Arch Biol Sci. 2024;76(2):151-160 https://doi.org/10.2298/ABS240306010S

Genetic variants in the retinoid X receptor gene contribute to osteoarthritis susceptibility

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Received: March 6, 2024; **Revised**: March 26, 2024; **Accepted**: March 27, 2024; **Published online**: April 19, 2024

Abstract: Osteoarthritis (OA) is a progressive disease of the joints that causes a gradual loss of function, resulting in limited mobility. Chronic inflammation is the main molecular process that triggers and propagates this disease. The retinoid X receptor (RXR), a member of the nuclear receptor family, is involved in modulating inflammatory pathways by influencing key procatabolic inflammatory cytokines, chemokines, and enzymes responsible for instigating and sustaining chronic joint inflammation. We evaluated the association between OA risk and genetic variants in the RXRα isoform. Compared to control individuals, a statistically significant difference in genotype distribution was detected for the rs7864987 polymorphism (P=0.008), while a positive inclination toward association was noted for rs3118523 (P=0.077). According to our findings based on the additive model, it appears that RXRα rs7864987 is linked to a higher risk of OA (adjusted odds ratio (OR)=1.846, P=0.012), whereas rs3118523 is associated with decreased risk of OA (adjusted OR=0.569, P=0.030). These results suggest that RXRα could be a significant inflammation-related gene involved in the complex network underlying the immunopathology of osteoarthritis. RXRα polymorphisms could potentially drive individualized retinoid therapy for OA based on genetic profile.

Keywords: retinoid X receptor (RXR), polymorphism, osteoarthritis, inflammation

Abbreviations: osteoarthritis (OA); retinoid X receptor (RXR); nuclear receptor (NR); vitamin D receptor (VDR); retinoic acid receptor (RAR); thyroid hormone receptor (TR); estrogen receptor (ER); glucocorticoids receptor (GR); retinoic acid response element (RARE); peroxisome proliferator-activated receptor (PPAR); liver X receptor (LXR); farnesoid X receptor (FXR); constitutive androgen receptor (CAR); nuclear factor kappa B (NF-κB); activator protein 1 (AP-1); genome-wide association studies (GWAS); interferon regulatory transcription factor (IRF); mitogen-activated protein kinase (MAPK); monocyte chemoattractant protein-1 (MCP-1); matrix metalloproteinase (MMP); vitamin D response element (VDRE); cyclo-oxygenase-2 (COX-2); inducible nitric oxide synthase (iNOS); all-*trans* retinoic acid (atRA); retinoic acid metabolism blocking agent (RAMBA)

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis in which the normal physiological and homeostatic metabolic functions in cartilage and related joint tissues are impaired, leading to a progressive loss of joint function [1]. The pathogenesis of OA involves a complex interplay of genetic, immunologic, metabolic, and etiological factors [2]. The exact molecular mechanisms that initiate a procatabolic state and contribute to the development of OA are still unknown. Proinflammatory cytokines, chemokines, and degradative enzymes are key players in the development and progression of OA, underscoring the immune system's significant contribution as a primary cause of the disease [3,4]. The increased production of proinflammatory mediators in an OA joint causes a chronic inflammatory state, resulting in cartilage degradation, subchondral bone remodeling, and synovial changes [4].

The retinoid X receptor (RXR) belongs to the nuclear receptor (NR) superfamily that comprises 48 members, including receptors for vitamin D (VDR), retinoic acid receptors (RARs), receptors for thyroid

hormones (TRs), estrogens (ERs), and glucocorticoids (GRs) [5]. RXR is crucial for NR signaling since it is involved in the downstream pathways of several NRs [6]. Three isoforms of RXR-RXRα (NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3) are encoded by three genes (9q34.3, 6p21.1-3, and 1q22-23, respectively), each of which has specific expression patterns, but overlapping functions [7]. RXRs modulate the transcription of target genes by binding to specific DNA sequences in the gene promoter known as response elements [8]. RXRs form dimer complexes with conventional hormone receptors (TR, VDR and RAR), metabolite or drug-sensing receptors (peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), farnesoid X receptor (FXR)), orphan receptors (Nur77, Nur1) and constitutive androgen receptor (CAR) [7]. The complexity of the RXR pathways arises from the diverse modes of RXR activation. The RXR can participate either actively or passively in the heterodimeric complex, depending on whether its ligand or the ligand of its heterodimeric partner activates it. However, it can also homodimerize and exert effects on its own [9]. The broad distribution of the RXR in human tissues reflects the importance of its signaling [10]. The activity of homo/heterodimeric RXRs significantly impacts the expression of numerous genes crucial for fundamental physiological processes such as cell development, differentiation, and proliferation [11,12]. Impaired signaling of the α-RXR isoform has significant effects on various cellular functions, including metabolic processes and homeostasis, suggesting that this isoform is a primary mediator of retinoid signaling [13].

