

Original article

Reprint

Associations of TNFSF11 gene polymorphisms with knee osteoarthritis in postmenopausal women

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Abstract:

Objective: to identify the role of rs9594738 and rs9594759 polymorphisms of the TNFSF11 gene in the development of osteoarthritis (OA) of the knee joints (KJ) in postmenopausal women.

Materials and Methods. Our case-control study involved 483 postmenopausal women. Of these, 157 were diagnosed with primary KJ OA. The remaining 326 women without signs of joint disease were included in the control group. Molecular genetic studies included the determination of rs9594738 and rs9594759 polymorphisms of the TNFSF11 gene using the real-time polymerase chain reaction method.

Results. Analysis of the distribution of genetic markers in two groups of women demonstrated a reduction in the frequency of occurrence of the TT genotype of the rs9594738 polymorphism among patients with OA (odds ratio 0.59; 95% confidence interval 0.36–0.97; $p=0.049$). The distribution of the alleles of this polymorphism, as well as the alleles and genotypes of the rs9594759 polymorphism of the TNFSF11 gene in the group of women with OA, did not differ significantly from the results of the molecular genetic examination of the control group ($p>0.05$).

Conclusion. We established an association between the rs9594738 polymorphism of the TNFSF11 gene and KJ OA in postmenopausal women. Further studies of the role of polymorphic variants of the TNFSF11 gene in the etiopathogenesis of KJ OA are needed to develop individual approaches to the prevention and treatment of this disease.

Keywords: women, postmenopause, osteoarthritis, polymorphisms, TNFSF11 gene

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Introduction

Osteoarthritis (OA) is among the most widespread disorders of the musculoskeletal system characterized by chronic progressive tissue damage to all joints. Aside from being the most common form of arthritis, OA is also the main cause of chronic pain and increasing loss of joint function, especially in elderly people and among postmenopausal women. Women suffer from this pathology 1.7–2.1 times more often than men [1].

Various joints, including knee joint (KJ), can be involved in the pathological process. When the KJ is damaged by degradation of articular cartilage and matching changes in the subchondral bone (SCB), the patient gradually loses the ability to work and lead an independent lifestyle. The disease inevitably results in a reduction of the quality of life, physical incapacity and disability of the patient. All of these are associated with high personal financial costs and a serious economic burden on the entire healthcare system. Since there is a tendency towards increasing life expectancy and aging of the population, it is assumed that the prevalence of OA of various localizations, including KJ OA, will increase more and more.

One of the means to reduce the incidence of KJ OA, including among postmenopausal women, is the use of personalized medicine methods, which suggests taking into

account individual characteristics of a patient when developing programs for prevention, examination, treatment, etc. The development and implementation of such approaches will ensure accurate prediction of the risk of developing this disease in each individual patient and will facilitate performing therapeutic and preventive measures based on individual programs long before the manifestation of pathology. Such strategy is already quite successfully employed for certain diseases in women to maintain their active longevity [2].

When developing personalized medicine methods for the effective prevention of OA, it should be taken into account that joint disease is multifactorial. Its occurrence and progression are triggered by various risk factors. These include female gender, as well as elderly and senile ages. The risk of OA is increased by excess weight and obesity, metabolic syndrome, excessive mechanical stress and joint injuries, alcohol consumption, smoking, and preceding joint diseases [1, 3].

It was also established that up to 68% of OA cases are genetically determined, and this was proven by the results of numerous epidemiological, family, and twin studies [4]. Molecular genetic studies (MGS) conducted in recent years also confirmed the important role of the genetic component in the etiopathogenesis of OA. Their results implied that the development of OA may depend on the function of tens and

even hundreds of genes [5]. However, to date, it has been established that the proportion of heritability associated with the identified OA risk loci is slightly over 20% [6]. This means that a large number of still unknown loci that can collectively determine the risk of OA have yet to be identified.

Among the candidate genes associated with OA, a group of genes that provide the formation of immune factors is distinguished. In particular, these include IL-1 α , IL-1 β , IL-1RN, IL-6, IL-10, IL-11, IL-17A, TGF-B1, TLR-4, TNFSF11, TNFRSF11B, and TNF, all of which are involved in the development of the immune response and inflammation [5, 7]. The possible role of the TNFSF11 gene in the pathogenesis of OA is explained by the fact that it encodes the ligand of the receptor activator of nuclear factor κ B (RANKL), a cytokine from the tumor necrosis factor family involved in the development of the inflammatory process in joints. Our research is the pioneering study in the Russian Federation investigating polymorphisms of this gene in OA.

