Original paper

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Osteoporosis and polymorphisms of osteoprotegerin gene in postmenopausal women – a pilot study

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Abstract

Objectives: Osteoprotegerin (OPG) has an important role in bone remodeling, and it has been proposed that the OPG gene might be a candidate gene for osteoporosis predisposition. Several studies have already assessed the connection between OPG gene polymorphism and bone mineral density (BMD). In this study we wanted to analyze the association of two polymorphisms in the OPG gene with BMD and bone turnover markers in women with and without osteoporosis.
Material and methods: In 22 postmenopausal women with osteoporosis (aged 65.6 ±12.6) and 59 women without osteoporosis (aged 60.8 ±8.7) we analyzed the association of two polymorphisms in the OPG gene with BMD, measured by dual energy absorptiometry and with bone turnover markers (crosslaps and osteoprotegerin). A163G, G209A, T245G and G1181C polymorphisms were determined.
Results: No significant differences in age, anthropometry, number of fractures, osteocalcin and cross-laps were found between women with and without osteoporosis. Women with osteoporosis were significantly longer in postmenopause. Significantly more women with osteoporosis had AG polymorphism (p = 0.038) compared to women without osteoporosis, while no significant difference was found in prevalence of TT and GG polymorphism between patients with and without osteoporosis. No relationship was found between investigated polymorphism and bone turnover markers. A significant negative correlation between total hip BMD and crosslaps (p = 0.046) as well as between total hip T score and crosslaps (p = 0.044) was found in women without osteoporosis
Conclusions: Postmenopausal women with osteoporosis had AG polymorphism more frequently than women without osteoporosis. Our results indicate that A163G polymorphism could have an impact on higher bone loss in postmenopausal women.

Key words: osteoprotegerin, osteoporosis, gene polymorphism, postmenopause, osteocalcin, crosslaps.

Introduction

The key role of RANK (receptor activator of nuclear factor)/RANKL (RANK ligand)/OPG (osteoprotegerin) in pathogenesis of postmenopausal osteoporosis, by regulating osteoclast differentiation and activation, is well known. Therefore, the genes which have a role in expression of any component of the RANK/RANKL/OPG pathway are possible candidate genes for osteoporosis [1, 2].

Several studies have assessed the connection between OPG gene polymorphism and bone mineral density (BMD), but the results were not consistent and were

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dependent on the type of examined polymorphism and ethnicity [3, 4]. Considering the importance of the osteoprotegerin gene in bone turnover, its role as a candidate gene for osteoporosis susceptibility is possible. Single nucleotide polymorphisms (SNP) discovered in the OPG gene were associated with BMD in postmenopausal women or in men and women with osteoporotic fractures [5, 6]. Several studies have analyzed the relationship between RANK and RANKL gene polymorphism, but the results were also inconsistent [7, 8].

The aim of the study was to analyze the association of two polymorphisms in the OPG gene with BMD and bone turnover markers in postmenopausal women with and without osteoporosis.

Material and methods

Subjects of the study were patients referred to the rheumatologists from one University Hospital and two Polyclinics for Rheumatology and Physical Medicine, due to rheumatic complaints, in the period between 2009 and 2010. A total of 81 postmenopausal women participated in the study: 22 women with osteoporosis (T score < –2.5 either at the lumbar spine, total femur or femoral neck) aged 65.6 ±12.6 and 59 women without osteoporosis (T score > –2.0) aged 60.8 ±8.7. There were 25 women with a T score between –1.0 and –2.0. Women who had diseases or used medication known to affect bone metabolism (hyperthyroidism, renal, hepatic and gonadal dysfunction, malignancy, malabsorption, corticosteroids, anticoagulants and antiepileptic drugs) were excluded from the study. Two women received estrogen replacement therapy. Information on previous fractures and smoking was collected. Twenty patients had one fracture, while six patients had two fractures. Among them, only one patient had a fragility vertebral fracture. The smoking index was calculated by multiplying the number of cigarettes by years of smoking. All subjects were Caucasians, born in Croatia, and their parents were born in Croatia, too.

