

# Analysis of OPG Gene Polymorphism T245G (rs3134069) in Slovak Postmenopausal Women

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**Abstract**—Osteoporosis is a common multifactorial disease with a strong genetic component characterized by reduced bone mass and increased risk of fractures. Genetic factors play an important role in the pathogenesis of osteoporosis. The aim of our study was to identify the genotype and allele distribution of T245G polymorphism in OPG gene in Slovak postmenopausal women. A total of 200 unrelated Slovak postmenopausal women with diagnosed osteoporosis and 200 normal controls were genotyped for T245G (rs3134069) polymorphism of OPG gene. Genotyping was performed using the Custom Taqman® SNP Genotyping assays. Genotypes and alleles frequencies showed no significant differences ( $p=0.5551$ ;  $p=0.6022$ ). The results of the present study confirm the importance of T245G polymorphism in OPG gene in the pathogenesis of osteoporosis.

**Keywords**—OPG gene, osteoporosis, Real-time PCR, T245G polymorphism.

## I. INTRODUCTION

OSTEOPOROSIS is a chronic complex disorder characterised by loss of bone mineral density (BMD), deterioration of bone micro-architecture and increasing risk for fractures. Osteoporosis is a common multifactorial disease with a strong genetic component characterized by reduced bone mass and increased risk of fractures. This is a complex disorder with multiple interactions between genetic effects and environmental factors. Current research in genetics of osteoporosis is focused on identification of responsible genes and polymorphisms. The heritability of bone mineral density (BMD) is 50-80% [9]. Genetic factors have been shown to be responsible for 40-75% of the individual variation [4]. DNA sequence variations might contribute to bone quality in part independently of BMD [14]. A number of single nucleotide polymorphisms in candidate genes for osteoporosis involved in bone physiology have been studied. A number of studies analyzing the association between different single nucleotide polymorphisms (SNPs) of the OPG-RANK-RANKL system genes and bone density have been carried out in the osteoporotic and general populations. The RANK/RANKL/OPG signaling pathway is essential for osteoclastogenesis. The binding of RANKL (produced by osteoblasts) to RANK, located on the surface of osteoclast

precursors, recruits the cytoplasmic adapter protein TNF factor receptor-associated factor 6 (TRAF6), leading to NF- $\kappa$ B activation and translocation to the nucleus; in the nucleus, the translocated NF- $\kappa$ B increases the expression of c-Fos; the interaction of c-Fos with nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) leads to increased expression of various osteoclastogenic genes; consequently, osteoclast formation is increased, thereby resulting in increased bone resorption. This signaling pathway is inhibited by the binding of OPG to RANKL. Finally, it is important to point out that the RANK/RANKL/OPG pathway is subjected to regulation by the canonical Wnt signaling in osteoblasts. A large number of genetic studies have been performed using different approaches to try to identify genetic variants responsible for susceptibility to disease and to understand better bone physiology at the molecular level. Among them, a few have been subjected to meta-analysis: the vitamin D receptor gene (VDR), estrogen receptors  $\alpha$  and  $\beta$  (ESR1 and ESR2), collagen 1  $\alpha_1$  chain (COL1A1), transforming growth factor beta (TGF- $\beta$ ), and more recently the LDL-receptor-related protein 5. Other genes coding for interleukin-6 (IL6) methylenetetrahydrofolate-reductase (MTHFR) and aromatase (CYP19) have also been extensively investigated.

Osteoprotegerin (OPG) gene is the most frequently analysed osteoporosis-related candidate gene playing a key role in the biological characteristics of bone. Some polymorphisms in the OPG gene had been reported to be associated with osteoporotic fractures. The promoter region of the human OPG gene contains various binding sites that may mediate the stimulation of OPG gene expression by different calcitropic factors. The human TNFRSF11B gene is a single copy gene found on chromosome 8q23-24 with five exons that spans 29 kb of the human genome. Numerous OPG gene polymorphisms have been studied in the association studies with osteoporosis and fracture risk. The locus harbouring the OPG gene was associated with low BMD and fracture risk by genome wide association studies [10], [12].

