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Association of the osteoprotegerin gene polymorphisms with bone mineral density in postmenopausal women

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Abstract

Objectives: Osteoprotegerin (OPG) is a recently discovered member of the tumour necrosis factor receptor superfamily. It plays a crucial role in the control of bone resorption and its gene could therefore be a good candidate gene for osteoporosis. The aim of our work was to find polymorphisms in the OPG gene and to investigate their possible contribution to the genetic susceptibility to osteoporosis by testing for their association with bone mineral density (BMD).
Methods: The whole OPG gene coding region was screened for the presence of polymorphisms in a group of 60 osteoporotic women by single-strand conformation polymorphism analysis (SSCP) approach. Association of the discovered polymorphisms with bone mineral density was investigated in 136 Slovenian postmenopausal women.
Results: We detected eight OPG gene polymorphisms that were confirmed by direct DNA sequencing, deletion 4752_4753delCT and nucleotide substitutions 1181G > C, 1217C > T, 1284G > A, 4501C > T, 6893A > G, 6950A > C and 8738T > A. Nucleotide substitutions 1284G > A and 8738T > A have not been previously described. Polymorphisms 4752_4753delCT, 6893A > G and 6950A > C were in complete linkage and the same was true for 1217C > T and 4501C > T. The association with BMD was found only for polymorphism 1181G > C. Subjects with genotype 1181GG had significantly lower lumbar spine BMD than subjects displaying 1181GC.
Conclusions: By our approach we detected eight polymorphisms in the OPG gene. According to our analysis polymorphism 1181G > C is associated with BMD and could therefore be considered as an element of genetic susceptibility to osteoporosis.

Keywords: Osteoprotegerin gene polymorphism; Bone mineral density; Osteoporosis; SSCP analysis; Heteroduplex analysis; RFLP analysis

1. Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase

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in bone fragility and susceptibility to fractures [1]. It is a complex disease in which not only environmental factors such as diet, smoking and exercise, but also genetic factors play a very important role. Namely, bone mass is the major component of bone strength and studies on twins and on families have shown that as much as 70–80% of the inter-individual variance in bone mineral density (BMD) at the spine and hip is genetically determined [2,3]. The genetic contribution to bone mass was observed even into old age, indicating that genes regulate both peak bone mass as well as the rate of bone loss. While the genetic influence on the attainment of peak bone mass is indisputable, direct evidence for the influence on bone loss is currently lacking due to insufficient studies [2–4]. Osteoporosis is a polygenic disease, which means that several different genes, each with a modest effect regulate bone mass and other determinants of fracture risk. Despite major efforts and different strategies used to find the responsible genes the majority still remain to be identified [2,3].

Osteoprotegerin (OPG) is a new member of the tumour necrosis factor receptor superfamily discovered in 1997 [5–7] and is a key regulator of bone remodelling. It protects bone from excessive resorption by inhibiting the terminal stages of osteoclastogenesis [5,7–9] suppressing mature osteoclast activation [9–12] and inducing their apoptosis [13]. These effects are a consequence of OPG acting as a soluble decoy receptor for receptor activator of nuclear factor kappa B (RANKL), a potent inducer of osteoclast differentiation and activation, when bound to its functional receptor RANK [10,11,14]. In addition to in vitro studies, there are also several in vivo studies confirming the bone protective role of OPG. OPG knock-out mice developed severe osteoporosis [15,16], while overexpression of OPG in transgenic mice resulted in osteopetrosis [5]. Consistent with this, administration of OPG prevented ovariectomy-induced bone loss in rats [5] and a single dose of OPG caused a rapid and sustained inhibition of bone resorption in postmenopausal women [17]. In addition, a rare human skeletal disorder idiopathic hyperphosphatasia or juvenile Paget's disease has been linked with defects in the OPG gene. In three related patients the disease was found to be caused by a 3-bp deletion in exon 3 of the OPG gene [18], whereas in two other patients with the same disease a homozygous deletion of the entire OPG gene has been discovered [19]. Its important role in the control of bone resorption ranks the OPG gene among the promising candidate genes for osteoporosis. The human OPG gene is a single copy gene located on chromosome 8, consists of five exons and spans 29 kb of the genome [20].

In our previous study [21] we screened the OPG gene promoter for polymorphisms in the group of postmenopausal osteoporotic women. We found four nucleotide substitutions and two of them, 209G > A and 245T > G, were significantly associated with lumbar spine BMD. The aim of our present study was to screen systematically the whole coding region of the OPG gene in a group of Slovenian postmenopausal women and to investigate the possible contribution of any polymorphisms discovered to the genetic susceptibility to osteoporosis by testing for their association with BMD.

2. Materials and methods

2.1. Patients

One hundred and thirty-six ambulatory, community-dwelling, postmenopausal female volunteers, aged 47–78 years (mean 61.7 years), were recruited. Excluded from the study were individuals with a history of hip fracture or recent acute onset of sudden low back pain. All subjects were examined clinically and routine biochemical tests were performed to exclude any disease (primary hyperparathyroidism, hyperthyroidism, Cushing syndrome, cirrhosis, kidney failure) or drugs (glucocorticoids, antiepileptics) known to affect bone metabolism. No subject had received antiresorptive medication (bisphosphonates or calcitonin) and no subject was treated with hormone replacement therapy (HRT).

The study was approved by the ethical committee and informed consent was obtained from all patients participating in the study.

2.2. DNA analyses

2.2.1. Polymerase chain reaction (PCR)

DNA was extracted from leukocytes of peripheral blood by the Miller salting-out procedure [22]. Oligonucleotide primers for all five exons were designed based on the sequences of the OPG gene available in GenBank (Accession no. AB008821 and