Objective: to determine the role of rs9594738 and rs9594759 polymorphisms of the TNFSF11 gene in the development of KJ OA in postmenopausal women.

Materials and Methods

The study was carried out within the framework of a comprehensive research at the S.I. Georgievsky Medical Academy, V.I. Vernadsky Crimean Federal University (CFU), and Donetsk State Medical University (DSMU), Russia. The study complied with all ethical requirements for scientific research and was approved by the Ethics Committee of the DSMU (protocol No. 27/5-1 of April 14, 2021).

The case-control study involved 483 postmenopausal women. Of these, 157 patients were diagnosed with primary OA of the KJ with varying degrees of functional impairment and varying radiographic stage sensu Kellgren – Lawrence. They formed the main group. The remaining 326 women without signs of joint disease were included in the control group.

Women were selected for the study via random sampling taking into account the inclusion and exclusion criteria. All subjects in both groups met the following inclusion criteria: female gender, postmenopause (absence of menstrual cycle for 12 months or more), and written voluntary informed consent. Moreover, before the onset of the examination, all women were informed about the objectives and nature of the study and had the opportunity to ask questions regarding all issues. The two groups of women differed by the presence (main group) or absence (control group) of KJ OA.

Exclusion criteria were as follows: male gender, secondary OA, diseases of the endocrine and immune systems, as well as rheumatic, mental, oncological and hematological pathologies, and chronic inflammatory diseases.

The two groups of women had similar characteristics in terms of their age and duration of the postmenopausal period ($p>0.05$). For instance, in women of the main and control groups, the median age and interquartile range were 61 [55; 67] and 61 [55; 67] years, respectively ($p=0.798$), and the duration of postmenopause was 12.0 [6; 19] years and 12.5 [6; 20] years ($p=0.545$).

After the clinical and instrumental examination, all women subjected to MGS. For this purpose, peripheral blood samples were taken into vacuum tubes with

ethylenediaminetetraacetic acid as an anticoagulant. Blood leukocytes were used for the analysis, the isolated DNA of which was tested for rs9594738 (C>T) and rs9594759 (C>T) polymorphisms of the TNFSF11 gene. To study these polymorphisms, the real-time polymerase chain reaction method was employed. DNA extraction and polymorphism detection were performed using commercial reagent kits, and reaction recording was performed using a DT-96 detection amplifier (all manufactured by DNA-Technology Scientific Production Association, Russia).

The obtained results were processed using the Medstat statistical software. Data on age and duration of the postmenopausal period were presented as a median and interquartile range (Me [Q1; Q3]). The Mann–Whitney U test was used for paired comparisons of the central values of two independent samples. The frequency of detection of polymorphic variants of the TNFSF11 gene in the samples was presented both as a count and as a percentage. The χ^2 criterion was employed to determine whether the distribution of the studied genotypes complied with the Hardy–Weinberg equilibrium. The statistical significance of differences in the distribution of genotypes and alleles between the groups was examined using χ^2 (contingency tables $k \times m$) as well. When assessing the associations of genotypes and alleles with the disease, we calculated the odds ratios and 95% confidence intervals. Differences in variables between the groups were assumed statistically significant at $p<0.05$.

Results

At the first stage of data processing, the frequencies of the studied polymorphic variants of the TNFSF11 gene were estimated in the general group of examined women ($n=483$). As a result, we confirmed that the distribution of genotypes of the rs9594738 and rs9594759 polymorphisms of the aforementioned gene among postmenopausal women complied with the Hardy–Weinberg law. The revealed genotype frequencies did not differ statistically significantly from the expected frequencies ($p>0.05$). The genotypes of the rs9594738 polymorphism CC, CT and TT were detected in 139 (28.8%), 240 (49.7%) and 104 (21.5%) individuals, respectively. Analysis of gene variants for the rs9594759 polymorphism demonstrated that 115 (23.8%) of the study subjects were homozygous for the C allele (CC), 127 women (26.3%) for the T allele (TT), whereas 241 individuals (49.9%) were heterozygous (CT).

