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Strong correlation between F2RL3 in the blood and osteoarthritis based on a retrospective cohort study

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Abstract

Osteoarthritis (OA) is a common joint disease that mainly damages articular cartilage and involves the whole joint tissue. The purpose of this study was to explore the relationship between F2R Like Thrombin Or Trypsin Receptor 3 (F2RL3) and OA, so as to provide a new direction for the treatment of bone and joint. A total of 234 patients with OA were recruited. Clinical data was recorded and the expression levels of ELOVL Fatty Acid Elongase 7, F2RL3, glycoprotein IX platelet and Integrin Subunit Alpha 2b were measured. Pearson chi-square test and Spearman correlation coefficient were used to analyze the relationship between OA and related parameters. Univariate and multivariate Logistic regression were used for further analysis. Pearson chi-square test showed that F2RL3 ($P < .001$) was significantly associated with OA; Spearman correlation coefficient showed that OA and F2RL3 ($\rho = -0.511$, $P < .001$) significantly correlated; Univariate Logistic regression analysis showed that F2RL3 (odds ratio = 0.104, 95% confidence interval: 0.057–0.189, $P < .001$) was significantly associated with OA; multivariate logistic regression analysis showed that F2RL3 (odds ratio = 0.098, 95% confidence interval: 0.053–0.182, $P < .001$) were significantly associated with OA. The expression of F2RL3 is low in patients with OA. The lower the expression of F2RL3, the higher the probability of OA.

Abbreviations: 95% CI = 95% confidence interval, ELOVL7 = ELOVL Fatty Acid Elongase 7, F2RL3 = F2R Like Thrombin Or Trypsin Receptor 3, GP9 = glycoprotein IX platelet, ITGA2B = integrin subunit alpha 2b, OA = osteoarthritis, OR = odds ratio, PCR = polymerase chain reaction.

Keywords: biomarker, F2RL3, odds ratio, osteoarthritis, potential targets

1. Introduction

Osteoarthritis (OA) is a kind of chronic painful arthritis, which is also the main cause of disability of patients.[1] Hundreds of millions of people around the world suffer from OA, and its incidence is increasing with the increase of risk factors such as population aging and obesity.[2] Disability due to OA is a huge cost to the global economy. Patients with OA often have many annoying complications that affect the quality of life, especially in the elderly.[3] OA remains the most challenging arthritic disease, with a high disease burden and no effective disease-modifying treatment.[4] Symptoms of OA include joint pain, stiffness and limited mobility,[5] mainly affecting the knee joint. However, the pathogenesis of OA is complex, with mechanical, genetic, metabolic and inflammatory pathways involved in its slow evolution.[6]

Sample F2R thrombin or trypsin receptor 3 (F2R Like Thrombin Or Trypsin Receptor 3 [F2RL3]) is a kind of protein-coding genes, the gene encoding protease activated receptor and members of the family. Gene ontology annotations associated with this gene include G protein-coupled receptor activity and thrombin activated receptor activity. The encoded receptor is proteollyzed to reveal the extracellular N-terminal tethered ligand, binding to and activating the receptor, which plays a role in blood clotting, inflammation and pain responses. Related studies have reported that soluble tissue factors promote inflammatory arthritis through a thrombine-dependent pathway, in which induced platelet activation is an important step.[7] F2RL3 is a protein-coding gene located on chromosome 19 with 2 exons. It encodes protease-activated receptor 4, which is involved in the pathophysiology of tumors and cardiovascular diseases.[8] F2RL3 gene has been shown to play a key role in mediating platelet activation, as well as a variety of signaling pathways, such as immune response, regulation of vascular endothelial cell activity, and inflammatory response.[9] Relevant studies have

Written informed consent was obtained from all patients.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This study was approved by the Ethics Committee of the Fuyang District First People's Hospital of Hangzhou.

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shown that epigenetic regulation of F2RL3 may increase platelet reactivity through protease activates receptor 4 function, thereby increasing the risk of morbidity and mortality of myocardial infarction.[10]

F2RL3 is also a functionally related gene that plays a role in platelet activation and cell signal transduction.[11] The F2RL3 gene encodes a protease activates receptor 4, which has been implicated in the pathophysiological processes of cardiovascular and tumor diseases.[12] F2RL3 gene methylation is closely associated with hypertension and mortality.[13] However, the relationship between F2RL3 and OA remains unclear.

