Serum osteoprotegerin (OPG) and the A163G polymorphism in the OPG promoter region are related to peripheral measures of bone mass and fracture odds ratios

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Abstract The purpose of this study is to investigate the association of serum osteoprotegerin (OPG) and the A163G polymorphism in the OPG promoter with peripheral measures of bone mass and with odds ratios for wrist and hip fracture in a case-control study of postmenopausal Danish women. The study included 66 women with lower forearm fracture, 41 women with hip fracture, and 206 age-matched controls. All had broadband ultrasound attenuation (BUA) and speed of sound (SOS) measured at the heel as well as bone mineral density (BMD) measured by DXA at the distal forearm. S-OPG was measured by ELISA. The A163G genotypes were determined by PCR-RFLP analysis. S-OPG levels correlated positively with age (r = 0.45; P < 0.0001) and negatively with distal forearm BMD (r = -0.31; P < 0.0001), heel BUA (r = -0.23; P < 0.0001), and heel SOS (r = -0.22; P < 0.0001). Comparing the highest quartile of S-OPG to the lowest, the odds ratio for osteoporotic fracture was 2.5 (95% CI, 1.3–4.7; P = 0.006). The G allele of the A163G was associated with significantly lower t-scores of both lower forearm BMD, heel BUA, and heel SOS as well as being significantly more frequent in the fracture patients compared to the controls. Patients with a combination of the highest quartile of S-OPG and presence of the G allele (n = 23) had a significantly elevated fracture odds ratio, 4.0 (95% CI, 1.7–9.9). A significant negative association between S-OPG with peripheral measures of bone mass and with increased fracture odds ratios was found. Furthermore, the A163G mutation in the OPG promoter had a significant influence on bone mass and fracture status independently of S-OPG level.

Key words osteoprotegerin · polymorphism · BUA · BMD · fractures

Introduction

In 1997, a novel secreted glycoprotein, osteoprotegerin (OPG), which is a member of the tumor necrosis factor (TNF) receptor superfamily, was discovered [1,2]. Together with RANKL (receptor activator of nuclear factor NF-κB ligand) and the cellular receptor RANK (receptor activator of nuclear factor NF-κB), OPG plays a key role in osteoclastogenesis. RANKL is expressed by osteoblastic lineage cells and stimulates its specific receptor, RANK, to promote differentiation, survival, fusion, and activation of osteoclasts as well as preventing osteoclast apoptosis [3]. OPG, on the other hand, acts as a soluble decoy receptor for RANKL, inhibiting its binding to RANK and thereby preventing osteoclast formation [4].

OPG can be measured in serum using enzyme-linked immunosorbent assay (ELISA) systems [5], and the possible association of serum OPG (S-OPG) with bone mineral density (BMD), biochemical markers of bone turnover and osteoporotic fractures have been investigated in several studies. In a study of middle-aged men (mean age, 56 years; n = 252), there was no association of S-OPG with BMD or markers of bone formation, while for the subgroup of men aged more than 40 years, a negative correlation between urinary excretion of deoxypyridinoline and S-OPG was found [6]. Similarly, in a study of elderly women (age, >65 years; n = 490), no significant correlation between BMD and S-OPG was present. There was, however, a significant inverse correlation between S-OPG and serum osteocalcin levels [7]. In the same study, a significant association between S-OPG and hip fractures was found, odds ratio 1.3 (95% CI, 1.0–1.7), whereas such an association could not be shown for all fractures combined or for wrist fractures alone [7]. In contrast to these findings, a Japanese study of 186 women (mean age, 65) and 56 men (mean age, 55) found increased S-OPG levels in postmenopausal women with osteoporosis as well as a
positive correlation between S-OPG and biochemical markers of bone turnover (serum bone-specific alkaline phosphatase and urinary excretion of pyridinoline and deoxypyridinoline) [8].