The osteoporosis candidate genes may be roughly categorized into several broad categories according to the function(s) of the coded molecules, mostly involved in metabolism of bone cells (osteoblasts and osteoclasts), structure and turnover of collagen and mineral (calcium and phosphorus), and regulatory/hormonal (obviously, sex-hormone) pathways. Multiple gene polymorphisms have been tested in association studies with BMD and/or fractures.

## II. MATERIALS AND METHODS

The aim of the study was to identify the distribution of

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T245G polymorphism within OPG gene in Slovak postmenopausal women. A total of 200 unrelated Slovak postmenopausal women with diagnosed osteoporosis and 200 normal controls were genotyped for T245G (rs3134069) polymorphism of OPG gene. Each patient was examined clinically and routine biochemical tests to exclude systematic and metabolic bone diseases other than primary osteoporosis were performed. Osteoporosis was defined according to the World Health organization criteria [14].

History and clinical examination were done to rule out secondary osteoporosis. Baseline blood hematology, biochemistry, and bone panel were also done. For the genetic analysis, 5 ml of whole blood was collected. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes using standard methodology. Real-time PCR allelic discrimination using the Custom Taqman® SNP Genotyping assay was used for genotyping of T245G polymorphism in the promoter region of OPG gene. PCR conditions were as follows: initial denaturation at 95°C for 5 min, then 30 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 60°C, and extension for 30 s at 72°C. The reaction was terminated by 7 min elongation at 72°C. Hardy-Weinberg equilibrium was tested for each SNP in groups of participants using the chi-square test. All tests were analyzed at significance level of  $\alpha' = 0.05$ . The normality of the population was tested using the non-parametric K-S test and goodness of fit  $\chi^2$  test. The chi-square test was used to compare observed genotype frequencies with those expected under Hardy-Weinberg and to test for differences in genotype frequencies between studied groups of individuals. The study was approved by the ethical committee and informed consent was obtained from all patients participating in the study.

### III. RESULTS AND DISCUSSION

The distribution of investigated genotypes of T245G polymorphism within OPG gene in the cohort of Slovak postmenopausal women with osteoporosis was as follows: TT (84.0%), TG (15.0%), GG (1.0%), in controls: TT (85.5%), TG (14.0%), GG (0.5%) (Tables I, II).

TABLE I  
GENOTYPES FREQUENCIES OF T245G POLYMORPHISM IN THE OPG GENE  
IN A COHORT OF 200 OSTEOPOROTIC SLOVAK POSTMENOPAUSAL WOMEN  
AND NORMAL CONTROLS

	Patients (n=200)			Controls (n=200)			$\chi^2$	p
	Genotype	Frequency	Percentage	Genotype	Frequency	Percentage		
Genotype frequencies	TT	168	84.0%	TT	171	85.5%	0.35	0.5551
	TG	30	15.0%	TG	28	14.0%		
	GG	2	1.0%	GG	1	0.5%		

TABLE II  
ALLELES FREQUENCIES OF T245G POLYMORPHISM IN THE OPG GENE IN A  
COHORT OF 200 OSTEOPOROTIC SLOVAK POSTMENOPAUSAL WOMEN AND  
NORMAL CONTROLS

	Patients (n=200)			Controls (n=200)			$\chi^2$	p
	Allele	Frequency	Percentage	Allele	Frequency	Percentage		
Allele frequencies	T	0.915		T	0.925		0.27	0.6022
	G	0.085		G	0.075			

In T245G polymorphism of OPG gene the risk G allele was more common among Slovak postmenopausal women with osteoporosis than in normal controls (8.5% versus 7.5%). Genotype and allele frequencies of T245G polymorphism within OPG gene among Slovak patients with osteoporosis and normal controls showed no significant differences ( $\chi^2=0.35$ ,  $p=0.5551$ ;  $\chi^2=0.27$ ,  $p=0.6022$ ). Accordingly, we suggest that in addition to the clinical risk factors, the T245G polymorphism (rs3134069) in TNFRSF11B gene are susceptibility genetic loci for osteoporotic fracture in postmenopausal Slovak women. Further studies are suggested to confirm these findings in other populations and to identify the biological mechanisms by which polymorphisms T245G polymorphism (rs3134069) affect BMD and the risk of osteoporotic fractures.

