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Research on correlation between GALNT3 gene and osteoporosis

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Abstract. – OBJECTIVE: The aim of this study was to explore the correlation between GALNT3 gene and osteoporosis.

PATIENTS AND METHODS: In this study, 184 cases of osteoporosis that were treated at our hospital from 2013 to 2014 were selected as research subjects in the observation group. In addition, 84 healthy people were selected as the control group from 2013 to 2014. The bone mineral density of the observation and control groups were detected by x-rays and the expression levels and differences of mRNA of the GALNT3 gene and protein in their body was detected using fluorescence quantitative polymerase chain reaction (qPCR), enzyme-linked immunoassay, and Western blotting.

RESULTS: X-ray results suggest that when compared to the healthy group, bone mineral density of patients in the observation group was significantly lower than that of research subjects in the control group, with significant differences. The fluorescence qPCR results suggest that the expression levels of mRNA of the GALNT3 gene in patients with osteoporosis were significantly lower than that in the healthy group ($p<0.05$). Enzyme-linked immunosorbent assay (ELISA) results suggest that the expression levels of the GALNT3 gene in patients with osteoporosis (1.26±0.32) μg/L was significantly lower than that in the healthy group (12.41±0.28) μg/L, with significant differences ($p<0.05$). The Western blotting results agreed with the ELISA results. We also found in our research that the bone mineral density of patients with osteoporosis significantly correlated with the expression levels of the GALNT3 gene ($r=0.95$).

CONCLUSIONS: Therefore, the GALNT3 gene significantly correlated with osteoporosis and the low expression of GALNT3 gene can promote the occurrence and deterioration of osteoporosis.

Key Words
GALNT3, Osteoporosis, Correlation, Enzyme linked immunoassay, Western-blotting.

Introduction

As we have entered the 21st century, the aging population has been accounting for an increasingly higher percentage of the total population year by year. According to statistical results in 2014, there are 230 million aging people (≥ 60 years old) in China[1]. This suggests that China has officially entered into an aging society[2]. Enhancing diagnosis and treatment of geriatric diseases has become an important topic for the improvement of the quality of life for the aging people. Statistical data[3] suggests that by 2014, aging patients that suffer from osteoporosis account for 78.36% of the total number of aging population. This indicates that osteoporosis has become an important disease that influences the quality of life of aging people[4]. Clinical research[5] suggests that osteoporosis is mainly manifested by wear, decay and backfall of bone joints in clinical practice and can influence normal movement, such as walking, and other daily behaviors of the human body. Research indicates that osteoporosis involves various causes. Bad diet habits, alcoholism and irregular living style all can lead to occurrence of osteoporosis. As people get older, their intake and storage capacity of calcium and other trace elements are reduced, which is also an important reason for the occurrence of osteoporosis[6]. Secondly, with development of molecular biotechnology in recent years, increasing research results have pointed that the occurrence of osteoporosis is not only influenced by external factors[7], but also significantly influenced by genetic factors. In addition, Joseph et al[8] suggested that expression of related genes in the notch1 signaling pathway in patients with osteoporosis are significantly lower than that in the healthy group and the notch1 signal pathway can get involved

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in the generation of osteoblasts and regulation of calcium levels in cytoplasm. Brambila-Tapia et al[9] suggested that the GALNT3 gene can encode UDP-N-acetyl-α-galactosaminyl-transferase in the human body and is mainly involved in the body bone metabolism and related processes. For example, research has suggested that the mutation of GALNT3 gene can cause recessive hereditary disease, which is mainly manifested by high serum phosphate and heterotopic ossification, etc.[10].

In this study, we have for the first time, discussed the correlation between the GALNT3 gene and osteoporosis and hope to provide certain theoretical and experimental references for diagnosis and treatment of osteoporosis.