RXRα has a regulatory role in inflammatory processes. Aberrant RXRα signaling influences the inflammatory response by recruiting immune mediators at the site of an infection [14]. RXRs are also involved in clearing apoptotic cells and activating proinflammatory macrophages [15]. PPAR-RXR signaling regulates the transcription of genes associated with adipocyte development, glucose/lipid metabolism, and inflammatory pathways in diabetes [16]. RXR signaling also has a role in the pathogenesis of neurodegenerative disorders such as Parkinson's disease as well as neuroinflammatory diseases such as Alzheimer's disease and multiple sclerosis [17]. Additionally, recent studies suggest that SNPs in the RXRα gene may influence the risk of several types of cancer [18–20].

The complex interaction between genetic factors and joint inflammation significantly influences the structure and function of cartilage, bone remodeling, and joint repair processes, contributing to the distinctive pathology of OA. Genome-wide association studies of OA aim to identify DNA sequence variations linked to the disease and identify potential genetic causes [21]. Nakajima et al. characterized OA as an inflammatory condition, highlighting the potential involvement of inflammatory pathways in its pathogenesis. They unveiled genetic variations in the HLA class II/III region associated with OA susceptibility [22]. The complexity of the genetic background of OA is also highlighted in a study by Cornelis et al., who showed that BMP and WNT signaling pathways could influence skeletal morphogenesis, joint malformations, and tissue homeostasis in articular cartilage and subchondral bone; these pathways may be involved in OA by affecting both chondrocyte cell death and extracellular matrix formation [23]. Bone mass and density, regulated by vitamin D, are factors closely related to OA. The RXR-VDR heterodimers play a crucial role in mediating the genomic effects of vitamin D and represent only a small part of the intricate network of RXR and NR signaling in the joint [24].

Given the insights into RXR function in inflammatory diseases, our aim was to investigate potential associations between common polymorphisms in the RXRα gene and their role as potential predisposing (causing) genetic factors in OA. Our study examined two different single nucleotide polymorphisms (SNPs) in the RXRα gene, rs3118523 and rs7864987, and their association with OA risk in the Caucasian-Serbian population.

MATERIALS AND METHODS

Ethics statement

The Ethics Committee of the Military Medical Academy in Belgrade, Serbia, granted the study approval Nos. 09/07/2014 and 3/4/2023. A signed informed consent form was obtained from each research participant.

Study participants

The study group consisted of 92 patients with primary OA who underwent complete hip or knee replacement surgery at the Clinic for Orthopedic Surgery and

Traumatology, Military Medical Academy, Belgrade, Serbia in the period from 2015-2018. Complete hip replacement surgery was performed in 64% of the respondents (59 patients), while 36% (33 patients) underwent complete knee replacement surgery. Most OA patients were women (64%), with a median age of 69. The control group comprised 91 sex- and age-matched healthy individuals recruited randomly in the institution. The following conditions excluded individuals from taking part in this research: secondary OA caused by trauma or joint structure surgery, congenital malformations, rheumatoid arthritis, developmental or hormone/metabolic abnormalities, gout, and infections. Subjects with a history of malignancy as well as OA or other systemic inflammatory or autoimmune conditions were not eligible to participate in the study as a control. The subjects were all Caucasian.

Biological samples, DNA isolation, and polymorphism genotyping

Following the manufacturer's instructions, genomic DNA for genotyping analysis was extracted from peripheral blood samples maintained at -20°C using the Extract Me Blood Kit (Blirt, Gdansk, Poland). RXRα gene polymorphisms rs3118523 and rs7864987 were genotyped by a 7500 Real-time PCR system using the commercially available TaqMan SNP Genotyping Assays (C__2002263_10, C__28976210_20, respectively) (Applied Biosystems, Foster City, CA, USA). TaqMan® SNP Genotyping Assay (20x), isolated genomic DNA, TaqMan® Universal PCR MasterMix (2×), and sterile distilled water were used for Real-time PCR reaction (total volume of 20 μL per sample). The characteristics of genotyped RXRα polymorphisms are presented in Supplementary Table S1. Real-time amplification was performed under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing/extension at 60°C for 60 s. SDS software (v.2.3) was used to perform allelic discrimination.