This study hypothesized that the lower the expression level of F2RL3, the higher the risk of OA during the development and progression of OA. Based on the above hypothesis, we recruited 234 patients with OA. These results may reveal F2RL3 as a potential molecular target of OA and provide new ideas for its molecular mechanism.

2. Methods

2.1. Patients and ethics

A total of 234 patients diagnosed with knee and tibial arthritis from March 2015 to December 2022 were selected.

Inclusion criteria: diagnosis of knee tibial arthritis. Normal cardiopulmonary function. Normal clotting function.

Exclusion criteria: poor lung, heart and liver function. Patients and their families did not agree to participate in the trial.

This study was approved by the Ethics Committee of the Fuyang District First People's Hospital of Hangzhou, and all patients signed written informed consent.

2.2. Research parameters

According to the clinical data of patients, they were classified according to sex (male/female), age (≤60/>60), ELOVL Fatty Acid Elongase 7 (ELOVL7) (low/high), glycoprotein IX platelet (GP9) (low/high), integrin subunit alpha 2b (ITGA2B) (low/high), and F2RL3 (low/high).

2.3. Detection of related parameters

Articular synovium tissue was taken from the tibial plateau of the knee of the patient, and the expression levels of ELOVL7, F2RL3, GP9 and ITGA2B were detected by PCR.

2.4. Polymerase chain reaction (PCR) detection

2.4.1. Total RNA extraction (gun head and centrifuge tube were sterilized by damp heat without RNA enzyme).

2.4.1.1. Sample preprocessing *Tissue*: Take homogenate tube, add 1ml RNA extraction solution, place on ice for pre-cooling. Take about 20 mg tissue and add it to homogenate tube. Grinds fully with homogenizer until no tissue is visible.

2.4.1.2. Separation of phase After sample pretreatment, supernatant was obtained by centrifugation at 12,000 rpm for 10 minutes. Add 250 μL trichloromethane, reverse the centrifuge tube for 15 seconds, mix well, let stand for 3 minutes, centrifuge at 4℃ at 12,000 rpm for 10 minutes, and transfer 400 μL supernatant 4 to a new centrifuge tube.

2.4.1.3. Precipitated RNA Add 0.8 times the volume of isopropyl alcohol and mix upside down. Place at –20℃ for 15 minutes. After centrifugation at 12,000 rpm at 4℃ for 10 minutes, the white precipitate at the bottom of the tube was RNA.

2.4.1.4. Washing RNA Remove the liquid, add 75% ethanol 1.5ml wash precipitation. Centrifuge at 4℃ at 12,000 rpm for 5 minutes to remove the liquid.

2.4.1.5. Dissolved RNA The centrifuge tube was placed on an ultra-clean table and blown for 3 minutes. 15 μL RNA solution was added to dissolve the RNA and incubated at 55℃ for 5 minutes.

2.4.1.6. RNA concentration determination RNA concentration and purity were detected by Nanodrop2000: After blank zero adjustment of the instrument, 2.5 μL RNA solution was taken on the detection base, the sample arm was put down, and the absorbance value was detected by software on the computer.

2.4.2. Reverse transcription (both gun head and PCR are sterilized by damp heat without RNase).

1. Preparation of reverse transcription reaction System.
2. Gently mixed and centrifuged.
3. Setup of reverse transcription program.

2.4.3. Quantitative PCR.

1. Take 0.1 mL PCR reaction plate, prepare the reaction system as follows, and make 3 multiple Wells for each sample.
2. PCR Amplification.

Table 1

Relevant characteristics of patients with osteoarthritis.

Characteristics			Osteoarthritis		*P*
			No	Yes	
Sex	Male	112	49 (20.9%)	63 (26.9%)	.480
	Female	122	59 (25.2%)	63 (26.9%)	
Age	≤60	109	55 (23.5%)	54 (23.1%)	.217
	>60	125	53 (22.6%)	72 (30.8%)	
ELOVL7	Low	116	53 (22.6%)	63 (26.9%)	.888
	High	118	55 (23.5%)	63 (26.9%)	
GP9	Low	116	55 (23.5%)	61 (26.1%)	.701
	High	118	53 (22.6%)	65 (27.8%)	
F2RL3	Low	123	27 (11.5%)	96 (41.0%)	<.001*
	High	111	81 (34.6%)	30 (12.8%)	
ITGA2B	Low	113	51 (21.8%)	62 (26.5%)	.762
	High	121	57 (24.4%)	64 (27.4%)	

Pearson chi-square test.

*$P < .05$.