The study was designed in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Institute for Medical Research and Occupational Health. All participants signed an informed consent form.

Height and weight were measured and body mass index (BMI) was calculated (weight divided by squared height; kg/m^2).

Bone mineral density (BMD; g/cm^2) was measured using dual energy X-ray absorptiometry (Lunar – Prodigy, Madison, WI). Measurements were made at the lumbar spine (L1–L4) and left proximal femur. BMD was also expressed as the T score, which represents the number of standard deviations with respect to the mean BMD of a control population between 20 and 40 years of age, using the manufacturer's reference values. A T score lower than –2.5 was diagnosed as osteoporosis.

Serum samples were collected in the morning after an 8-hour fasting period. Osteocalcin was measured by radioimmunoassay (RIA) (DiaSource, Belgium) and β-crosslaps in sera was measured by ELISA test (IDS GmbH, Germany).

The QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) was used for DNA purification from blood. Polymerase chain reaction (PCR) was used to make copies of DNA segments and to amplify RANK gene fragments: A163G, T245G and G1181C. PCR products were cut using AseI/HinfI restriction endonucleases in order to determine A163G, G209A, T245G and G1181C polymorphisms.

Data were analyzed using statistical software Statistica, version 12 (StatSoft Inc., Tulsa, OK). The results are shown as mean values +/– standard deviation (SD). The distribution of variables was tested with the Kolmogorov-Smirnov test. All variables were normally distributed. The differences between two mean values were calculated with the Student *t*-test. The significance level for the difference between two proportions was computed based on the z-value for the respective comparison. The association between two variables was analyzed by the linear correlation test. *P*-values less than 0.05 were considered statistically significant.

Results

There were no significant differences in age, BMI, smoking index, number of fractures, osteocalcin and crosslaps between groups of patients with and without osteoporosis (Table I). Women with osteoporosis were significantly longer in postmenopause ($p = 0.008$). BMD at all measured sites was significantly lower in patients with osteoporosis ($p < 0.001$). There were no significant differences in bone density at all measure sites between patients with or without fractures and between smokers and non-smokers.

We performed an analysis of A163G and T245G OPG polymorphism. Distribution of OPG A163G polymorphism in postmenopausal women with osteoporosis was 9.5% AA, 52.4% AG and 38.1% GG, and in women without osteoporosis it was 1.7% AA, 29.3% AG and 69.0% GG. Frequency distribution of OPG T245G polymorphism in postmenopausal women with osteoporosis was 9.5% TG and 90.5% TT, and in women without osteoporosis it was 119% TG and 88.1% TT.

Significantly more women with osteoporosis had AG ($p = 0.038$) and GG ($p = 0.017$) genotype compared to women without osteoporosis (Table II). There was no

Table I. Characteristics of women with osteoporosis (N 22) and women without osteoporosis (N 59)

	Women with osteoporosis (mean ± SD)	Women without osteoporosis (mean ± SD)	*P*-value*
Age (years)	65.6 ±12.6	60.8 ±8.7	NS
Height (cm)	161.1 ±5.8	162.2 ±5.3	NS
Weight (kg)	70.4 ±10.5	73.1 ±12.4	NS
BMI (kg/m^2)	27.1 ±4.0	27.7 ±4.4	NS
Duration of postmenopause (years)	18.6 ±12.2	11.7 ±9.4	0.008
Smoking index	169.3 ±178.4	129.1 ±188.6	NS
Spine BMD (g/cm^2)	0.918 ±0.103	1.129 ±0.160	< 0.001
Femoral neck BMD (g/cm^2)	0.761 ±0.094	0.912 ±0.099	< 0.001
Total femur BMD (g/cm^2)	0.796 ±0.087	0.971 ±0.114	< 0.001
Crosslaps (0.142–1.351 ng/ml)	0.340 ±0.221	0.445 ±0.221	NS
Osteocalcin (5–25 ng/ml)	9.308 ±9.374	8.792 ±6.552	NS

