CD38 is associated with premenopausal and postmenopausal bone mineral density and postmenopausal bone loss

Received: November 17, 2004 / Accepted: August 15, 2005

Abstract One goal of osteoporosis research is to identify the genes and environmental factors that contribute to low bone mineral density (BMD) and fracture. Linkage analyses have identified quantitative trait loci (QTLs), however, the genes contributing to low BMD are largely unknown. We examined the potential association of an intronic polymorphism in CD38 with BMD and postmenopausal bone loss. CD38 resides in 4p15, where a QTL for BMD has been described. CD38/−/− mice display an osteoporotic phenotype at 3 months, with normalization of BMD by 5 months. The CD38 polymorphism was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in 457 postmenopausal and 173 premenopausal Caucasian women whose spine and hip BMD was measured by dual energy X-ray absorptiometry (DXA). Influence of the CD38 polymorphism on bone loss was analyzed in 273 postmenopausal women over a follow-up of 2.94 ± 1.50 years. The CD38-PvuII polymorphism was significantly associated with premenopausal and postmenopausal (P = 0.001) lumbar spine BMD. Women homozygous for the G allele had >14% lower spinal BMD than women with GC/CC genotypes. An allele dose effect was observed at the spine in premenopausal (P = 0.002) and postmenopausal (P < 0.001) cohorts. The CD38-PvuII polymorphism was significantly associated with femoral neck BMD in pre- and postmenopausal women (P = 0.002 and P = 0.011, respectively). However, significance was lost following adjustment of hip BMD for covariates in the postmenopausal cohort (P = 0.081). The CD38-PvuII polymorphism was weakly associated with bone loss at the spine (P = 0.024), in postmenopausal women not taking hormone replacement therapy. We suggest that the CD38-PvuII polymorphism may influence the attainment and maintenance of peak BMD and postmenopausal bone loss.

Key words CD38 polymorphism · bone mineral density · association · lumbar spine · resorption

Introduction

Osteoporosis is a common disease of the elderly, causing significant morbidity and mortality. It is a skeletal disorder characterized by compromised bone strength [bone mineral density (BMD) and bone quality], predisposing a person to an increased risk of fracture [1]. The risk of osteoporosis is determined by peak bone mass (achieved by the third decade of life) and the rate of age-related bone loss. Both genetic and environmental factors influence peak BMD and age-related bone loss, but heritability accounts for 40%–90% of variation in BMD at the spine and hip [2–5]. To date, many linkage and association studies have been performed in an attempt to identify the genes influencing BMD. A number of quantitative trait loci (QTL) influencing BMD have been identified, including 1p36 [6–8], 1q21–23 [9], 2p22–25 [8,10], 3p22–21.1 [8,11], 4p15 [12], and 11q12–13 [8,9,13] however, the genes affecting BMD within these loci largely remain to be defined. Association studies have investigated the influence of a number of candidate genes on BMD, including the vitamin D receptor (VDR) [14], type I collagen alpha 1 (COLIA1) [15], estrogen receptor (ER) [16], calcitonin [17] and more recently osteoprotegerin (OPG) [18], lysyl hydroxylase (PLOD1) [19], low density lipoprotein receptor-related protein 5 (LRP5) [20], and the brain natriuretic peptide gene (BNP).
The effect of allelic variants in these genes on BMD has been inconsistent, and current evidence suggests that they account for only a small portion of the population variance in BMD [22,23]. CD38 is a cellular enzyme that catalyzes the cyclization of the metabolite nicotinamide adenine dinucleotide (NAD⁺) to cyclic ADP-ribose (cADPr) [24]. cADPr is a second messenger that activates Ca²⁺ release from ryanodine receptor (RyR)-gated intracellular Ca²⁺ stores [25]. CD38 also functions as a NAD⁺ glycohydrolyase and an ADP-ribosyl hydrolase [25,26]. CD38 is widely expressed in many cells [27] including osteoblasts and osteoclasts [28]. This enzyme has been shown to have important roles in many cellular processes including apoptosis [29], T-lymphocyte signaling [30], insulin release from pancreatic beta cells [31], and neutrophil migration [32]. Sun et al. reported a novel role for CD38 in the regulation of bone resorption [28]. Activation of CD38 in the osteoclast triggers Ca²⁺ release through RyR2 activation. This increase in cytosolic Ca²⁺ is accompanied by an elevation in interleukin-6 (IL-6) release and inhibition of bone resorption. Following deletion of CD38, CD38−/− mice display a significantly reduced BMD at the femur, tibia, and lumbar spine at 3 months of age [33]. At 4 months of age only the lumbar spine BMD of CD38−/− mice was significantly reduced compared to their wild-type littermates. Full normalization of BMD was restored at all sites by 5 months of age. Thus, in the absence of CD38, bone resorption was stimulated. In addition, osteoclast formation was elevated, indicating that CD38 also controls osteoclastogenesis. These data imply that CD38 plays a crucial role in osteoclast formation and bone resorption. It is unknown what mechanism is responsible for the restoration of BMD; however, CD157, a closely related cyclase, may be up-regulated to adopt the function of CD38 [34,35].