At the second stage of data processing, the frequencies of genetic markers in the main and control groups were examined. Table 1 presents the distribution of genotypes of the rs9594738 polymorphism of the TNFSF11 gene, which showed just a tendency close to statistical significance to a reduction in the frequency of detection among patients with OA of the TT genotype ($p=0.054$). However, whenever we pooled together carriers of the CC and CT genotypes from both healthy individuals and women with KJ OA into one subgroup (CC+CT), and formed another subgroup from individuals with the TT genotype (Table 1), this trend achieved statistical significance (odds ratio 0.59; 95% confidence interval 0.36–0.97; $p=0.049$). Analysis of the frequencies of the C and T alleles of the TNFSF11 gene rs9594738 polymorphism yielded comparable results in the main and control groups ($p=0.401$).

The distribution of genotypes of the rs9594759 polymorphism of the TNFSF11 gene did not differ statistically significantly ($p=0.188$) between women with KJ OA and individuals in the control group (Table 2). The frequency of C and T alleles of this polymorphism did not differ between the two groups as well ($p=0.153$).

Discussion

When examining postmenopausal women, we detected a rarer TT genotype of the rs9594738 polymorphism of the TNFSF11 gene among individuals with KJ OA. The distribution of the alleles of this polymorphism, as well as the alleles and genotypes of the rs9594759 polymorphism of the TNFSF11 gene, in the group of women with OA did not differ statistically significantly from MGS data of individuals in the control group ($p>0.05$).

The TNFSF11 gene encodes RANKL, which is a proinflammatory cytokine of the tumor necrosis factor superfamily. This cytokine was described in the 1990s and, along with two other mediators, was included in the RANK/RANKL/OPG system, where RANK and RANKL are, respectively, the activator of the receptor of the nuclear factor κ B and its ligand, while OPG is osteoprotegerin. The last decade has been marked with rapid progress in the field of osteoimmunology, clarifying the close mechanisms of interaction between the musculoskeletal and immune systems. The RANK/RANKL/OPG cytokine system has become one of the striking examples of such interaction [8, 9]. This cytokine system is a key system in the regulation of osteoclastogenesis, and its disorders may eventually lead to the development of skeletal pathology. The role of these cytokines has been best studied in conditions of osteoporosis. It has been proven that in the skeletal system, osteoblasts and osteocytes serve as the source of RANKL. Through this cytokine, these cells control osteoclast function and bone resorption. Excessive synthesis of RANKL results in increased resorption properties of osteoclasts and the formation of osteoporosis. Insufficient level of this cytokine leads to a reduction in osteoclast activity and the development of osteopetrosis. Therefore, mutations in the TNFSF11 gene capable of causing quantitative and/or structural and functional changes in RANKL molecules can have an adverse effect on bone tissue [10].

Table 1. Frequency of occurrence of genotypes and alleles in the rs9594738 polymorphism of the RANKL gene among postmenopausal women with knee joint osteoarthritis

Genotype and allele	Group				$p(\chi^2)$
	control, $n=326$		main, $n=157$		
	count	%	count	%	
CC	96	29.5	43	27.4	0.054 (5.85)
CT	151	46.3	89	56.7	
TT	79	24.2	25	15.9	
CC+CT	247	75.8	132	84.1	0.049 (3.85)
TT	79	24.2	25	15.9	
C	343	52.6	175	55.7	0.399 (0.71)
T	309	47.4	139	44.3	

Table 2. Frequency of occurrence of genotypes and alleles in the rs9594759 polymorphism of the RANKL gene among postmenopausal women with knee joint osteoarthritis

Genotype and allele	Group				$p(\chi^2)$
	control, $n=326$		main, $n=157$		
	count	%	count	%	
CC	75	23.0	40	25.5	0.188 (3.34)
CT	157	48.2	84	53.5	
TT	94	28.8	33	21.0	
C	307	47.1	164	52.2	0.153 (2.04)
T	345	52.9	150	47.8	

It should be pointed out that RANKL can be produced not only by osteoblasts and osteocytes. An incomplete list of cells producing this cytokine includes T cells and B lymphocytes, epithelial cells of the mammary gland, vascular endothelial cells, and periodontal tissue cells [11]. In this regard, the identified associations of TNFSF11 gene polymorphisms with an increased risk and more severe course of not only osteoporosis, but also multiple myeloma [12], breast cancer [13], cardiovascular diseases [14], periodontitis [15], etc., become quite comprehensible.

