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TNFRSF11B Gene Polymorphisms A163G and G11811C in Prediction of Osteoporosis Risk

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***Abstract*—**Osteoporosis is a complex health disease characterized by low bone mineral density, which is determined by an interaction of genetics with metabolic and environmental factors. Current research in genetics of osteoporosis is focused on identification of responsible genes and polymorphisms. TNFRSF11B gene plays a key role in bone remodeling. The aim of this study was to investigate the genotype and allele distribution of A163G (rs3102735) osteoprotegerin gene promoter and G1181C (rs2073618) osteoprotegerin first exon polymorphisms in the group of 180 unrelated postmenopausal women with diagnosed osteoporosis and 180 normal controls. Genomic DNA was isolated from peripheral blood leukocytes using standard methodology. Genotyping for presence of different polymorphisms was performed using the Custom Taqman®SNP Genotyping assays. Hardy-Weinberg equilibrium was tested for each SNP in the groups of participants using the chi-square (χ^2) test. The distribution of investigated genotypes in the group of patients with osteoporosis were as follows: AA (66.7%), AG (32.2%), GG (1.1%) for A163G polymorphism; GG (19.4%), CG (44.4%), CC (36.1%) for G1181C polymorphism. The distribution of genotypes in normal controls were follows: AA (71.1%), AG (26.1%), GG (2.8%) for A163G polymorphism; GG (22.2%), CG (48.9%), CC (28.9%) for G1181C polymorphism. In A163G polymorphism the variant G allele was more common among patients with osteoporosis: 17.2% versus 15.8% in normal controls. Also, in G1181C polymorphism the phenomenon of more frequent occurrence of C allele in the group of patients with osteoporosis was observed (58.3% versus 53.3%). Genotype and allele distributions showed no significant differences (A163G: χ^2=0.270, p=0.605; χ^2=0.250, p=0.616; G1181C: χ^2= 1.730, p=0.188; χ^2=1.820, p=0.177). Our results represents an initial study, further studies of more numerous file and associations studies will be carried out. Knowing the distribution of genotypes is important for assessing the impact of these polymorphisms on various parameters associated with osteoporosis. Screening for identification of "at-risk" women likely to develop osteoporosis and initiating subsequent early intervention appears to be most effective strategy to substantially reduce the risks of osteoporosis.

***Keywords*—**Osteoporosis, Real-time PCR method, SNP polymorphisms.

I. Introduction

OSTEOPOROSIS is a complex health disease characterized by low bone mineral density, which is determined by an interaction of genetics with metabolic and environmental factors. Osteoporosis is characterized by a combination of low bone mass and deteriorated microarchitecture of the bone [4]. Several studies proved that genetic factors play an important role in the pathogenesis of osteoporosis [3], [2], [5]. The genetic of osteoporosis represents one of the most active areas for research in bone biology. A number of candidate genes have been investigated be associated with BMD and osteoporosis, such as TNFSF11B gene, vitamin D receptor (VDR), collagen type 1a1 (COLIA1). DNA sequence variations of these genes might contribute to bone quality in part independently of BMD [8]. Due to its important role in bone biology, TNFRSF11B gene, coding for osteoprotegerin (OPG) has been considered as a candidate gene for osteoporosis [6]. TNFSF11B gene plays a crucial role in the control of bone resorption. Numerous TNFRSF11B gene polymorphisms have been studied in the association studies [2], [3]. The aim of the present study was to investigate the distribution of two informative polymorphisms: A163G and G1181C in the osteoprotegerin gene in Slovak postmenopausal women.

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II. Methods

The genotyping was carried out in a cohort of 180 postmenopausal unrelated women and 180 normal controls of Caucasian origin. The samples were screened for informative A163G polymorphism in osteoprotegerin gene promoter and G1181C polymorphism in osteoprotegerin first exon. Each patient was examined clinically and routine biochemical tests to exclude systematic and metabolic bone diseases other than primary osteoporosis. Osteoporosis was defined according to the World Health Organization criteria [7]. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes using standard methodology. Real-time PCR allelic discrimination TaqMan assay was used for genotyping analyses of A163G and G1181C polymorphisms of TNFRSF11B gene. Hardy-Weinberg equilibrium was tested for each SNP in the groups of participants using the chi-square $(\chi)^2$ test. The study was approved by the ethical committee and informed consent was obtained from all patients participating in the study.

III. Results

The distribution of investigated genotypes in the cohort of osteoporotic postmenopausal women were as follows: AA (66.7%), AG (32.2%), GG (1.1%) for A163G polymorphism; GG (19.4%), CG (44.4%), CC (36.1%) for G1181C polymorphism. The distribution of genotypes in normal controls were follows: AA (71.1%), AG (26.1%), GG (2.8%)

for A163G polymorphism; GG (22.2%), CG (48.9%), CC (28.9%) for G1181C polymorphism (Tables I, II).

