ISSN 2304-3415, Russian Open Medical Journal
2021. Volume 10. Issue 1 (March). Article CID e0101
DOI: 10.15275/rusomj.2021.0101

Original article

Investigating the role of osteoprotegerin gene polymorphic variants in osteoporosis

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Received 26 June 2020, Revised 9 February 2021, Accepted 12 February 2021

Abstract: In recent genome-wide association studies (GWAS), several polymorphic loci of the osteoprotegerin (*OPG*) gene were significantly associated with bone mineral density (BMD) and fractures in men over 50 years of age and postmenopausal women. The objective of our study was to search for associations of rs3102735, rs3134069, rs2073617, rs2073618, rs3102734 and rs7844539 of the *OPG* gene with the risk of osteoporotic fractures and the level of BMD in individual and comorbid conditions in men and women from the Volga-Ural region of Russia.
Material and Methods — 828 women and 496 men of various ethnic groups (Russians, Turks) were examined using two-energy x-ray absorptiometry (DEXA) in the femoral neck and lumbar spine. 1324 deoxyribonucleic acid (DNA) samples were genotyped using a fluorescent endpoint genotyping system, after that we searched for associations of these polymorphic loci with fractures and low BMD levels of various localizations.
As a *result*, there was a significant association of rs3134069 and rs3102734 with fractures in general and in the peripheral parts of the skeleton, as well as rs7844539 and rs3102734 in women and rs2073618 in men with low BMD. Another significant association of rs3102734 and rs2073618 with low bone mineral density in the femoral neck was found in both genders.
Conclusion — Polymorphic variants rs3134069, rs3102734, rs7844539 and rs3102734 are potential markers of the risk of osteoporetic fractures and the formation of low BMD in men and women from the Volga-Ural region of Russia.

Keywords: osteoprotegerin, osteoporosis, polymorphism, gene, genotyping.

Cite as Yalaev BI, Tyurin AV, Mirgalieva RI, Khusnutdinova EK, Khusainova RI. Investigating the role of osteoprotegerin gene polymorphic variants in osteoporosis. *Russian Open Medical Journal* 2021; 10: e0101.

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Introduction

Osteoporosis is a multifactorial metabolic disease that increases the risk of bone fracture, reduces the level of bone mineral density (BMD), and disrupts the architectonics of bone tissue [1]. According to the international osteoporosis foundation (IOF), about 200 million people are affected by osteoporosis [2], mostly located in the hip neck, spine, and forearm bones. So, at the point of 2000, there were 1.6 million fractures on the femur, 1.7 million on the forearm bones and 1.4 million on the spine bones among 9 million detected cases of fractures. According to forecasts, the number of osteoporotic fractures worldwide may increase by 310% in men and 240% in women by 2050 [3].

Genome-wide studies and studies of candidate genes have shown that there are several single nucleotide variants (SNP) of the osteoprotegerin genes (*OPG*) that are associated with fractures and low BMD [4-5]. Osteoprotegerin is a key element of the bone remodeling system. This system is responsible for the activation and proliferation of osteoclasts – bone resorption cells. *OPG* protects bone tissue from excessive resorption by competitively binding to the RANK receptor (*Editor's comment:* RANK is the receptor for RANK-Ligand) and is therefore a factor of increasing BMD [6].

It was found that an increased risk of osteoporetic fractures in postmenopausal women is associated with changes in *OPG* gene expression and with SNPs in the promoter, intron, and exon of this gene. In particular, the meta-analysis of Yuqin Peng and co-authors shows the relationship between the GG and GC genotypes of the rs2073618 polymorphic variant (c. 9C>G, p. Asn3Lys) and the AA genotype of the rs3102735 polymorphic variant (c.-1010A>G) with a decrease in the BMD of the lumbar vertebrae and hip neck, as well as a decrease in the BMD of the lumbar spine in the genotypes AA rs2073617 (c.-223C>T) in postmenopausal women. However, the strength of associations of these genotypes with the level of BMD varied greatly in European and Asian populations [7]. Shuhui Qin and colleagues found that the slowdown in osteoclast differentiation in cell cultures with wild and mutant OPG gene variants is associated with changes in the promoter and coding sites. The authors noted that the mechanism for reducing inhibition remains unclear and requires further research [8].