2.5. Statistical methods

Data is expressed as a percentage of the total. Pearson Chi-square test and Spearman correlation coefficient were used to analyze clinical parameters and OA. Univariate and multivariate logistic regression analysis was used to calculate odds ratio (OR) values of each variable of OA. All statistical analyses were performed using SPSS software, version 21.0 (IBM, Armonk, NY). $P < .05$ was considered statistically significant.

3. Results

3.1. Pearson chi-square test was used to analyze the association between OA and related factors

The relationship between OA and related clinical factors was summarized by Pearson chi-square test. F2RL3 was significantly associated with OA ($P < .001$). However, there was no significant correlation between sex ($P = .480$), age ($P = .217$), ELOVL7 ($P = .888$), GP9 ($P = .701$), ITGA2B ($P = .762$), and OA (Table 1).

3.2. Spearman's correlation coefficient analyzed the association between vertebral compression fractures and related factors

Further analysis of Spearman's correlation coefficient showed that OA was significantly correlated with F2RL3 ($\rho = -0.511$, $P < .001$). However, sex ($\rho = 0.046$, $P = .482$), age ($\rho = 0.081$, $P = .219$), ELOVL7 ($\rho = 0.009$, $P = .888$), GP9 ($\rho = 0.025$, $P = .703$), and ITGA2B ($\rho = -0.020$, $P = .763$) had no significant correlation with OA (Table 2).

Table 2

Relationship between patient characteristics and osteoarthritis.

	Osteoarthritis	
Characteristics	ρ	*P*
Sex	−0.046	.482
Age	0.081	.219
ELOVL7	−0.009	.888
GP9	0.025	.703
F2RL3	−0.511	<.001*
ITGA2B	−0.020	.763

Spearman correlation analysis.
*$P < .05$.

3.3. Univariate Logistic regression analysis of OA and related factors

Binary Logistic regression was used to determine the relationship between relevant parameters and OA, OR, and 95% confidence interval (95% CI). Table 3 describes the OR and 95% CI of the study subjects at the level of univariate Logistic regression, and the results show that F2RL3 (OR = 0.104, 95% CI: 0.057–0.189, $P < .001$) is significantly correlated with OA. However, sex (OR = 0.831, 95% CI: 0.496–1.390, $P = .480$), age (OR = 1.384, 95% CI: 0.825–2.319, $P = .218$), ELOVL7 (OR = 0.964, 95% CI: 0.576–1.611, $P = .888$), GP9 (OR = 1.106, 95% CI: 0.661–1.849, $P = .702$) and ITGA2B (OR = 0.924, 95% CI: 0.552–1.545, $P = .762$) were not significantly associated with OA (Table 3).

3.4. Multivariate logistic regression analysis of OA related factors

The study uses multivariate Logistic regression to describe the OR and 95% CI of the study objects at the multivariate level. F2RL3 was significantly correlated with OA (OR = 0.098, 95% CI: 0.053–0.182, $P < .001$). Sex (OR = 0.739, 95% CI: 0.394 1.387, $P = .346$), age (OR = 1.495, 95% CI: 0.800–2.795, $P = .208$), ELOVL7 (OR = 1.406, 95% CI: 0.751–2.632, $P = .286$), GP9 (OR = 1.096, 95% CI: 0.591–2.031, $P = .772$), and ITGA2B (OR = 0.757, 95% CI: 0.403–1.423, $P = .387$) were not significantly associated with OA (Table 4).

4. Discussion

Pearson's chi-square test, Spearman's correlation coefficient and Logistic regression analysis showed that F2RL3 was significantly associated with OA.

OA is the most common musculoskeletal disease and the largest cause of disability in the world.[14] OA is a chronic disease that progresses gradually. Its main symptom is joint pain, usually pain at rest. It is manifested as pain after rest, which is relieved after a short period of activity, but the pain is aggravated after too much activity. Another symptom is joint stiffness, which often occurs when you get up in the morning or after your joints have been in a certain position for a long time during the day.

Table 3

Influence of relevant parameters on osteoarthritis based on univariate logistic regression analysis.

			Osteoarthritis		
Parameter			OR	95% CI	*P*
Sex	Male	112	1		.480
	Female	122	0.831	0.496–1.390	
Age	≤60	109	1		.218
	>60	125	1.384	0.825–2.319	
ELOVL7	Low	116	1		.888
	High	118	0.964	0.576–1.611	
GP9	Low	116	1		.702
	High	118	1.106	0.661–1.849	
F2RL3	Low	123	1		<.001*
	High	111	0.104	0.057–0.189	
ITGA2B	Low	113	1		.762
	High	121	0.924	0.552–1.545	

95% CI = 95% confidence interval, OR = odds ratio.
*$P < .05$.