Patients and Methods

Sample Selection

In this study, there were 184 cases of osteoporosis that were treated at our hospital from 2013 to 2014 were selected as research objects. In the observation group, there were 89 male cases and 95 female cases, with average age of 68.3±4.2years old. There were 84 healthy people selected as the control group from 2013 to 2014, including 38 male cases and 46 female cases, with an average age of 67.2±3.7 years old. The RNA extraction kit (TaKaRa, Dalian, China), extraction kit for animal cell total protein (Axygen, Tewksbury, MA, USA), GALNT3 monoclonal first antibody of goat anti-rabbit (Acris, New York, NY, USA), and horseradish-peroxidase (HRP) marked second antibody of rabbit anti-human (AVM, Beijing, China). The main equipment include fluorescence quantitative PCR (ABI, Foster City, CA, USA), protein electrophoresis apparatus (Beijing Liuyi, Beijing, China), multifunctional enzyme mark instrument (Bio-Rad, Hercules, CA, USA), and bone mineral density determinator (Hologic, Bedford, MA, USA).

Methods

Fluorescence Quantitative Polymerase Chain Reaction (qPCR) RNA Extraction

The lesion tissues of patients in different groups were used as research subjects. Equivalent tissue samples were exactly weighed and the operations were conducted according to instructions on the RNA extraction kits. The concentration of total RNA extracted was detected by microscale nucleic acid basis weight instrument and existence of its degradation was judged by gel electrophoresis.

Fluorescence Quantitation

The total RNA extracted was taken as the template and the original reverse transcription RNA was 500 ng. The complementary deoxyribose nucleic acid (cDNA) was obtained by reverse transcription and then the operations were conducted according to instructions. The total volume was 10 μL and the primers are shown in Table I[11].

Enzyme Linked Immunoassay

The total protein extraction was taken as the research object and the total protein levels of various research samples were detected using Coomassie brilliant blue staining. The total protein in the enzyme-linked immunosorbent assay (ELISA) experiment process was 2.5 μg. During the experimental process, 100 μL of sample were detected and the 35 μL detection liquid were added into the 96 orifice. Operations were conducted according to instructions on kits and the absorption value was determined at 455 nm[12].

Protein Western Immunoblotting

In this study, we used the extraction kits for animal cell total protein from Axygen (Tewksbury, MA, USA) in order to extract protein from animal cells (specific operations were conducted according to instructions). 0.5 mg of the research sample was exactly weighted and taken from each group. After extraction of total protein, the quantitation was conducted using Coomassie brilliant blue staining. 20 μL of the processed sample was taken for protein sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, and then for transmembrane processing, and finally for closed treatment for 2 h. The first antibody was diluted according to the ratio of 1:1000 for usage, and then incubated at room

Table I. Fluorescence quantitative PCR primer.

Primer name	Primer sequence
ganlt1-F	CGTCGTGCTGACAGCTAGCGCTC
galnt1-R	CGTAGTCGATCGATCGACCGTCG
GAPDH-F	CGTCGTCGGGACAGCTAGCTGAG
GAPDH-R	CGTAGTCGACAGCTGATCGCAGCTG

Table II. Detection of bone mineral density of the observation group and the control group.

Group	N	Lumbar spine	Proximal femur bone	*p*	T
Observation group	84	0.960±0.12	0.937±0.11	0.00	8.29
Control group	184	0.681±0.11	0.793±0.12	0.00	8.04

temperature for 2 h. After the addition of the secondary antibody, the sample was incubated at room temperature for 2 h. After washing with the eluent (10 min/once, 5 times), the colored solution was added for color development.

Detection of Bone Mineral Density by X-Ray

Detection was conducted according to the X-ray Diagnosis and the experiment conducted by Rahimi et al[13].

Immunohistochemistry Experiment

In this study, we conducted a conventional antibody incubation and staining in the hippocampal CA3 area of mice by the affinity between streptomycin and peroxidase (S-P). The judgment standard of immunohistochemistry was as follows: the membrane staining < 10% or the only membrane was stained or > 10% staining could be observed as positivity[14].

Statistical Analysis

The results were analyzed using Statistical Product and Service Solutions (SPSS) 10.0 statistical software (SPSS Inc., Chicago, IL, USA). Data were expressed by mean ± standard deviation and $p<0.05$ was of statistical significance.