Statistical analysis and bioinformatics

ENSEMBL database (http://www.ensembl.org/) and the HaploReg v4.2 platform [25] were used to analyze the potential effects of the investigated polymorphisms on transcription factor binding motifs and/or enhancers. Statistical analysis was performed with the SPSS program (version 20.00, SPSS Ins., Chicago, IL, USA). The difference in genotype frequencies between the patient cohort and control group and the association of RXRα gene polymorphisms with demographic and etiological variables was assessed using either the non-parametric Chi-square test (χ^2) or Fisher's exact test. The crude and adjusted odds ratios (OR) with a 95% confidence interval (CI) adjusted for age and sex were computed using unconditional binary logistic regression to analyze the link between RXRα polymorphisms and OA susceptibility. The analysis included additive, dominant, recessive, and overdominant models. In the additive model, which compares homozygous genotypes of wild type (wt), heterozygous (het), and mutant (mut) genotypes, every genotype contributes equally to the phenotype. According to a dominant model that compares wt vs. het+mut, the dominant wt homozygote has the most influence on the phenotype. A recessive model presupposes that homozygous mutations have the greatest impact on the phenotype. In an overdominant paradigm, the heterozygote, which distinguishes between het and the combination of wild type and mutant (wt+mut), exerts the greatest influence on the phenotype. Associations were considered significant if P values were less than 0.05 ($P<0.05$).

RESULTS

According to the ENSEMBL database, rs7864987 is the regulatory region variant in the RXRα promoter, while rs3118523 is the binding site of transcription factor CCCTC-binding factor (CTCF). According to our analysis using the HaploReg v4.1 platform, RXRA polymorphism rs7864987 could influence the motif for potential binding of AP-1, NF-Y, and TATA transcription factors, whereas rs3118523 might impact the binding of the Maf oncogene family (Supplementary Table 1).

Demographic and clinicopathologic characteristics of the studied OA cohort

All patients recruited for the study had end-stage OA and underwent total joint arthroplasty. OA-related clinical characteristics (sex, age, BMI, previous injuries, family history, physical activity, smoking, menopause, and early onset of OA) were monitored. Most patients (64%) were diagnosed with hip OA and 36% with knee OA. The demographic and OA-related clinical

characteristics of patients are given in Table 1. Our study showed a significant difference in genotype distribution between patients with OA and age- and sex-matched controls for RXRα rs7864987 polymorphism (P=0.008). The trend toward a statistically significant difference in the frequency of genotypes was noted for the second analyzed RXRα polymorphism, rs3118523 (P=0.077) (Table 1).

Association between clinicopathologic characteristics, genotype distribution of RXRα gene variants, and OA

Analysis of the association between RXRα variants and demographic and clinicopathological factors for OA revealed an association between RXRα rs7864987 polymorphism and age (P=0.035) (Table 2). We also observed a trend toward an association between higher body mass index (BMI) for the same gene variant, rs7864987 (P=0.084). There was no significant association between any of the observed demographic and clinicopathological characteristics of OA patients and the rs3118523 RXRα variant.

Analysis of genotype distribution of the studied RXRα gene variants in the control and OA groups and logistic regression analysis of the RXRα gene variants and OA risk

The association between the RXRα gene variants and the risk of OA was analyzed using binary logistic regression analysis. Age and sex were included as confounding variables as they are known risk factors for primary OA. According to different genetic models employed in the analysis, both investigated RXRα variations are associated with OA risk. The frequencies of the analyzed RXRα variants in OA patients and the control group are presented in Table 3. The additive genetic model showed an association of the RXRα rs3118523 genetic variant with a reduced risk of OA (OR=0.569, P=0.030). Consistent with this finding, the recessive model also indicated that the mutant (GG) genotype of rs3118523 was associated with a reduced risk of OA compared to the combined wild type (AA) and heterozygous (AG) genotypes (OR=1.909, P=0.042).