OPG gene is a key transcription factor in chondrogenesis. Several recent publications have addressed the hypothesis that polymorphisms in the regulatory region at the 5' end of the OPG gene may contribute to the genetic regulation of various bone phenotypes [16], [6]. Furthermore, several studies demonstrated that through gene-gene interactions, OPG gene affects bone mineral density (BMD) together with some others genes, like vitamin D receptor gene and TNF superfamily member 11 genes [7]. Osteoprotegerin is a decoy receptor for receptor activator of nuclear factor kappa-B ligand (RANKL) and a competitive inhibitor of osteoclast recruitment and activity. It inhibits osteoclast differentiation and function by preventing the interaction of RANKL with its receptor RANK. The OPG gene encodes a 44 kDa protein with homology to members of the tumor necrosis factor (TNF) receptor superfamily. Both in vitro and in vivo OPG acts as a soluble inhibitor of osteoclast maturation and osteoclast activation.

A number of single nucleotide polymorphisms in candidate genes for osteoporosis involved in bone physiology have been studied in different populations. Conflicting results were however obtained [1]-[4], [8], [17]. Discordant results may be due to different populations studied/due to racial differences in BMD and fracture risk.

Postmenopausal osteoporosis has become a major epidemic of the last millennium and is expected to be a problem for health care providers in the present millennium as well. Osteoporosis is a disease in which the net loss of bone exceeds bone formation and it occurs in women after estrogen loss in postmenopause. Postmenopausal osteoporosis is a major public health epidemic worldwide. Most women with postmenopausal osteoporosis present with a fracture as the first indication of the disease. The most serious complication of osteoporosis is hip fracture, which increases patients' morbidity and mortality rates. Osteoporotic hip fracture usually requires hospitalization and surgery and may result in lengthy or permanent disability or even death. From family histories, twin studies, and molecular genetics, it is quite evident now that some of a patient's predisposition for osteoporosis can be inherited.

The most recent WHO and European guidelines for the management of osteoporosis underscore the contribution of clinical risk factors in addition to bone mineral density in

order to evaluate the individual probability of a fragility fracture. Among those clinical risk factors, a woman whose mother has had a hip fracture has twice the fracture probability of women without such family history. This pertains to the inheritance of phenotypical traits of bone mineral mass and bone quality (micro-architecture essentially) that define osteoporosis. By comparing BMD between monozygotic and dizygotic twins, and between parents and offspring, studies over the last four decades have documented the major contribution of genetic factors to osteoporosis. The susceptibility to fractures indeed depends on many factors, including non-skeletal factors such as propensity to fall, diminished soft-tissue cushion, and more broadly on the physical environment. Moreover, some heritability of fracture is notably independent of BMD, and it is confirmed by the findings of SNPs that contribute to variation in BMD but do not always contribute to osteoporotic fractures. Some studies reported differences in BMD heritability between pairs of the same sex and the opposite sex, likely reflecting (in utero) imprinting effect on the expression of osteoporosis genes.

A variety of studies have been performed on OPG polymorphisms, mostly focusing on the A163G, T245G, and G1181C variants. The promoter region of the human OPG gene contains various binding sites that may mediate the stimulation of OPG gene expression by different calciotropic factors. Polymorphisms in this region may contribute to the genetic regulation of bone mass as suggested by several recent publications [5].

A large number of genetic studies using different approaches were performed to understand bone physiology at the molecular level. In numerous studies polymorphism T245G of OPG gene was associated with BMD [1], [4], [6], [17]. The present study investigated the prevalence of informative T245G polymorphism which is located in the promoter region of OPG gene. Our data from Slovak postmenopausal women has shown that in T245G polymorphism the variant G allele was more common among patients with osteoporosis than in controls. Genotypes and alleles frequencies of T245G OPG polymorphism showed no significant differences. The results of the present study are compatible with results of others studies [4], [17]. In the study of Langdahl et al. genotypes with the rare G allele A163G and T245G have significantly prevailed in patients with vertebral fractures in comparison with controls [4]. These findings implicate that OPG polymorphisms might be associated with bone quality parameters.