The objective of this study is to find the significance of polymorphic variants rs3102735 (c.-10A>G), rs3134069 (c.-928T>G), rs2073617 (c.-223C>T), rs2073618 (c.9C>G, p.Asn3Lys), rs3102734 (c.30+15C>T) and rs7844539 (c.817+8A>C) of the osteoprotegerin gene (*OPG*, *TNFRSF11B*) in the formation of a low

level of bone mineral density and the development of osteoporotic fractures of various localities in men and women from the Volga-Ural region of Russia.

Material and Methods

Structure of study sample

We used a collection of DNA samples from 1,324 people, including 828 postmenopausal women (61.9±7.0 years) and 496 men (62.0±10.8 years) over 45 years old. Ethnic composition of the studied sample: 67% of Russians and 31% of Turks, 2% of other nationalities and crossbred men: 72% of Russians, 28% of intermarriage of Russians and Turks. The samples were divided into comparison groups according to presence of fractures, osteoporosis and osteopenia, and normal BMD indicators. Patients with fractures were divided into several comparison groups depending on their location: group 1 – with fractures of the lumbar spine, group 2 – with fractures of peripheral bones, including the femoral neck, lower leg, hands, femur, etc., group 3 – with combined fractures of the lumbar spine and peripheral bones of the skeleton, and group 4 – with atypical fractures in locations that are usually least susceptible to osteoporotic fractures (toes, ankles, ribs, etc.). The characteristics of male and female samples are shown in *Tables* 1 and 2. The study material was DNA extracted from peripheral blood by phenol-chloroform extraction (Mathew C.G., 1985) [9]. The pathogenesis of osteoporosis depends significantly on gender and differs in men and women both in age of manifestation of the disease and in clinical manifestations, which is primarily determined by sexual dimorphism in the hormonal regulation of bone metabolism. For this reason, a comprehensive analysis of the clinical and genetic aspects of osteoporosis in the study sample of men and women was conducted separately. The analysis of six polymorphic variants of the osteoprotegerin gene was carried out considering the presence of fractures in general, various parts of the skeleton, the level of BMD in general, as well as variability in the level of BMD of the spine and hip neck.

Exclusion criteria

When forming the sample, rheumatological diseases of connective tissue, taking medications that can negatively affect the level of bone and muscle metabolism, active bacterial or viral infection, pregnant or nursing women, and refusal to participate in the study were selected as exclusion criteria. All subjects signed an informed consent to participate in the study in accordance with the standards developed by the Helsinki Declaration of the world medical association (WMA) "Ethical principles of scientific medical research involving people as subjects of research" and with the approval of the local bioethical Committee of the Institute of Biochemistry and Genetics of the Ufa Federal Research Centre of the Russian Academy of Sciences.

Table 1. Characteristics of comparison groups in a sample of men

Phenotype of the studied trait	*Number of subjects studied (N)*	*Compared group*
No fractures	345	
With fractures	151	Fractures in general
Total	496	
Vertebral fractures	50	
Fractures of the periphery (hip neck, radius and shoulder bones, ribs, hands)	76	Fractures depending on the location
Combined fractures (of the periphery and spine)	18	
Atypical fractures (clavicle, little finger, foot, etc.)	7	
Osteoporosis	137	By the level of BMD according to the T-criterion
Osteopenia	164	
Normal	141	
Total	442	

Table 2. Characteristics of comparison groups in a sample of woman

Phenotype of the studied trait	*Number of subjects studied (N)*	*Compared group*
No fractures	477	
With fractures	350	Fractures in general
Total	828	
Vertebral fractures	29	
Fractures of the periphery (hip neck, radius and shoulder bones, ribs, hands)	263	Fractures depending on the location
Combined fractures (of the periphery and spine)	11	
Atypical fractures (clavicle, little finger, foot, etc.)	47	
Osteoporosis	162	By the level of BMD according to the T-criterion
Osteopenia	150	
Normal	96	
Total	408	