Analysis of the additive model for RXRα rs7864987 indicated that this genetic variant is associated with an increased risk of OA (OR=1.846, P=0.012). The mutant C allele of the RXRα rs7864987 is a predisposing factor for OA (OR=2.855, P=0.01), as evidenced by the dominant model, which revealed that the combined heterozygous (TC) and mutant (CC) genotypes increase the risk of disease development (OR=2.620, P=0.002). The overdominant model revealed a significant association between the TC genotype and the risk of OA when compared to the combined wild-type (TT) and mutant (CC) genotypes (OR=2.633, P=0.002) (Table 3).

Table 1. Baseline characteristics of the studied cohort of OA patients

Variable		Controls (N=91)	%	Total OA (N=92)	%	P
Sex	**Male**	37	41	33	36	0.545
	Female	54	59	59	64	
Age* (years)	<69	49	54	44	48	0.461
	>69	42	46	48	52	
RXRα (rs3118523)	AA	53	58	67	73	0.077
	AG	30	33	22	24	
	GG	8	9	3	3	
RXRα (rs7864987)	TT	56	61	36	39	**0.008**
	CT	28	31	48	52	
	CC	7	8	8	9	

OA – Osteoarthritis; BMI – body mass index; *age according to the median age of 69; bold values show statistically significant P values (P<0.05).

DISCUSSION

The regulation of RXR signaling is unique due to the complexity resulting from heterodimeric interactions of RXR with various ligands, each of which plays a specific role in different biological processes [26]. RXR can potentially engage in heterodimeric interactions with one-third of the currently known NRs [13]. The exact mechanisms of RXR biological activity remain elusive due to its ability to function both as a homodimer and as a partner in heterodimeric complexes. RXR can be characterized as a unique NR due to its involvement in independent biological processes and signaling pathways [27].

As far as we know, our study is the first to associate genetic RXRα polymorphisms with susceptibility to OA. Our findings indicate a significant association between the RXRα rs7864987 gene variant and heightened risk of OA, and age tends to be associated with higher BMI. Conversely, the second analyzed RXRα variant, rs3118523, was identified as a protective factor for OA. These data clearly

Table 2. RXRα gene variants' associations with demographic and clinicopathological features in OA patients

Demographic and risk factors		OA N=92	RXRα (rs3118523)				RXRα (rs7864987)			
			AA	AG	GG	P	TT	TC	CC	P
Sex	**Male**	33	25	6	2	0.367	13	17	3	0.993
	Female	59	42	16	1		23	31	5	
Age* (years)	<69	44	33	10	1	0.837	13	29	2	**0.035**
	>69	48	34	12	2		23	19	6	
Smoking	Yes	18	13	4	1	0.823	11	6	1	0.103
	No	74	54	18	2		25	42	7	
High physical activity	Yes	20	16	3	1	0.531	9	9	2	0.768
	No	72	51	19	2		27	39	6	
History of injury	Yes	31	23	8	0	0.448	11	17	3	0.872
	No	61	44	14	3		25	31	5	
Family history	Yes	39	31	7	1	0.621	16	20	3	0.965
	No	53	37	14	2		21	27	5	
Early onset	<55	51	38	12	1	0.726	19	27	5	0.870
	>55	41	29	10	2		17	21	3	
BMI (kg/m²)	≤25	32	22	9	1	0.767	17	13	2	0.084
	25-30	39	28	9	2		12	21	6	
	>30	21	17	4	0		7	14	0	
Menopause	Yes	56	39	16	1	0.527	22	29	5	0.813
	No	3	3	0	0		1	2	0	
Early menopause (<45 years)	Yes	15	10	5	0	0.758	4	8	3	0.161
	No	41	29	11	1		18	21	2	

*age according to the median age of 69; BMI – body mass index; bold values show statistically significant P values (P<0.05).

