28] TGF-β is important for maintaining the cartilage. Low TGF -β is associated with increased risk of OA.[29,30]

The occurrence of OA is influenced by genetic factors. SMAD3 gene polymorphisms

Table 1. Baseline characteristics in knee OA patients and controls

Variable	Cases	Controls	p
Age (years)	53.8 $\pm$ 6.8	54.3 $\pm$ 6.2	0.837
Gender			
Female	45 (56.3%)	56 (56%)	0.973
Male	35 (43.7%)	44 (44%)	
Body mass index	26.2 $\pm$ 4.4	24.1 $\pm$ 3.5	<0.001
Kellgren-Lawrence grading			
2	39 (48.8%)	–	
3	36 (45%)	–	
4	5 (6.2%)	–	

Table 2. Association between SMAD3 gene rs6494629 C/T polymorphism and knee OA

Genotype/ Allele	Knee OA n (%)	Control n (%)	Total n (%)	p	OR (95% CI)
TT	17 (68)	8 (32)	25 (100)	0.055	1.72 (1.01 -2.49)
CT	34 (43)	45 (57)	79 (100)	0.671	1.03 (0.71-1.5)
CC	29 (38.2)	47 (61.8)	76 (100)		1 (ref.)
TT+CT	51 (49)	53 (51)	104 (100)	0.320	1.15 (0.82-1.61)
CC	29 (38.2)	47 (61.8)	76 (100)		1 (ref.)
TT	17 (68)	8 (32)	25 (100)	0.056	1.5 (1.05-2.15)
CT+CC	63 (40.6)	92 (59.4)	155 (100)		1 (ref.)
Allele T	68 (52.7)	61 (47.3)	129 (100)	0.078	1.41 (0.98 – 1.56)
Allele C	92 (39.8)	139 (60.2)	231 (100)		1 (ref.)

were reported to be associated with OA development, but these results were controversial. [17-22] Gene polymorphisms are strongly influenced by ethnicity so they showed different results. The study of Valdes in Europe showed SMAD3 polymorphism rs6494629C/T rs266335G/A, rs12901499A/G, and rs2289263A/C were significantly associated with knee OA, but only rs12901499A/G was associated with hip OA.[18] A study by Zhang et al dan Liying et al in Chinese population and Sharma et al in India found that SMAD3 rs12901499 polymorphism was responsible for the occurrence of knee OA.[20,22,31] Kang et al in China showed that patients with TT genotype and T allele of rs12102171, GG genotype and G allele of rs2289263 polymorphisms significantly increased the risk of having OA compared to control group.[8] However, Su et al in Taiwan reported no association of SMAD3 polymorphism (rs6494629 C/T and rs12901499A/G) and knee OA.[19] The GG genotype of rs12901499 might lower the risk of developing knee OA compared with the AA genotype.[22] On the contrary Sharma et al in India and Liying et al in northeastern China showed that SMAD3 rs12901499 polymorphism, GG and GA genotype, were significantly associated with the knee OA.[20,31]

Table 3. Association between SMAD3 gene rs6494629 C/T polymorphism and knee OA by age group

Genotype/ Allele	Knee OA n (%)	Control n (%)	Total n (%)	p	OR (95% CI)
Age ≤50 years					
TT	9 (90)	1 (10)	10 (100)	0.001*	3.6 (1.64 -7.91)
CT	17 (40.5)	25 (59.5)	42 (100)	0.234	1.62 (0.7-3.76)
CC	5 (25)	15 (75)	20 (100)		1 (ref.)
TT+CT	26 (50)	26 (50)	52 (100)	0.055	2 (0.89-4.48)
CC	5 (25)	15 (75)	20 (100)		1 (ref.)
TT	9 (90)	1 (10)	10 (100)	0.002*	2.54 (1.71-3.76)
CT+CC	22 (35.5)	40 (64.5)	62 (100)		1 (ref.)
Allele T	35 (56.5)	27 (43.5)	62(100)	0.005*	1.71 (1.17 – 2.5)
Allele C	27 (32.9)	55 (67.1)	82 (100)		1 (ref.)
Age >50 years					
TT	8 (53.3)	7 (46.7)	15 (100)	0.469	1.24 (0.71 -2.18)
CT	17 (45.9)	20 (54.1)	37 (100)	0.769	1.07 (0.68-1.7)
CC	24 (42.9)	32 (57.1)	56 (100)		1 (ref.)
TT+CT	25 (48.1)	27 (51.9)	52 (100)	0.691	1.02 (0.72-1.53)
CC	24 (42.9)	32 (57.1)	56 (100)		1 (ref.)
TT	8 (53.3)	7 (46.7)	15 (100)	0.840	0.94 (0.49-1.78)
CT+CC	41 (44.1)	52 (55.9)	93 (100)		1 (ref.)
Allele T	33 (49.3)	34 (50.7)	67 (100)	0.442	1.13 (0.83 – 1.53)
Allele C	65 (43.6)	84 (56.4)	149 (100)		1 (ref.)