Genotypes with the rare G allele (A163G and T245G) have significantly prevailed in patients with vertebral fractures. In the same cohort, these genotypes have not been related to BMD or biochemical markers of bone turnover. Takacs et al. found an association between the rs3102735 polymorphism and BMD, but did not find any effect on the fracture risk [13]. There are several possible explanations for these discrepancies. BMD and fracture risk are complex traits, and the contribution of a single gene is expected to be relatively small and is influenced by other genes and environmental factors. Inconsistency in results could therefore arise from

differences in the ethnic background, age, calcium intake, and sites of BMD measurements. Moreover, because osteoporosis is a multifactorial disease, other genes, especially genes that participate in the RANKL/RANK/OPG pathway, should be examined to determine their potential contributions to BMD. Stein et al. (2014) suggest that in addition to trabecular and cortical bone loss, changes in plate and rod structure may be important mechanisms of fracture in postmenopausal women with osteopenia [11].

A number of single nucleotide polymorphisms in osteoporosis candidate genes involved in bone physiology have been studied and associated with BMD or fracture risk in different populations. Conflicting results were, however, obtained [4], [1], [2], [11], [15]. Several hypotheses have been reported to explain conflicting results between OPG genotypes and BMD in different populations, such as linkage disequilibrium with functionally relevant genetic variants and environmental factors modifying the genotype effect on BMD.

The genetic influences differ between osteoporotic and non-osteoporotic post-menopausal women, suggesting that some polymorphisms might not cause osteoporosis, but can modify the degree of osteoporosis. These differences could have a great clinical impact, since even small changes in BMD may result in large changes of fracture risk. Diverse genetic influences were also observed in the pre- and post-menopausal women. The differences could be a result of numerous genetic interactions that were partly also addressed in our study. The association may also be modified by the influence of other osteoporosis related polymorphisms and several environmental factors, such as dietary calcium and vitamin D intake, physical activity. Genetic variation at the human OPG gene locus has been associated with colorectal, prostate cancer risk and bipolar disorders, also. Environmental factors that influence bone density include dietary factors such as intakes of calcium, alcohol, and caffeine and lifestyle factors such as exercise and smoking. Ethnic differences in the propensity to nontraumatic bone fracture suggest that genetic factors are important. Osteoporosis is classified into many types, but postmenopausal osteoporosis is 80% of all cases. Menopausal changes mainly relate to the decline in the ovarian function and it's hardly controlled under the hypothalamo-pituitary-ovarian axis. Osteoporosis is now recognized as one of the major problems facing postmenopausal women and osteoporosis induced fracture cause people to become bedridden with serious complication.

As BMD is under polygenic control, other candidate genes have been tested, as well as their gene by-gene interactions, or their potential relation to bone mass. With an increase in the ageing population worldwide, late onset chronic disorders such as osteoporosis, heart disease and diabetes become a greater economic burden on society. Osteoporosis is the most common metabolic bone disorder worldwide. Systematic search for the genes for osteoporosis has been done by genome-wide linkage studies with pedigrees, which have shown some hotspots linked to BMD. Further fine mappings were required to specify the genes contributing to the pathophysiology of osteoporosis and consequent analyses of

their functions in bone biology. Prevention of osteoporotic fractures is the major clinical goal of osteoporosis therapy, and the incidence of osteoporotic fractures should be an ideal phenotype used in the genetic studies searching the genes for osteoporosis.

The importance of OPG gene as a candidate gene for the development of osteoporosis has been confirmed in several different studies. In our study, we did not fully cover all genetic variation in the OPG gene, further investigations on different and larger populations, interaction with other genes and association studies will be carried out. Additional approaches, such as sib-pair analysis, will probably be necessary in the future to identify the important genetic determinants of osteoporosis. The genetic effects of the OPG gene SNPs need further functional and clinical confirmation.

#### IV. CONCLUSION

Our findings further highlighted the importance of the OPG gene and provide more information to the understanding of the genetic architecture of osteoporosis. Further studies are required to utilize the results of genetic studies for the advancement of osteoporosis practice.

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