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MMP-1 gene polymorphism in osteoporosis

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Abstract. – OBJECTIVE: To investigate the correlation of osteoporosis (OP) with matrix metalloproteinase-1 (MMP-1) gene polymorphism.

PATIENTS AND METHODS: A total of 199 patients as observation group (OP) and 180 healthy subjects (control group) were enrolled in the study, and the general data, the expression levels of serum calcium, serum phosphate and MMP-1 were collected and determined. The bone mineral density was determined using a bone sonometer, and the method of TaqMan-MGB probe was adopted for the detection of gene polymorphism of MMP-1 rs494379.

RESULTS: The level of serum calcium and bone mineral density were lower in observation group than those in control group ($p>0.05$), while the levels of serum phosphate and MMP-1 were higher than those in control group ($p>0.05$). There were differences in rs494379 genotype and allele in MMP-1 gene between the two groups ($p<0.05$). In the analysis of the genetic model, the dominant models between the two groups were different ($p<0.05$), while there were no differences in the recessive model and additive model ($p>0.05$). The MMP-1 level was higher in rs494379 AA genotype than that in GG, GT genotype in MMP-1 gene.

CONCLUSIONS: Gene polymorphism of MMP-1 rs494379 has a correlation with the occurrence of OP.

Key Words:
Osteoporosis, Matrix metalloproteinase-1, Single nucleotide polymorphism.

Introduction

Osteoporosis (OP) is a common systemic bone disease characterized by the decline in bone mass, microstructural damage of bone tissues, increase in bone fragility, and an increased risk for fractures. According to the National Epidemiological Survey of China in 2018, the overall morbidity rate of OP among populations aged above 50 years old reached 19.2%, in which that among females reached 32.1% while that among females aged over 65 years old was up to 51.6%. OP mostly attacks postmenopausal women and elderly men, which causes enormous distress to the patient as well as a heavy economic burden to the family and the society. In recent years, some research has shown that matrix metalloproteinases (MMPs) are involved in the occurrence and development of OP[1-4]. During the bone remodeling process, matrix metalloproteinase-1 (MMP-1) is closely related to degradation of bone matrix, startup of bone resorption and formation of bones, which is a coupling factor that reflects mutual regulation of bone resorption and bone formation[5-8]. Therefore, MMP-1 gene was selected as the candidate gene in the present study. The polymorphism of MMP-1 rs494379 was detected among OP patients collected in our department using the method of TaqMan-MGB probe to explore the correlation of MMP-1 gene polymorphism with the occurrence of OP, thus providing theoretical support for the genetic polymorphism of OP.

Patients and Methods

Research Objects

OP patients who were admitted in our hospital from January 2017 to December 2018 were enrolled in the present study. Inclusion criteria: (1) Patients who accorded with diagnostic criteria in Guidelines for the Diagnosis and Treatment of Primary Osteoporosis (2017), and (2) patients who had a good compliance and complete data. Exclusion criteria: (1) Patients who took drugs

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that affected bone metabolism (such as vitamin D, glucocorticoids, and calcium), (2) patients who suffered from dysfunction of major organs such as heart, kidney, and liver, or (3) patients complicated with mental disease or other cognitive disorders that may affect the degree of cooperation. According to the above criteria, a total of 199 OP patients with an average age of (60.68 ± 4.80) years old were enrolled in observation group. Meanwhile, 180 healthy subjects in the physical examination center of our hospital during the same period were included in control group, whose average age was (61.21 ± 5.20) years old. All research subjects were unrelated Chinese Han population and all signed the informed consent. This investigation was approved by the Ethics Committee of the First People's Hospital of Jingzhou City, China.

Collection of General Clinical Data

The name, age and gender of the research objects were collected. A total of 5 mL venous blood was extracted from the elbow of the patients and centrifuged at 4°C and 800 g for 5 min. Serum was collected into 0.5 mL Eppendorf (EP) tubes (Hamburg, Germany) (200 μL/tube) and stored at -80°C for standby application. The levels of serum calcium, serum phosphate and MMP-1 were determined using enzyme-linked immunosorbent assay (ELISA, Novus Biologicals, Littleton, CO, USA) and the bone mineral density was detected *via* a bone sonometer.

DNA Extraction

Deoxyribonucleic acid (DNA) was extracted from 1 mL of venous blood collected from the elbow of the research objects using a medium whole blood genomic DNA isolation kit (Beijing Bioteke Corporation, Beijing, China) in accordance with the instructions of the kit. Genotype analysis of samples was carried out with TaqMan®SNP Genotyping Assays Kit (Thermo, Waltham, MA, USA). The probe information of specific gene locus is shown in Table I.