Results

Detection of Bone Mineral Density of the Observation Group and the Control Group

In this study, we used the bone mineral density determinator in order to detect the bone mineral density of the lumbar spine and proximal femur bone of healthy people and patients with osteoporosis. According to Table II, the bone mineral density of the lumbar spine and proximal femur bone of healthy people, (0.960±0.12, 0.937±0.11) g/cm^3, was significantly higher than that of patients with osteoporosis (0.681±0.11, 0.793±0.12) g/cm^3.

Expression Differences of mRNA of GALNT3 Gene in the Observation Group and the Control Group

In order to detect expression differences of mRNA of the GALNT3 gene in research samples of various groups, we used the total RNA extracted from healthy people and patients with osteoporosis as research subjects. Fluorescence qPCR was used to detect expression differences of GALNT3 gene in research samples and the results of the extracted RNA electrophoresis are shown in Figure 1. We can see from the picture that extracted RNA showed no significant degradation. The 28S brightness was about two times the 18S brightness, which suggests that extracted RNA could be applied for a later test. We can see from Figure 2 that the expression level of mRNA of the notch1 gene in research samples from the observation group were significantly lower than that in the control group ($p<0.05$). These results suggest that after different processing, expression of the notch1 gene varies among gastric cancer cells.

Expression Differences of Protein of GALNT3 Gene in the Observation Group and the Control Group

We took total protein from healthy people and patients with osteoporosis as research subjects. The enzyme-linked immunoassay was used to quantitatively determine expression differences of the protein of GALNT3 gene in the observation group and the control group. Results were shown in Figure 3.

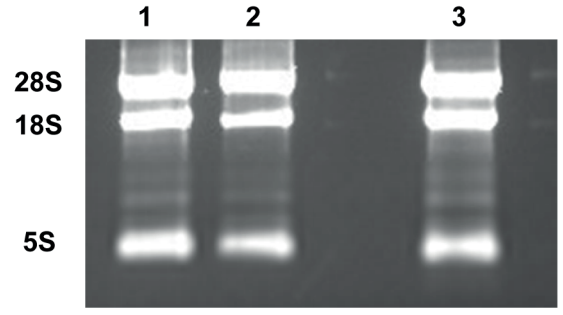

Figure 1. Extraction results of RNA in different samples. 1: electrophoresis of RNA from healthy people; 2-3: electrophoresis of RNA from patients with osteoporosis.

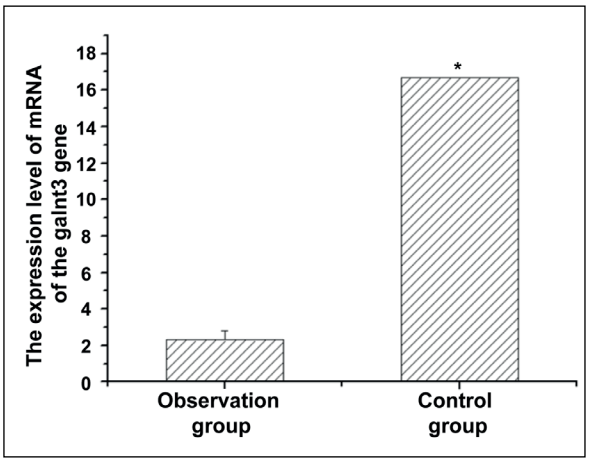

Figure 2. Expression differences of the mRNA of GALNT3 gene in the observation group and the control group. *p<0.05.

The expression levels of protein of GALNT3 gene in research samples from the observation group, (1.26±0.32) μg/L, were significantly lower than that in the control group (12.41±0.28) μg/L (p<0.05) (Figure 3). These results suggest that there was certain correlation between expression levels of protein of GALNT3 gene and osteoporosis.

Expression Differences of Protein of GALNT3 Gene in the Observation Group and the Control Group as Detected by Western-blotting

We obtained total protein from healthy people and patients with osteoporosis as research subjects. The Coomassie brilliant blue was used to quantitatively determine total protein. The 2.5 μg total protein was extracted for SDS-PAGE electrophoresis. After transmembrane incubation, the expression differences of protein of GALNT3 gene in the observation group and the control group were determined (Figure 4). The expression levels of protein of GALNT3 gene in the observation group were significantly lower than that in the control group (Figure 4). Quantitative detection of the Western-blotting results was shown in Figure 5, which agreed with results of ELISA and Western-blotting.