*p<0.05

In this study found that there was no significant association between SMAD3 gene rs6494629 C/T polymorphism and knee OA. Although subgroup analysis of age showed positive association between this SNP and knee OA risk in the younger age groups (≤ 50 years). Patients with TT genotype and T allele were at risk for developing knee OA ($p < 0.05$), but this association appeared in the ≤50 years age group. Our findings suggest that the role of SMAD3 rs6494629 genotypes with TT genotype and T allele may play a significant role in knee OA in younger patients but not in older patients (>50 years) where other comorbidities and environmental factors such as obesity, lack of physical activity, bone density, and trauma may contribute to the development of OA.

The limitations of our study include the small sampel size which might make our study underpowered, as well as this study was not assess the role of SMAD3 rs6494629 C/T polymorphism against the expression of TGF-β. This study was a hospital-based case-control study, and selection bias might happened.

CONCLUSION

SMAD3 gene rs6494629 C/T polymorphism, predisposes to development of OA in younger age groups, whereas knee OA in older age was more influenced by other factors. Further studies are necessary to examine other SNPs that may affect the occurrence of knee OA and also identify the association of this polymorphism with other joints such as hip OA and spine OA.

Competing Interests: The authors have declared no conflict of interests present.

Funding: This research did not receive any financial support.

REFERENCES

1: Murray CJ, Atkinson C, Bhalla K, et al. The state of US health, 1990-2010: Burden of diseases, injuries, and risk factors. JAMA. 2013;310:591–608. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5436627/)

2: Chaganti RK, Lane NE. Risk factors for incident osteoarthritis of the hip and knee. Curr Rev Musculoskelet Med. 2011;4:99-104. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3261259/)

3: Yucesoy B, Charles LE, Baker B, Burchfiel CM. Occupational and genetic risk factors for osteoarthritis: A review. Work. 2015;50: 261–273. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4562436/)

4: Haq I, Murphy E, Dacre J. Osteoarthritis. Postgrad Med J. 2003;79:377–383. (Full text available at http://pmj.bmj.com/content/postgradmedj/79/933/377.full.pdf)

5: Du H, Chen SL, Bao CD, Wang XD, Lu Y, Gu YY. Prevalence and risk factors of knee osteoarthritis in Huang- Pu District, Shanghai, China. Rheumatol Int. 2005;25:585–590.

6: Silverwood V, Blagojevic-Bucknall M, Links C, Jordan JL, Protheroe J, Jordan KP. Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage. 2015;23: 507-515. (Full text available at https://www.oarsijournal.com/article/S1063-4584(14)01342-9/fulltext)

7: Loughlin J. The genetic epidemiology of human primary osteoarthritis: current status. Expert Rev Mol Med. 2005;7: 1-12.

8: Kang B, Zhao F, Zhang X, Deng X, He X. Association between the interaction of SMAD3 polymorphisms with body mass index and osteoarthritis susceptibility. Int J Clin Exp Pathol. 2015;8:7364-7370. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4525971/)

9: Arai T, Akiyama Y, Okabe S, Ando M, Endo M, Yuasa Y. Genomic structure of the human SMAD3 gene and its infrequent alterations in colorectal cancers. Cancer Lett. 1998; 122: 157-163.

10: Roberts AB, Russo A, Felici A, Flanders KC. Smad3: a key player in pathogenetic mecha-nisms dependent on TGF-beta. Ann N Y Acad Sci. 2003; 995: 1-10.

11: Brown KA, Pietenpol JA, Moses HL. A tale of two proteins: differential roles and regulation of Smad2 and Smad3 in TGF-beta signaling. J Cell Biochem. 2007; 101: 9-33.