Table 3. Genotype frequencies and logistic regression analysis for RXRα gene variants and OA risk

Gene/ SNP	Genotype	Controls		OA Cases		Adjusted OR# [95% CI]	P*
		N=91	%	N=92	%		
RXRα rs3118523	**AA**	53	58	67	73	1.000 (Referent)	
	AG	30	33	22	24	0.577 [0.299-1.117]	0.103
	GG	8	9	3	3	0.314 [0.079-1.252]	0.101
	Additive model [a]					0.569 [0.342-0.947]	**0.030**
	Recessive model [b] mut vs. wt+het (*Refrent*)					0.542 [0.281-0.976]	**0.042**
	Dominant model [c] het+mut vs. wt (*Refrent*)					0.370 [0.094-1.456]	0.155
	Overdominant model [d] het vs wt+mut (*Refrent*)					0.632 [0.330-1.212]	0.167
RXRα rs7864987	**TT**	56	62	36	39	1.000 (Referent)	
	TC	28	31	48	52	2.855 [1.500-5.434]	**0.001**
	CC	7	8	8	9	1.774 [0.588-5.352]	0.309
	Additive model [a]					1.846 [1.143-2.980]	**0.012**
	Recessive model [b] mut vs. wt+het *(Refrent)*					1.133 [0.391-4.805]	0.818
	Dominant model [c] het+mut vs. wt (*Refrent*)					2.620 [1.428-4.805]	**0.002**
	Overdominant model [d] het vs wt+mut (*Refrent*)					2.633 [1.413-4.907]	**0.002**

– odds ratio (OR) adjusted for sex and age; [a] Additive model – wt vs. het vs. mut; [b] Recessive model – mut vs. wt+het; [c] Dominant model – wt vs. het+mut; [d] Overdominant model – het vs. wt+mut.

point to the involvement of RXRα in OA susceptibility, implying the distinct contribution of each RXRα variant to this disease. Furthermore, our findings highlight the complex interaction of genetic and environmental factors in the development of OA and pave the way for a more comprehensive understanding of the multifactorial etiology of the disease.

Both investigated variations, rs7864987 and rs3118523, are non-coding, often leading to alterations in the structure of the receptor's ligand-binding region. This change may alter receptor function and affect disease susceptibility [28]. According to our analysis of the ENSEMBL database and HaploReg v4.1, a platform for studying enhancer histones and binding motif changes, the examined polymorphisms could lead to chromatin changes and allele-specific binding of transcription factors [25]. According to the ENSEMBL database, rs7864987 is a variant of the regulatory region in the RXRA promoter that could affect its transcription, whereas rs3118523 is the predicted binding site of the transcription factor and chromatin state modulator CTCF, whose binding sites were recently linked to the osteoarthritis in a genome-wide association study [29]. HaploReg v4.1 analysis showed that both RXRA polymorphisms could influence chromatin changes in different immune cells. Furthermore, rs7864987 could influence the motif for possible binding of the transcription factors AP-1, NF-Y, and TATA, while rs3118523 could affect the binding of the Maf oncogene family.

Previously, polymorphisms in the RXRα gene (rs7861779 and rs12004589) have been reported

as being involved in anti-tumor immune response in colorectal cancer [19], ovarian cancer (rs749759) [20], head and neck squamous cell cancer (rs3118570) [30], and renal cancer [31]. In addition, five RXRα polymorphisms, rs881658, rs11185659, rs10881583, rs881657, and rs7864987, were found to be correlated with diminished disease-free survival in breast cancer patients treated with chemotherapy, whereas rs10881583 and rs881657 were associated with enhanced disease-free survival in breast cancer patients undergoing hormone treatment [18]. The integrative analysis of genome-wide association studies (GWAS) data revealed the potentially important role of the RXR signaling pathways and their genetic variations in the antiviral immune response to the smallpox vaccine [32] and hepatitis C virus infection [33]. Although RXR polymorphisms have not been studied in OA, severe OA of the hand has recently been associated with genetic polymorphisms in the ALDH1A2 gene, which encodes the key enzyme for the synthesis of all-*trans* retinoic acid (atRA) [34].