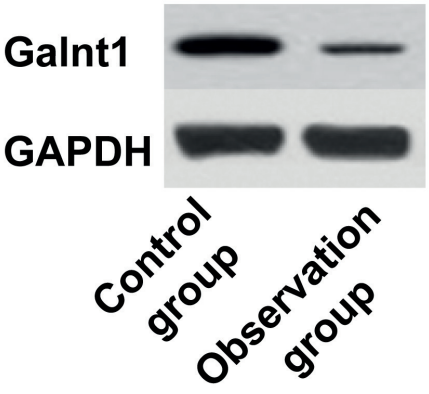

Figure 4. Expression differences of protein of GALNT3 gene in the observation group and the control group as detected by Western-blotting.

Expression Differences of Protein of GALNT3 Gene in the Observation Group and the Control Group as Detected by Immunohistochemistry

Samples from the observation group and the control group were used as research subjects. Expression differences of the GALNT3 gene in different samples were detected using immunohistochemistry (Figure 6). The expression levels

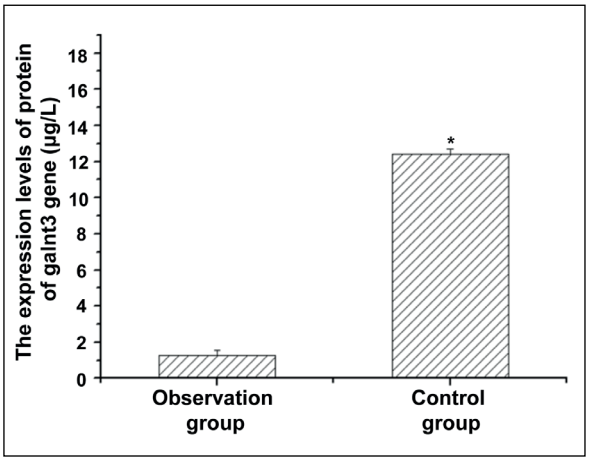

Figure 3. Expression differences of protein of GALNT3 gene in the observation group and the control group. *p<0.05.

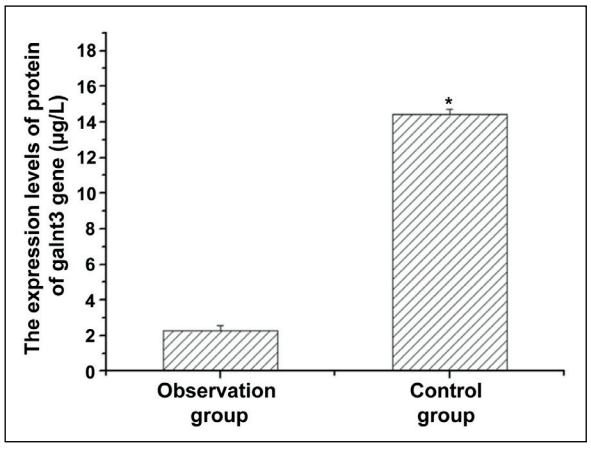

Figure 5. Quantitative detection of expression differences of protein of GALNT3 gene in the observation group and the control group by Western-blotting. *p<0.05.

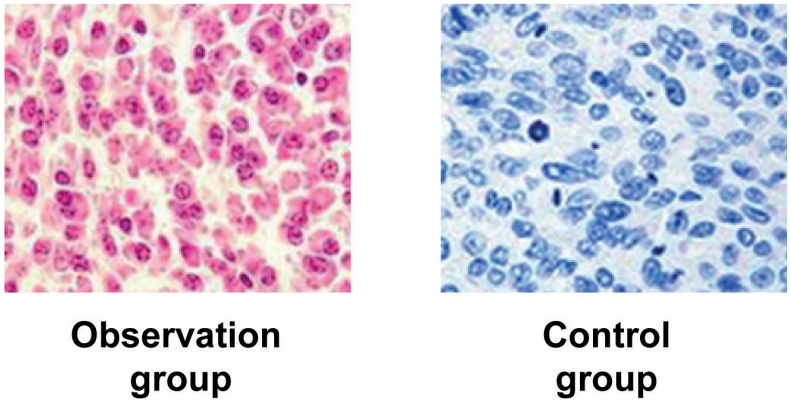

Figure 6. Expression levels of GALNT3 gene in the observation group and the control group as detected by immunohistochemistry.