TABLE I
A163G POLYMORPHISM IN THE TNFRSF11B GENE: GENOTYPE AND ALLELE FREQUENCIES IN A COHORT OF 180 OSTEOPOROTIC POSTMENOPAUSAL WOMEN AND NORMAL CONTROLS

		Osteoporotic postmenopausal women		*Normal controls*		χ^2	p
Genotype distribution	AA	120	(66.7%)	128	(71.1%)	$\chi^2 =$ 0.270	**p = 0.605**
	AG	58	(32.2%)	47	(26.1%)		
	GG	2	(1.1%)	5	(2.8%)		
Allele frequency	A	82.8%		84.2%		$\chi^2 =$ 0.250	**p = 0.616**
	G	**17.2%**		**15.8%**			

TABLE II
G1181C POLYMORPHISM IN THE TNFRSF11B GENE: GENOTYPES AND ALLELES FREQUENCIES IN A COHORT OF 180 OSTEOPOROTIC POSTMENOPAUSAL WOMEN AND NORMAL CONTROLS

		Osteoporotic postmenopausal women		*Normal controls*		χ^2	p
Genotype distribution	GG	35	(19.4%)	40	(22.2%)	$\chi^2 =$ 1.730	p = 0.188
	CG	80	(44.4%)	88	(48.9%)		
	CC	65	(36.1%)	52	(28.9%)		
Allele frequency	G	41.7%		46.7%		$\chi^2 =$ 1.820	**p = 0.177**
	C	**58.3%**		**53.3%**			

In A163G polymorphism the variant G allele was more common among patients with osteoporosis: 17.2% versus 15.8% in normal controls. Also, in G1181C polymorphism the phenomenon of more frequent occurrence of C allele in the group of patients with osteoporosis was observed (58.3% versus 53.3%). The genotype and alleles frequencies of A163G and G1181C polymorphism of TNFRSF11B gene among patients with osteoporosis and normal controls showed no significant differences (A163G: χ^2=0.270, p=0.605; χ^2=0.250, p=0.616; G1181C: χ^2= 1.730, p=0.188; χ^2=1.820, p=0.177).

IV. DISCUSSION

Osteoporosis is widely recognized as an important health problem because its complications, including fractures, are associated with significant morbidity and mortality [1]. TNFRSF11B gene is an important candidate gene in the pathogenesis of osteoporosis. Some polymorphisms in the TNFRSF11B gene had been reported to be associated with osteoporotic fractures. Regarding the A163G polymorphism in TNFRSF11B gene, it was reported that a large proportion of patients with bone fractures have the OPG (163) G allele [3]. The promoter region of the human TNFRSF11B 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 [4].

The present study investigated the prevalence of informative A163G polymorphism located in osteoprotegerin gene promoter and G1181C polymorphism in osteoprotegerin first exon. Our data from Slovak postmenopausal women has shown that in A163G polymorphism the variant G allele was more common among patients with osteoporosis (17.2% versus 15.8%). Also, in G1181C polymorphism the phenomenon of more frequent occurrence of G allele in the group of patients with osteoporosis was observed (58.3% versus 53.3%). Genotypes and alleles frequencies of A163G and G1181C polymorphism showed no significant differences. The results of the present study are compatible with results of others study [3], [9]. In the study of Ling-xia et al. (2011) the G allele of A163G polymorphism was significantly more frequent among patients with osteoporosis (12.3%) than in normal individuals (6.5%) [4]. Also, in a study of Langdahl et al. (2002) genotypes with the rare G allele A163G and T245G have significantly prevailed in patients with vertebral fractures in comparison with controls [3]. These findings implicate that TNFRSF11B gene polymorphisms might be associated with bone quality parameters. 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 [6], [8]. 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 [5]. In the majority of studies from different populations, individuals with GG genotype of G1181C SNP had increased risk for the development of osteoporosis [3], [6].

The importance of TNFRSF11B gene as a candidate gene for the development of osteoporosis has been confirmed in several studies. In our study, we did not fully cover all genetic variation in the TNFSF11B gene, since only two common SNPs were studied. Our results represents an initial study, further investigations on different and larger populations, interaction with other genes and association studies will be carried out. The genetic effects of TNFRSF11B gene SNPs need further functional and clinical confirmation.

V. CONCLUSION

Knowing the distribution of genotypes is important for assessing the impact of these polymorphisms on various parameters associated with osteoporosis. Screening for the identification of “at-risk” women likely to develop osteoporosis and initiating subsequent early intervention appears to be most effective strategy to substantially reduce the risks of osteoporosis.

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