CD38 has been mapped to 4p15 [36]. Kammerer et al. reported a QTL for forearm BMD at 4p15 (maximum LOD score 4.33) [12]. CD38 is therefore a strong positional and functional candidate gene for the influence of BMD. On the basis of these observations we investigated the effect of a biallelic polymorphism in intron 1 of the CD38 gene [34] on BMD cross-sectionally in a cohort of 173 premenopausal and 457 postmenopausal women and longitudinally in 273 postmenopausal women.

Materials and methods

Subjects

Women with normal BMD, osteopenia, and osteoporosis, identified from records at the bone densitometry unit (BDU) of Cork University Hospital, Ireland, were asked to participate in this study. Approximately 85% of patients were referred to the BDU by general practitioners, and the remainder were self-referrals. Blood and serum samples were collected from all participants and coded for anonymity. All subjects were screened by nurse-led questionnaires and biochemical testing for celiac disease [37]. Participants with secondary causes of osteoporosis – premature menopause (<40 years of age), oophorectomy, hyper- or hypothyroidism, hyperparathyroidism, Paget’s disease, chronic liver disease, chronic renal failure, malabsorption, celiac disease, anorexia nervosa, rheumatoid arthritis, steroid use >6 months, chronic diuretic usage, cancer – were excluded from the study. Individuals taking hormone replacement therapy (HRT) or bisphosphonates prior to their BMD measurement were also excluded. Altogether, 457 postmenopausal and 173 premenopausal women were included in this study. The mean ages of the pre- and postmenopausal women were 43.90 ± 6.26 and 60.26 ± 8.71 years, respectively.

In addition, 273 postmenopausal women who had two or more dual energy X-ray absorptiometry (DXA) scans over a mean follow-up time of 2.94 ± 1.50 years were randomly recruited from postmenopausal women attending the BDU for a subsequent scan. The same exclusion criteria listed above was applied to this group; however, women taking HRT following the first scan were included in this cohort to investigate whether the CD38 polymorphism influences response to HRT. Of the 273 women, 145 were not taking any medication, 98 were currently using HRT, and 30 were past users of HRT. The height and weight of each participant was measured at each visit. There was an overlap of 19 women between the cross-sectional and longitudinal analysis.

Ethics approval for this study was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Informed written consent was obtained from all participating women.

Bone mineral density measurements

All participants had BMD determined at the lumbar spine (L2–L4) and the left femoral neck. BMD values were assessed on at least one occasion by DXA (Lunar DPX1Q). BMD was recorded as grams per centimeter squared, and a T-score and a Z-score for each individual and subjects were determined according to World Health Organization (WHO) criteria. The rate of change in BMD was calculated as percentage annualized change. The in vivo reproducibility was 0.5% for the lumbar spine and 1.0% for the femur calculated from repeated scans performed on 10 individuals. Machine calibration using the phantom spine was performed daily prior to doing any scan to ensure the precision of the machine. All follow-up scans were performed on the same machine.

CD38 genotyping

Genomic DNA was isolated using the phenol-chloroform extraction method. The CD38 intron 1 polymorphism [34] was screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based methods. PCR amplification was performed using the primers 5′-CCGGTGTTGGGTAGGAGCAGGAGT-3′...