of GALNT3 gene in the observation group were significantly lower than that in the control group (Figure 6). Statistical results of the number of cells with positive glant1 gene in different research samples found that the positive rate of glant1 gene in healthy people (97.5%) was significantly higher than that in patients with osteoporosis (5.1%) (Table III).

Correlation Between Bone Mineral Density and Expression Level of GALNT3 Gene

In this study, we took the expression levels of protein of GALNT3 gene and data of bone mineral density as the research subjects. The correlation between the expression levels of protein of GALNT3 gene and the bone mineral density were detected using SPSS software. Results suggested that their correlation was 0.95, which indicated that the expression levels of protein of GALNT3 gene and the bone mineral density share significant positive correlation. That is, a reduction in the expression levels of protein of the GALNT3 gene can to a great extent, induce the occurrence of osteoporosis.

Discussion

Osteoporosis, as a type of orthopedic diseases, is characterized by reduction of bone mineral density of the whole body and abnormality of bone microstructure. Clinically, it manifests by a reduction of bone hardness, including waist pain, soreness of knees joints, shoulder lassitude and other symptoms[15]. Statistical survey results of epidemiology in 2014 suggest that there are about 80 million osteoporosis patients in China. Among them, the elderly account for about 85-89% of the population. Therefore, osteoporosis has become an important disease that influences the quality of life of elderly people. Current research results suggest[16] that osteoporosis is the combined consequence of dietary habits and genetic factors. Clinical statistical data suggest[17] that the main reason of osteoporosis is the loss of phosphorous components in bone. However, research suggests that phosphorous, as an important trace of human body, is very important for the structure of body organs and the development of bone. Research results in recent years suggest that in the body of osteoporosis patients, genes related to regulation of phosphorous metabolism and protein levels show a reduction to different levels compared with healthy people. For example, research suggests[18] that the FGF23 protein, a phosphorous regulation factor, shares close correlation with bone phosphorous metabolism in the human body, but mutation and loss of PHEX, DMP1 and GALNT3 genes in this signal pathway can lead to hereditary metabolism bone disease in different muscle striations[19]. The protein encoded by the GALNT3 gene, located on the No. 2 human chromosome, is mainly involved in the O-glycosylation of FGF23. Therefore, it is considered as an important protein, which prevents

Table III. Positive cell rates of galnt1 gene in the observation group and the control group as detected by immunohistochemistry.

Group	Cell number (n)	Galnt1 positive cell number	Galnt1 positive cell rate (%)	Galnt1 negative cell number	Galnt1 negative cell rate (%)
Observation group	400	390	97.5	10	2.5
Control group	400	20	5.1	380	94.9

the degradation of the FGF23 protein and promotes this function[20]. In this research, we detected the expression levels of mRNA and protein of the GALNT3 gene in healthy people and patients with osteoporosis. Results suggest that expression levels of mRNA and protein of the GALNT3 gene in healthy people and patients with osteoporosis were observed with significant differences. That is, the expression levels of mRNA and protein of the GALNT3 gene in patients with osteoporosis were significantly reduced, which indicates that mRNA and protein of the GALNT3 gene share close correlation with osteoporosis. In addition, we also found that the levels of mRNA and protein of GALNT3 gene share significant positive correlation with bone mineral density.

Conclusions

A reduction of expression levels of mRNA and protein of the GALNT3 gene can lead to a reduction of bone mineral density and further lead to occurrence of osteoporosis. However, we have not discussed in detail how GALNT3 gene induce reduction of bone mineral density and further induce osteoporosis.

Conflict of Interests:

The authors declared no conflict of interest.

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