Table 4

Multivariate logistic regression analysis of the characteristics and influence of osteoarthritis.

	Osteoarthritis		
Characteristics	**OR**	**95% CI**	***P***
Sex	0.739	0.394–1.387	.346
Age	1.495	0.800–2.795	.208
ELOVL7	1.406	0.751–2.632	.286
GP9	1.096	0.591–2.031	.772
F2RL3	0.098	0.053–0.182	<.001*
ITGA2B	0.757	0.403–1.423	.387

95% CI = 95% confidence interval, OR = odds ratio.

*$P < .05$.

The treatment of OA should adopt a variety of comprehensive treatment, step treatment method to effectively relieve pain, and the development of end-stage surgery is required.[15] OA is easy to occur in the knee joint, hand joint, cervical vertebra, lumbar vertebra, and some small joints deteriorate with age, and small joint hyperplasia will occur, leading to the occurrence of OA. Due to today's lifestyles, higher rates of obesity and higher average life expectancy, its prevalence is on the increase and is highly prevalent worldwide, resulting in a huge economic burden.[14] Although OA is often referred to as a joint disease with cartilage damage and loss, OA is a more diversified disease with a complex pathogenesis that affects all tissues within the joint.[16] The pathogenesis of OA largely depends on the imbalance between pro-inflammatory and anti-inflammatory mediators, leading to low-grade inflammation, cartilage degradation, bone remodeling and synovial hyperplasia.[17]

The pathological driving force for OA development involves more than simple mechanical "wear and tear." Inflammatory mechanism plays an important role in tissue response to joint injury, which may lead to the occurrence of post-traumatic joint OA.[18] The inflammation of OA is different from that of rheumatoid arthritis and other autoimmune diseases in that the inflammation is chronic, relatively low grade and mainly mediated by the innate immune system. Clinically, many OA patients have symptoms related to joint inflammation, such as morning stiffness, fever, pain, and joint effusion partially caused by synovial thickening or synovial effusion.[19] Chondrocytes and synovial cells in OA produce or overproduce many inflammatory mediators that are characteristic of inflammatory arthritis. A variety of soluble inflammatory mediators, including cytokines, chemokines, growth factors, adipokines, prostaglandins and leukotrienes, have been found in OA joint tissues and body fluids. Relevant studies have determined that the chronic low-grade inflammation found in OA contributes to the development and progression of the disease. In the progression of OA, the entire synovial joint, including cartilage, subchondral bone and synovial membrane, is involved in the inflammatory process.[20] Synovitis is considered to be an important feature in patients with OA and is associated with symptoms and structural progression. In addition, there is growing evidence that OA is associated with platelet dysfunction.[21,22]

F2RL3 functions to encode thrombin protease-activated receptor-4, a protein expressed in various tissues throughout the body, including blood leukocytes and lung tissues, and a key regulator of platelet reactivity.[23] F2RL3 gene has been found to play a role in mediating platelet activation and many signaling pathways related to endothelial cell function and vascular inflammatory response.[24] F2RL3 is a key gene in immune cell recruitment, behavior and coagulation.[25] The F2RL3 gene is associated with IL-18, possibly due to the unique properties of IL-18 as a cytokine, which enhances cell-mediated cytotoxicity and immune responses to T-cofactor 1 (pro-inflammatory) and T-cofactor 2 (anti-inflammatory).[26] F2RL3 is a cardiovascular related gene, and relevant studies have shown that,[27,28] F2RL3 has a causal relationship with cardiovascular pathology through its role in endothelial physiology and platelet activation. Other studies have shown that F2RL3 is involved in the mechanism by which metabolic syndrome affects the occurrence of OA, and may be a potential biomarker for the diagnosis of OA.[29]

However, there are some flaws in this study. Although clinical data have been examined and analyzed, the molecular mechanism of F2RL3 expression levels on OA has not been validated in animal models. Therefore, future studies should focus on animal experiments to explore the molecular pathway and mechanism of F2RL3 in OA.

5. Conclusion

There is a significant correlation between the expression level of F2RL3 and the incidence of OA. Low expression of F2RL3 in OA, the lower the expression, the greater the probability of OA. As a potential target of OA, F2RL3 provides a new idea for the molecular mechanism of its occurrence and development.

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Author contributions

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