A specific modulatory function of RXRs in the immune responses is well documented. Though the expression of each isotype varies by cell type, RXRs are expressed to varying degrees in nearly all tissues [26]. Studies have revealed that three RXR isoforms are expressed differentially across various immune cells, playing a crucial role in maintaining homeostasis and the inflammatory response [17,26]. RXR has profound effects on proinflammatory mediators, alone or in combination with a heterodimeric partner since it acts as a negative regulator of NF-kB or activator protein 1 (AP-1) [8]. Several heterodimeric RXR partners, including ERs, VDRs, and LXRs, are associated with suppressing proinflammatory genes [35]. RXR also has an important role in initiating inflammation by regulating the chemoattractant potential of the chemokines CCL6 and CCL9 at sites of inflammation or injury [14]. It has been shown that both NF-κB [36] and interferon regulatory transcription factor 3 (IRF3) mediate the repression of RXR and other nuclear receptor target genes [35]. RXR also modulates immunological self-tolerance by affecting macrophages' phagocytic activity and the uptake of cellular debris [15]. Macrophages are recruited to the inflamed OA synovium through the influence of monocyte chemoattractant protein-1 (MCP-1) [37]. These findings that RXRα's may control the transcription of gene-related inflammation triggered by the presence of self-antigens can serve as a future OA therapeutic approach based on the ability of RXR and NR to enhance apoptotic cell removal and self-tolerance preservation [15]. The RXRα isoform is expressed at significantly reduced levels in OA cartilage [38], and this deficiency is likely a contributing factor to the procatabolic state of an OA joint [39]. The inflammatory reaction driven by the heightened activity of proinflammatory cytokines is widely recognized as the foundation for the onset and progression of OA. The expression of TNF-α, which collaborates strongly with IL-1 and governs most of the procatabolic processes in OA, has been reported as upregulated in the synovial fluid, synovial membrane, cartilage, and subchondral bone of OA joints [3,40]. TNF-α also regulates the production of cellular stress-related molecules from synovial cells, chondrocytes, and necrotic bone cells, initiating the inflammatory process [3]. Furthermore, TNF-α controls the expression of matrix metalloproteinases (MMP-1, MMP-3, and MMP-13) in chondrocytes that degrade structural components of the ECM, leading to cartilage remodeling in OA [41]. Retinoids that selectively activate RXR homodimers but not RAR-RXR heterodimers inhibit the expression of TNF-α, IL-6, IL-8, and MCP-1 and lower the expression of MMP-1 [42,43]. It has also been shown that RXR signaling via the p38 mitogen-activated protein kinase (MAPK)/NF-κB pathway positively regulates the expression of anti-inflammatory cytokines [41]. Moreover, various nuclear receptors (NRs), such as VDR, ER, and PPARγ, have been suggested as potential contributors to OA. [38]. One of the most important interactions of RXR is its dimerization with VDR. RXR is among the proteins that regulate the biological effects of the active form of vitamin D, 1.25-dihydroxyvitamin D3 [1.25(OH)2D3], which has a crucial role in human bone metabolism. Transcription of several 1.25(OH)2D3-regulated genes is triggered by the heterodimerization of VDR-RXR by interaction with the vitamin D response element (VDRE) in the promoter region of these genes [44]. Another heterodimeric partner of RXR within the nuclear receptor superfamily is LXR, which plays a protective role against cartilage degradation in OA [38]. Activation of LXR suppresses the inflammatory mediators cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) that regulate MMP activity in chondrocytes [45]. Furthermore, it was found that the expression of LXR is significantly decreased in human OA cartilage, just like RXR [38]. These findings revealed the involvement of RXRs in the etiology of

OA, which is consistent with RXR's involvement in chronic inflammation. In addition, RXR also has the ability to prevent cellular senescence that can trigger the development and contribute to the progression of OA by altering the tissue microenvironment and shifting the balance between ECM synthesis and degradation [46,47]. Due to the pleiotropic activity of RXR activation, it is a challenge to determine the direct physiological effects of RXR gene variants.