12: Chen CG, Thuillier D, Chin EN, Alliston T. Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. Arthritis Rheum. 2012;64: 3278-3289. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3544176/)

13: Li TF, Gao L, Sheu TJ, et al. Aberrant hypertrophy in Smad3-deficient murine chondrocytes is rescued by restoring transforming growth factor beta-activated kinase 1/activating transcription factor 2 signaling: a potential clinical implication for osteoarthritis. Arthritis Rheum. 2010;62: 2359-2369. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2921996/)
14: Yang X, Chen L, Xu X, Li C, Huang C, Deng CX. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol. 2001;153:35-46. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2185521/)
15: Ferguson CM, Schwarz EM, Reynolds PR, Puzas JE, Rosier RN, O'Keefe RJ. Smad2 and 3 mediate transforming growth factor-beta1-induced inhibition of chondrocyte maturation. Endocrinology. 2000;141:4728-4735. (Full text available at https://academic.oup.com/endo/article/141/12/4728/2988591)
16: Blaney Davidson EN, Vitters EL, van der Kraan PM, van den Berg WB. Expression of transforming growth factor-beta (TGF-β) and the TGF-β signalling molecule SMAD-2P in spontaneous and instability-induced osteoarthritis: role in cartilage degradation, chondrogenesis and osteophyte formation. Ann Rheum Dis. 2006;65:1414-1421. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1798346/)
17: Xiao JL, Meng JH, Gan YH, Zhou CY, Ma XC. Association of GDF5, SMAD3 and RUNX2 polymorphisms with temporomandibular joint osteoarthritis in female Han Chinese. J Oral Rehabil. 2015;42:529-536.
18: Valdes AM, Spector TD, Tamm A, et al. Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. Arthritis Rheum. 2010;62: 2347-2352. (Pdf available at http://twinsuk.ac.uk/wpcontent/uploads/2012/03/Valdes_ArthtirisRheum_2010.pdf)
19: Su SL, Yang HY, Lee HS, et al. Gene-gene interactions between TGF-beta/ Smad3 signalling pathway polymorphisms affect susceptibility to knee osteoarthritis. BMJ Open. 2015;5:e007931. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4466616/)
20: Liying J, Yuchun T, Youcheng W, et al. A SMAD3 gene polymorphism is related with osteoarthritis in a Northeast Chinese population. Rheumatol Int. 2013;33:1763-1768.
21: Liva E, Panagiotou I, Palikyras S, et al. Candidate gene investigation of spinal degenerative osteoarthritis in Greek population. Spine J. 2017;17:1881-1888.
23: Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum. 1986;29: 1039–1049.
24: Sanghi D, Avasthi S, Mishra A, Singh A, Agarwal S, Srivastava RN. Is radiology a determinant of pain, stiffness, and functional disability in knee osteoarthritis? J Orthop Sci. 2011;16:719-725.
25: Paradowska-Gorycka A, Romanowska-Prochnicka K, Haladyi E, Manczak M, Maslinski S, Olesinska M. Association of the Smad3 and NFATc2 gene polymorphisms and their serum levels with susceptibility to rheumatoid arthritis in Polish cohorts. Clin Exp Immunol. 2015;179:444-453.
26: Li TF, Darowish M, Zuscik MJ, et al. Smad3-deficient chondrocytes have enhanced BMP signaling and accelerated differentiation. J Bone Miner Res. 2006;21:4-16. (Full text available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2649698/)
27: Tardif G, Reboul P, Dupuis M, et al. Transforming growth factor-beta induced collagenase-3 production in human osteoarthritic chondrocytes is triggered by Smad proteins: cooperation be-tween activator protein-1 and PEA-3 binding sites. J Rheumatol. 2001;28: 1631-1639.
28: van der Kraan PM, Blaney Davidson EN, Blom A, van den Berg WB. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. Osteoarthritis Cartilage. 2009;17:1539-1545. (Full text available at https://www.oarsijournal.com/article/S1063-4584(09)00167-8/fulltext)
29: Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. Osteoarthritis Cartilage. 2007;15:597-604. (Full text available at https://www.oarsijournal.com/article/S1063-4584(07)00065-9/fulltext)
30: Finnson KW, Parker WL, Chi Y, Hoemann CD, Goldring MB, Antoniou J. Endoglin differentially regulates TGF-beta-induced Smad2/3 and Smad1/5 signalling and its expression correlates with extracellular matrix production and cellular differentiation state in human chondrocytes. Osteoarthritis Cartilage. 2010;18:1518–1527. (Full text available at https://www.oarsijournal.com/article/S1063-4584(10)00299-2/fulltext)
31: Sharma AC, Srivastava RN, Srivastava SR, Pamar D, Singh A, Raj S. Association between Single Nucleotide Polymorphisms of SMAD3 and BMP5 with the Risk of Knee Osteoarthritis. J Clin Diagn Res. 2017;11(6):GC01-GC04. (Full text available at https://www.ncbi.nlm.nih.gov/pubmed/28764194)