Statistical Analysis

The statistical analysis was completed using Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY, USA). The results of measurement data were presented as $\bar{x} \pm s$, and compared using the independent-samples *t*-test. Chi-square test was employed for the comparison of enumeration data. The likelihood ratio χ^2-test was adopted to analyze whether the distribution of different genotypes accorded with Hardy-Weinberg equilibrium. Genotypes and gene frequency between the two groups were compared using the R×C chi-square test. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparison of Basic Data

The level of serum calcium and bone mineral density were lower in observation group than those in control group ($p>0.05$), and the levels of serum phosphate and MMP-1 were higher than those in control group ($p>0.05$) (Table II).

Genetic Equilibrium Test

The likelihood ratio χ^2-test was performed on actual frequency and theoretical frequency of 3 genotypes in observation group and control group. The distribution frequency of rs494379 genotype in MMP-1 gene in observation group and control group accorded with Hardy-Weinberg genetic equilibrium ($p>0.05$), so they were comparable (Table III).

Comparison of Genotype Distribution Frequency

AA, AG, GG genotype distribution frequencies were 64.82%, 30.15%, and 5.03%, respectively, in observation group, and 55.56%, 33.33%, and 11.11%, respectively, in control group. The genotype distribution frequency between the two groups was different ($p<0.05$) (Table IV).

Comparison of Allele Distribution

Table I. TaqMan®-MGB probe information at rs494379 genetic locus in MMP-1 gene.

SNP Reference	rs494379
Assay ID	C____632712_10
SNP Type	Transition Substitution, Intron, Intragenic
Context Sequence	GTACTCCATGGTCTTTTTGAAAAAG[A/G]CTGGTTCTGATGGTCATAAAGTGCT

Table II. Comparison of general information between the two groups.

Group	N	Age (year)	Male/ female (n)	Serum calcium (mmol/L)	Serum phosphate (mmol/L)	Bone density (g/cm^2)	MMP-1 (μg/L)
Observation group	199	60.68 ± 4.80	80/119	2.07 ± 0.27	1.13 ± 0.26	0.70 ± 0.10	4.32 ± 1.42
Control group	180	61.21 ± 5.20	89/91	2.35 ± 0.17	1.22 ± 0.24	1.07 ± 0.12	3.61 ± 1.21
t/χ^2		0.420	3.268	3.670	5.272	3.731	3.461
p		0.520	0.071	0.043	0.013	0.038	0.045

Table III. Detection of genetic equilibrium of rs494379 genotype in MMP-1 gene.

Group	No.	AA		AG		GG		χ^2	p
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	199	129	127.04	60	63.92	10	8.04	0.75	0.69
Control group	180	100	93.89	60	72.22	20	13.89	5.16	0.08

Table IV. Comparison of distribution conditions of rs494379 genotype in MMP-1 gene between the two groups.

Group	N	Genotype [n (%)]			χ^2	p
		AA	AG	GG		
Observation group	199	129 (64.82)	60 (30.15)	10 (5.03)	6.069	0.048
Control group	180	100 (55.56)	60 (33.33)	20 (11.11)		

Frequency

A, G allele distribution frequencies were 79.90% and 20.10% in observation group, and 72.22% and 27.78% in control group. The allele distribution frequency between the two groups was different (p<0.05) (Table V).

Analysis on rs494379 Genetic Model in MMP-1 Gene

In the analysis of genetic model, there was difference in the dominant model between the two groups (p<0.05), while there were no differences in recessive model and additive model (p>0.05), indicating that the dominant model is available for describing the genetic model of rs494379 in OP MMP-1 gene (Table VI).

Comparison of MMP-1 Level Between Different Genotypes of rs494379 in MMP-1 in Observation Group

According to the comparison of general information and genotypes, correlations of three genotypes of rs4943797 in MMP-1 gene in observation group with MMP-1 level were further analyzed. It was found that the MMP-1 level of rs494379 AA genotype was higher than AG, GG genotype in MMP-1 gene (p<0.05) (Table VII).

Discussion

Table V. Comparison of distribution conditions of rs494379 A/G allele in MMP-1 gene between the two groups.

Group	N	Allele [n (%)]		χ^2	p
		A	G		
Observation group	199	318 (79.90)	80 (20.10)	6.153	0.013
Control group	180	260 (72.22)	100 (27.78)		

Table VI. Analysis of rs494379 genetic model in MMP-1 gene between the two groups [case (%)].