Table 3. Found associations of alleles and genotypes of the studied loci with osteoporetic and fractures and low BMD levels

Polymorphic loci	*Genotype or allele*	*Gender*	*Feature*	χ^2	*OR (odds ratio)*	*CI (confidence level)*	*p-level*	*FDR*
rs3134069 (c.-928 T>G)	T	woman	fractures in general	11.463	1.968	1.322-2.929	0.0007	0.0125
rs3102734 (c. 30+15 C>T)	T	woman	fractures in general	8.797	1.789	1.213-2.639	0.003	0.0130
rs3102734 (c. 30+15 C>T)	TT	woman	fractures in general	11.003	2.002	1.321-3.033	0.0009	0.010
rs3134069 (c.-928 T>G)	TT	woman	peripheral bone fractures	8.676	1.957	1.245-3.076	0.003	0.0129
rs3102734 (1217 C>T)	T	woman	peripheral bone fractures	6.424	1.724	1.127-2.638	0.011	0.045
rs7844539 (c. 817+8A>C)	C	woman	lowBMD in the lumbar spine	5.071	1.981	1.084-3.623	0.024	0.073
rs2073618 (c. 9C>G, p. Asn3Lys)	CG+GG	man	lowBMD in the lumbar spine	4.201	1.732	1.021-2.937	0.040	0.120

Method for measuring bone density

All patients underwent the bone mineral density measurement by two-phase absorption X-ray densitometry (DEXA) using the QDR 4500A device ("Hologic", USA) with the determination of bone mineral density in standard locations (axial femur, lumbar spine), determination of total bone, muscle and fat mass. The overall sample was divided according to the T-criterion - at a value of T more than -1.0, normal BMD was determined, at values from -1.0 to -2.5, osteopenia was diagnosed, and at values less than -2.5, osteoporosis was diagnosed (according to WHO recommendations).

Methods for genotyping DNA samples

Genotyping of the studied loci was performed using polymerase chain reaction (PCR) "in real time" using TaqMan technology on the platform of the CFX96 device (Biorad Touch Real-Time PCR Detection System).

Statistical analysis

Statistical processing of the research results was performed using the Microsoft Excel 2019 and Statistica 6.0 software package. When comparing frequencies of alleles and genotypes in groups of patients and controls as well as patients with different diagnoses (according to T-criterion) the Pearson $\chi 2$ analysis was used. Correction for the multiplicity of comparisons was performed by calculating the value of FDR (Fals discovery rate – the average percentage of false deviations of hypotheses, among all deviations), exactly with the Benjamin-Hochberg method using the online calculator FA Discovery Rate Online Calculator (https://tools.carbocation.com/FDR). In the case of statistically significant differences, the strength of associations was evaluated in the odds ratio (OR), OR>1 was considered as a positive association with an allele or genotype (increased risk factor), and OR<1 – as a negative association (reduced risk factor). All statistical tests were performed for a two-way significance level. Differences were considered statistically significant at $p<0.05$.

Results

As a result, we found the T allele of the polymorphic variant rs3134069 (c.-928T>G), as well as the T allele and the TT genotype of the rs3102734 (c. 30+15C>T) polymorphic variant in women significantly associated with osteoporetic fractures in general compared to controls. Differences in men do not reach statistical significance (*Table* 3). When considering fractures by group, we found the TT genotype of the rs3134069 (c.-928T>G) in women associated with fractures of the peripheral parts of the skeleton, in which most fractures occur on the femoral neck, radius and humerus. We also found the T allele of the rs3102734 (1217 C>T) significantly associated with peripheral bone fractures compared to the control (patients without fractures and low BMD) in women. However, we did not find a statistically significant association of alleles and genotypes of these loci with other fracture groups.