Our understanding of RXR modulators is limited because of their extensive crosstalk with other NRs. Several clinical studies suggest that RXR-specific synthetic ligands are promising therapies for treating cancers such as cutaneous T-cell lymphoma, non-small cell lung cancer, acute myeloid leukemia, breast cancer, and thyroid cancer [48]. Two RXR agonists, alitretinoin and bexarotene, are already widely used in treating Kaposi's sarcoma and cutaneous T-cell lymphoma [13]. RXR signaling and potential therapeutic effects are of particular interest in neurodegenerative diseases. Numerous studies reported bexarotene as an effective anti-inflammatory agent in murine models of Alzheimer's disease [49], Parkinson's disease [50], amyotrophic lateral sclerosis (ALS) [51], multiple sclerosis (MS) [52], and stroke [53]. These findings have significant implications for the treatment of other inflammation-related diseases. A novel therapeutic strategy for OA could target RXRα and its ligands by limiting the inflammatory cytokines, chemokines, and degradative enzymes that cause cartilage degradation and associated joint tissue deterioration. Talarozole (retinoic acid metabolism blocking agent, RAMBA), a selective inhibitor of atRA previously used to treat psoriasis and acne, has been shown to exert anti-inflammatory effects on cartilage in animal models [34]. *Ex vivo* treatment of porcine cartilage with talarozole leads to changes in the expression of inflammatory genes *via* stimulation of the RXR-PPAR heterodimer, which binds to regulatory elements in promoters of inflammatory genes [54]. Talarozole suppressed the expression of inflammatory genes in articular cartilage 6 h after destabilization of the mouse knee joint, reducing cartilage degradation and osteophyte formation [34]. Increasing the level of all-trans retinoic acid with this drug showed an anti-inflammatory effect in an experimental OA model and slowed the disease's development. In addition, RNA sequencing of the articular cartilage of patients with OA of the hand showed a reciprocal relationship between ALDH1A2 and inflammatory gene expression. Mechanoinflammation cartilage, as a possible primary trigger of OA, was associated with a decrease in atRA-inducible genes, which was reversed by treatment with talarozole [34]. In addition, bexarotene prevented articular ECM degradation by inhibiting advanced glycation end product (AGE) [55]. The RXR modulator K-80003 reduced articular cartilage degeneration, synovial inflammation, and osteoarthritic pain in rats by suppressing the activation of proinflammatory NF-κB signaling [56]. These results identify agents that modify retinoic acid metabolism as potential disease-modifying drugs for OA. Therefore, RXR modulators may play an important anti-inflammatory role and serve as potential therapeutic molecules against OA.

Although our data associated RXRA polymorphism rs7864987 with age above the median but not with early-onset OA, a distinct assessment of early-onset OA as a separate entity is required to detect the early stages of the degenerative processes. The crucial timeframe during which OA may still be treatable to halt the disease's progression is gaining broader recognition [57,58].

While our findings may offer valuable insights into the inflammatory aspect of OA, it's important to acknowledge certain limitations in this study that warrant consideration. Firstly, the sample size was relatively small, possibly leading to biased conclusions. In addition, the observed association of RXR with susceptibility to OA in the studied Serbian population may not apply to all Caucasians. Our study focused on two polymorphisms in the RXRα gene, and additional research is needed to comprehensively grasp the potential involvement of other RXR genetic variants in susceptibility to OA, either directly or in conjunction with its heterodimeric effects with other NRs. Research on the genetic variants of the RXRα gene is minimal, and additional research on the functional impacts of its genetic variants on immunomodulation of complex diseases, including OA, is warranted with a larger sample size and different ethnic background.

CONCLUSIONS

Our study is the first to demonstrate the possible association between the RXRα polymorphism rs7864987

as a risk factor and rs3118523 as a protective factor for OA. Genetic polymorphisms in RXR-encoding genes and enzymes involved in the synthesis of atRA may be an important component of the complex network underlining the immunopathology of OA. Novel genetic, biochemical, and imaging biomarkers are needed to improve the identification of patients who would benefit from therapies targeting the diverse aspects of OA pathogenesis, such as inflammation, cartilage matrix degradation, and bone remodeling. RXR agonists and other medications that alter retinoic acid metabolism demonstrate anti-inflammatory effects, suggesting their potential as novel therapeutics for OA. Polymorphisms in RXRα and other genes involved in retinoic acid metabolism may pave the way for personalized retinoid therapy for OA tailored to an individual's genetic profile. Additional research with a larger sample size and diverse ethnic backgrounds is needed to investigate the functional effect and potential pharmacogenetic implications of RXR gene variations in osteoarthritis.

Funding: The authors received no funding for this work.

Author contributions: Conceptualization, DS and GS.; investigation, DS, VV, NI, and GS; data curation, DS, VV, NI, BBN, and GS; writing/original draft preparation, DS and GS; writing/review and editing, DS, VV, NI, BBN, and GS; supervision, GS All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: For studies involving human participants (privacy or ethical issues), clinical data are not publicly available but can be obtained upon personal request through contact with the corresponding author.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Characteristics of genotyped RXR polymorphisms.

Gene	Location	rs number	SNP change	Variant Type, Region	HaploReg v4.1 prediction of motifs changes
RXRα	9q34.2	rs3118523	A > G	Intron 4	Maf
		rs7864987	T > C	Intron 1	AP-1, NF-Y, TATA