	Item	Observation group	Control group	χ^2	*p*
Recessive model	AA *vs.* AG+GG	129 (64.82)/70 (35.18)	100 (55.56)/80 (44.44)	3.395	0.065
Dominant model	AA+AG *vs.* GG	189 (94.97)/10 (5.03)	160 (88.89) 20 (11.11)/ 20 (11.11)	4.803	0.028
Additive model	AA *vs.* AG *vs.* GG	129 (64.82)/60 (30.15)/ 10 (5.03)	100 (55.56)/60(33.33)/	6.069	0.048

With the development of economy, changes in living habits and the increasing aging population, the morbidity of OP has been on a rise year by year and patients have become younger in average age[9,10]. According to current research, the occurrence of OP is correlated with environmental factors, dietary habits, endocrine secretion, heredity, and some other factors. Meanwhile, lack of mineral substances such as calcium, phosphorus in human body may also affect the occurrence and development of OP[11,12]. The general conditions of research objects from the two groups were analyzed in the present study, and it was found that the level of serum calcium and bone mineral density were lower in observation group than those in control group, while the level of serum phosphate was higher than that in control group. It indicated that OP may result in metabolic disturbance of calcium and phosphorus and decreased bone mineral density. Bone metabolism in healthy individuals can maintain a balance state in destruction and regeneration by supplement of minerals. But when the mineral supplement is insufficient, this balance will be broken, leading to the destruction of the bone trabecula, and finally causing decreased bone mineral density and occurrence of OP. Therefore, calcium supplements and other treatment methods should be given to OP high-risk populations in the early stage.

MMPs are a kind of extracellular proteinase compromised by zinc ion-dependent enzyme that can degrade extracellular matrix, which are considered to be the most important category in various proteolytic enzyme families that can degrade extracellular matrix[13]. Bone is a special connective tissue made up of various cells and extracellular matrixes which is called bone matrix in bone tissue. Bone matrix is closely related to the integrity of cellular and histological structure in bone tissue. In normal tissues, the bone matrix is in a dynamic equilibrium of continuous generation and degradation. The degradation of bone matrix mainly depends on MMPs. The surface of normal bone tissue is covered by a layer of barrier comprised by osteoblasts and type I collagen that is not mineralized to prevent contact of osteoclasts and mineralized bone matrix. After being stimulated by bone resorption factor, osteoblasts will secrete MMPs to degrade type I collagen, activate osteoclast and start bone resorption[14-17]. MMP-1 is a matrix metalloproteinase in MMPs family that mainly degrades type I collagen. Recent studies have shown that MMP-1 secreted by osteoblasts starts bone resorption and bone formation by degrading bone matrix such as type I collagen during the process of bone remodeling. In the present study, the MMP-1 level of research objects from the two groups was compared and analyzed, and the results showed that it was higher in observation group than that in control group, suggesting that the expression of MMP-1 in OP patients is increased, so the degradation of type-I collagen is enhanced, the signal of starting up bone resorption is reinforced, and the degradation of bone matrix and loss of bone mass are aggravated, ultimately bringing about OP.

With the increasingly advanced molecular biological techniques and continuous development of genomics, people's understanding about diseases has gradually shifted to the genetic level. In recent years, research[18-20] about OP has also found that the onset of OP is closely related to genetic inher-

Table VII. Comparison of MMP-1 levels between different genotypes of rs494379 in MMP-1 gene.

Item	AA	AG	GG	*t*	*p*
MMP-1 (μg/L)	4.56 ± 1.47	4.21 ± 1.32	4.19 ± 1.40	6.420	0.040

itance. Therefore, it was speculated that MMP-1 protein formed after transcription and translation of MMP-1 gene may be correlated with the occurrence and development of OP. In the present study, polymorphic site rs494379 (A/G) in MMP-1 was selected and TaqMan-MGB probe method was adopted to analyze genotype frequency and allele frequency of observation group and control group. It was found that there were differences in the distribution frequency of rs494379 (A/G) genotype and allele in MMP-1 gene, indicating that rs494379(A/G) polymorphism in MMP-1 gene is correlated with onset risk of OP. rs494379(A/G) genetic models in MMP-1 gene were further analyzed and it was found that there was a difference in the dominant model between the two groups, indicating that the dominant model is available for describing the genetic model of rs494379 in MMP-1 gene of OP patients. According to the comparison of MMP-1 level and genotype, correlations of three genotypes of rs4943797 in MMP-1 gene in observation group with MMP-1 level were further analyzed. The results revealed that the MMP-1 level of rs494379 AA genotype was higher than that in AG, GG genotypes in MMP-1 gene, suggesting that the mutation of rs494379 AA gene in MMP-1 gene of OP patients may be related to an increased MMP-1 level in OP patients. It can be speculated that mutation in MMP-1 gene leads to an increased expression of MMP-1, so the degradation of type-I collagen is enhanced, the signal of starting up bone resorption is reinforced, and the degradation of bone matrix and loss of bone mass are aggravated, ultimately bringing about OP.

Conclusions

In summary, gene polymorphism of MMP-1 rs494379 has a correlation with the occurrence of osteoporosis (OP).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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