Further, we analyzed the distribution of frequencies of alleles and genotypes in comparison groups according to T-criteria. As a result, it was found that the C allele of the rs7844539 (c. 817+8A>C) in women and the sum of the CG+GG genotypes of the rs2073618 (c. 9C>G, p. Asn3Lys) in men were significantly associated with low levels of BMD in the lumbar spine compared to controls, but the associations were not corrected for the multiplicity of the Benjamin–Hochberg comparisons (*Table* 3).

Similarly, it was found that the C allele of the rs3102734(c.30+15C>T) was significantly associated with hip osteopenia in women ($\chi 2=3.988$, OR=1.946, CI=1.003-3.776, p=0.046 (0.137 FDR)), the CG genotype of the rs2073618 (c.9C>G, p.asn3lys) was associated with hip osteopenia ($\chi 2=4.543$, or=1.644, CI=1.039-2.599, p=0.033 (0.099 FDR), but both associations did not survive after FDR correction. In addition, we performed a comparative analysis of the relationship of polymorphic variants of the *OPG* gene depending on the comorbid states of the BMD level according to *Table* 1 and 2.

In this case, the control group of subjects with normal BMD was compared with the group in which osteoporosis or osteopenia were diagnosed simultaneously in the lumbar spine and in the femoral neck. No statistically significant differences were found in this category of patients.

Discussion

A feature of the clinical and epidemiological characteristics of primary osteoporosis is heterogeneity, multi-factoricity and significant hereditary component, while the pathogenetic mechanisms of this disease may differ significantly depending on the gender and location of fractures. The found differential relationship of the studied polymorphic loci with endophenotypes of osteoporosis indicates these differences, but it is necessary to continue the research in this direction.

Bonfa A.C. and his colleagues showed that the TT genotype of the rs3134069 polymorphic variant (245 T>G) is significantly associated with a low level of BMD [10], which is consistent with our results. However, at the same time, another paper discusses the inconsistency of results in various studies, where some studies indicate the G allele as a risk, and others indicate the T allele [11].

In postmenopausal women from China, the relationship was found between the G allele of the *OPG* gene (A163G and T245G polymorphic variants) and the risk of osteoporosis [12]. In the study by Simona Mencej-Bedrac and colleagues in European populations of postmenopausal women, it was found that the GG and TG genotypes of the rs3134069 polymorphic variant (245 T>G) were significantly associated with low BMD [13]. However, these associations are not observed in the study of a large group of Australian women [14]. Conflicting results from these studies may be related to different frequencies of allelic combinations in different populations.

Information about the impact of the rs3102734 on the risk of osteoporosis and osteoporetic fractures is quite small, however, in the work of Sheng X. and his colleagues, a positive association of the risk allele of this locus with osteoporetic fractures was found (p=0.004), which is also consistent with the results of our study [15].

There is also little information about the association of the rs7844539 polymorphic variant with endophenotypes of osteoporosis in the scientific literature, but this polymorphic variant has been studied in several papers. For example, Christopher V. and colleagues conducted work on sequencing the *OPG* gene and searching for functional connections of polymorphic variants with clinical manifestations of the disease, but authors did not find a statistically significant association [16].

Currently, the osteoprotegerin gene is actively studied not only in connection with bone remodeling, but also in terms of the pathogenesis of other diseases since it is involved in the central systems of cellular signaling pathways. The obtained results show a significant association of the gene polymorphism of this ligand with osteoporosis, and therefore actualize the need for further research of its role in the pathogenesis of multifactorial diseases of the musculoskeletal system.

Conclusion

The analysis of polymorphic variants of the *OPG* gene revealed several significant relationships that showed a heterogeneous effect within the samples depending on the gender and phenotype of the disease. Polymorphic variants rs3134069 and rs3102734 were significantly associated with peripheral bone fractures in women, and polymorphic variants rs7844539 and rs2073618 were associated with low levels of BMD in the femoral neck in women and men, respectively. In addition, we found an association of the rs3102734 polymorphic variant with low BMD in men and women, which showed the significance of this locus in the development of both fractures and low BMD.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

The reported study was funded by RFBR, project number 19-315-90083.

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