Finally, in a recent study of randomly chosen men (n = 346; age range, 23–90 years) and women (n = 304; age range, 21–93), there was a trend for S-OPG levels to be associated positively with bone resorption markers and inversely with BMD in the men but not in the women [9]. Whether there is a clinical utility for measuring serum OPG in terms of diagnosing individuals at risk of osteoporosis thus does not yet seem clear from the relatively few available studies, which report conflicting results for several of the relevant parameters.

Twin and family studies indicate a strong genetic influence on BMD [10–12], and several polymorphisms in candidate genes such as the vitamin D receptor, the estrogen receptor, collagen type I alpha 1, and several of the cytokines have been studied, although most often with conflicting results in different studies [13]. As much as 60%–80% of the variance in bone phenotype measurements is thought to be under genetic control [10]. However none of the candidate genes that have been studied so far can explain this high level of genetic influence on bone mass. Given the many complex molecular and cellular processes involved in bone remodeling, it is most likely that a combination of several genes are involved in the observed hereditability of bone mass.

As OPG has an important role as an inhibitor of osteoclast differentiation, polymorphisms in the gene coding for OPG (located on chromosome 8; GenBank accession number U94332) might influence the bone remodeling process, and OPG could thus be a candidate gene for identifying individuals at risk of developing low bone mass or osteoporosis. A single nucleotide polymorphism in the promoter region of OPG (A163G) was identified in a study by Kusk [14], in which postmenopausal women carrying the G allele had lower bone mass than women homozygotic for the A allele. In a subsequent examination of the OPG gene, Langdahl et al. identified 12 polymorphisms, of which 3 (2 in the promoter region and 1 in exon 1) had an impact on bone mineral density and osteoporotic fractures in their study of 50 normal and 50 osteoporotic patients [15]. This study similarly showed the G allele of the OPG A163G polymorphism to be more common in osteoporotic patients.

The purpose of this study was thus to investigate the association of S-OPG and the A163G polymorphism with peripheral measures of bone mass and the odds ratios for wrist and hip fracture in a case-control study of postmenopausal women.

**Subjects and methods**

During a 10-month period, patients with lower forearm fracture or hip fracture admitted to the Department of Orthopaedic Surgery at Hvidovre University Hospital were screened consecutively for inclusion in a case-control study [16]. The inclusion criteria were postmenopausal women (at least 1 year postmenopausal) and written informed consent. The exclusion criteria were: systemic diseases or treatments (presently or previously) known to influence the calcium metabolism or physical or mental inability to complete the study procedures. A total of 76 patients with lower forearm fracture and 47 patients with hip fracture were included in the study, corresponding to 25% of the screened patients. Of the 123 included fracture patients, S-OPG and the A163G genotype were determined in a total of 107 patients. Blood samples and bone mass measurements were performed with minimal delay after the fracture was sustained.

To identify the reference population, questionnaires were mailed to a group of women with the same age profile as the fracture patients (using the Danish central population register). These were screened for the inclusion and exclusion criteria with the added criterion, as compared to the fracture patients, of never having sustained a postmenopausal fracture. A total of 231 women were included as controls (details of the selection process are given elsewhere [16]). There was no significant difference between the age distribution of the lower forearm fracture patients and the total reference population. The hip fracture patients, however, were significantly older than the reference population. To compensate for this, 172 controls were omitted using an automatic algorithm aiming at obtaining a similar age distribution. The resulting subgroup (n = 59) of the reference population was used for all comparisons with the hip fracture group. In the total control group, S-OPG and the A163G genotype were determined in 206 subjects (and from 52 of the 59 in the subgroup). The population characteristics are shown in Table 1. The study was approved by the Ethics Committee for Copenhagen and Frederiksberg municipalities.

**Ultrasound measurements**

The heel ultrasound measurements were performed on the DTU-one (Osteometer MediTech, Hawthorne, CA, USA). The two parameters measured were broadband ultrasound attenuation (BUA) (dB/MHz) and speed of sound (SOS) (m/s). The DTU-one uses an imaging system making it possible to determine a reproducible region of interest on the calcaneus, thus improving the in vivo precision [17]. In this population, the coefficient of variation (CV) was 2.7% for BUA and 0.2% for SOS.