Synovial fibroblasts and chondrocytes are also a source of RANKL. These cells, along with activated lymphocytes, participate in the pathogenesis of joint diseases by enforcing the local synthesis of various proinflammatory cytokines, including RANKL. To date, evidence has been obtained of the participation of RANKL in the pathogenesis of rheumatoid arthritis. The pathogenetic role of RANKL in rheumatoid arthritis is confirmed by the identified associations of TNFSF11 gene polymorphisms with the risk of developing this disease [16, 17].

In recent years, increasing attention has been paid to the study of the effects of RANKL, as well as the entire RANK/RANKL/OPG system, in the development of OA [18, 19]. Furthermore, an opinion has been expressed about the key role of RANKL in the pathogenesis of OA [18]. Activation of the RANK/RANKL/OPG pathway that occurs in OA is a complex process determining the onset and progression of the disease. In OA, RANKL expression in the cartilage matrix increases, thereby aggravating its damage. It should be noted that chondrocytes express not only RANKL, but also its RANK receptors. Consequently, through its own receptor, this cytokine triggers a signaling cascade with the activation of multiple genes in chondrocytes, which is accompanied by disorders in the production of extracellular matrix components, in particular collagen and proteoglycans, and degradation of the cartilage structural integrity. Changes in cartilage structure are also triggered by increased chondrocyte apoptosis caused by RANKL molecules. A reduction in the number of these cells intensifies degenerative processes in cartilage tissue.

An equally important component of OA pathogenesis is damage to the SCB [18]. The RANK/RANKL/OPG cytokine system also plays a leading role in this process. Stimulation of RANKL production triggers increased activity of osteoclasts, which induce accelerated bone resorption, especially in the SCB. Impaired bone remodeling results in structural abnormalities in the subchondral region characteristic of OA, such as the loss of trabecular bone mass, formation of cystic lesions and sclerotic changes. Along with structural changes in the SCB due to increased RANKL synthesis, it is assumed that there is also stimulation of axon growth in sensory

nerves. The presence of these sensitive nerve fibers in the SCB may contribute to elevated pain sensitivity characteristic of OA.

It has been established that patients with OA are characterized by a significant increase in RANKL expression in synovial tissue [18]. Moreover, this property of synovial tissue may be of decisive importance in the initiation of the disease and maintaining chronic inflammation. By binding to its RANK receptor, RANKL triggers a sequence of intracellular signaling events, the culmination of which is the formation of persistent inflammation with increased production of both RANKL per se and other proinflammatory cytokines (such interleukin 6, tumor necrosis factor, etc.).

All of the above implies a noteworthy contribution of the RANKL cytokine to the pathogenesis of OA. Solid confirmation of this fact was obtained by L. Pan et al. [20]. The authors of the study identified five immunoregulatory genes that are critically important in the pathogenesis of OA. Among these, they assumed the leading role of the TNFSF11 gene, a reduction in the expression of which can decelerate the progression of destructive processes in cartilage tissue in OA, while an increase in its expression, on the contrary, can induce an inflammatory component, accelerate the destruction of joint tissues and contribute to aggravation of clinical symptoms.

It is important to point out that the TNFSF11 gene is included in the GeneCards database as a candidate gene associated with KJ OA [7]. However, to date, we do not have unambiguous evidence of the involvement of this gene polymorphisms in the etiopathogenesis of the disease. The list of polymorphisms of the gene in question that are associated with the risk of development and severity of KJ OA has not been clarified yet. Hence, it is advisable to conduct further studies of the genetic aspects of OA, including the study on the role of polymorphic variants of the TNFSF11 gene. At the same time, additional observations of patients with KJ OA are needed performed on women of different ages and on men, as well as on patients with primary and secondary OA.

Conclusion

MGs of postmenopausal women revealed a rarer occurrence of the TT genotype of the rs9594738 polymorphism of the TNFSF11 gene among individuals with KJ OA. The distribution of alleles of this polymorphism, as well as alleles and genotypes of the rs9594759 polymorphism of the TNFSF11 gene in the group of women with OA, did not differ statistically significantly from the results of MGs of individuals in the control group ($p > 0.05$). Further studies of the role of polymorphic variants of the TNFSF11 gene in the etiopathogenesis of KJ OA both in postmenopausal women and among other groups of individuals are needed to develop individual approaches to the prevention and treatment of the disease.

Conflict of interest. None declared by the authors.

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