**Differences between groups were tested with t-test; NS – not significant*

Table II. Differences in proportion of women with and without osteoporosis according to different genotypes of OPG *A163G* and OPG *T245G* polymorphism

Gene polymorphism	Women with osteoporosis *n* (%)	Women without osteoporosis *n* (%)	*P*-value*
OPG *A163G:*			
AA	2 (9.5)	1 (1.7)	NS
AG	11 (52.4)	17 (29.3)	0.038
GG	7 (38.1)	40 (69.0)	0.017
OPG *T245G:*			
TT	18 (90.5)	51 (88.1)	NS
TG	2 (9.5)	7 (11.9)	NS

**Differences in sample proportions were tested with 2-sample Z-test; NS – not significant*

difference in prevalence of TT and GG polymorphism between patients with and without osteoporosis.

There was a significant negative correlation between total hip BMD and crosslaps (r = –0.419; p = = 0.046) as well as between total hip T score and crosslaps (r = –0.423; p = 0.044) in women without osteoporosis (data not presented in tables). No significant correlation between BMD and bone markers was found in women with osteoporosis.

Discussion

Our study on A163G and T245G OPG polymorphisms in postmenopausal women with normal bone and osteoporosis indicated that A163G could be a possible candidate gene for prediction of osteoporosis in postmenopausal women. It is possible that the A163G gene has an influence on higher bone loss in postmenopausal women.

Our investigation comprised women of the same ethnicity. This is important since the ethnic and genetic characteristics of subjects have an impact on results. Association between A163G, G209A, T245G polymorphisms has been determined in studies from different countries [5, 9], and consequently T245G was selected as a representative gene for analysis. In the Chinese population, A163G OPG polymorphism was strongly associated with BMD at different skeletal sites in adult men, but not in women [8]. Arko et al. [4] and Zajíčková et al. [9] also reported the association between OPG T245G polymorphism and bone mass. However, our results differ from a Danish study [3] where an association between A163G and T245G polymorphisms with bone mass was not found. Different associations were found between G1181C OPG polymorphisms and BMD in different countries and populations. While no association was found in postmenopausal women from Ireland and Malta [6, 10], the OPG gene G1181C polymorphism was identified as a genetic factor associated with BMD of the lumbar spine in Korean women [11]. In elderly Australian women, who were relatively homogeneous and of Caucasian origin, no significant relationship was found between variations in the OPG gene (G1181C, T950C and A163G) and bone-related biochemistry or bone mass [12]. Although the results from different studies indicate that

the impact of G1181C OPG polymorphisms on BMD could differ across populations, dissimilar study designs and methods may distort those conclusions.

In our study, the levels of bone turnover markers, osteocalcin and β crosslaps did not differ between women with and without osteoporosis. We did not find a relationship between investigated polymorphism of the OPG gene and bone turnover markers. That is similar to studies by Ueland et al. [12] and Zupan et al. [13].

The limitation of the study is the relatively small number of women. Our patients with osteoporosis were older, although non-significantly, and they had a longer postmenopausal period, which may alter the relation between analyzed polymorphism and osteoporosis. However, our conclusions are limited by the scope of the pilot study, and possible imprecision could be inherent in data from small samples.

Conclusions

In our study group we found that postmenopausal women with osteoporosis had AG polymorphism more frequently than women without osteoporosis. This indicates the potential role of the A163G gene in prediction of osteoporosis. A larger population would be necessary in order to yield more consistent results and to compare them adequately with other studies, although the differences in population ethnicity, region of DXA measurements, and size of study population create difficulties in appropriate comparison of different studies.

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The authors declare no conflict of interest.

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