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Genetic and environmental determinants of bone mineral density in Chinese women

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Abstract

BMD is a complex trait determined by genetic and lifestyle factors. To assess the genetic and environmental determinants of BMD in southern Chinese women, we studied a community-based cohort of 531 pre- and postmenopausal southern Chinese women and assessed the influence of 12 candidate gene loci and lifestyle risk factors on spine and hip BMD. The candidate genes studied include estrogen receptor alpha (ESR1) and beta (ESR2), calcium sensing receptor (CASR), vitamin D receptor (VDR), collagen type Iα1 (COLIA1), and LDL receptor-related protein 5 (LRP5). Social, medical, reproductive history, dietary habits and lifestyle factors were determined using a structured questionnaire. Single nucleotide polymorphisms (SNPs) of the COLIA1 and LRP5 gene in Chinese were determined by direct sequencing. Nucleotide (nt) -1363C/G and -1997 G/T of COLIA1, nt 266A/G, 2220C/T and 3989C/T of LRP5 gene were analyzed.

Using stepwise multiple linear regression analyses, body weight was the strongest predictor for BMD in premenopausal women ($n = 262$), which accounted for 15.9% of the variance at the spine, 20% at femoral neck, 17.1% at trochanter, 24.3% at total hip and 10.9% at the Ward's triangle. Other significant predictors were ESR1 Ivs1-397T/C genotype (2.2% at the spine); LRP5 2220C/T genotype (1.3% at the spine, 1.6% at the trochanter); LRP5 266A/G genotype (1.1% at Ward's triangle); age at menarche (1.3% at trochanter) and age (2.0% at Ward's triangle). As for postmenopausal women ($n = 269$), body weight (~25% at various sites) and age (~16% at femoral neck, trochanter, total hip and Ward's triangle sites) were the strongest predictors of BMD. Other significant predictors were age at menarche (4.4% at spine, 0.7% at femoral neck, 1.4% at trochanter, and 1.4% at Ward's triangle); weight bearing physical activity (2.1% at trochanter and 1% at total hip); calcium intake (1.1% at femoral neck, 0.9% at trochanter, and 1.7% at total hip) ; height (0.7% at trochanter); and ESR2 1082A/G genotype (0.8% at trochanter). We conclude that BMD at various sites and at different time span of a woman is modified by different genetic and lifestyle factors, suggesting that BMD is highly dependent on gene–environmental interactions.

Keywords: Candidate genes; Risk factors; BMD; Chinese Women

Introduction

Osteoporosis, a disease characterized by low bone mass and porous bone structure, is a major public health problem worldwide because it incurs significant costs, morbidity and mortality [1]. Epidemiological studies have consistently shown that bone mineral density (BMD) is a primary predictor of osteoporotic fractures; with each standard deviation reduction in BMD being associated with a 1.5- to 2.5-fold increase in fracture risk for both women and men [2]. Development of osteoporosis is strongly regulated by genetic factors. BMD is a complex trait determined by the peak bone mass achieved during adulthood and the subsequent rate of bone loss with age. The importance of genetic factor is illustrated by the results of twin and family studies indicating that heredity accounts for 50–80% of the variance in BMD, depending on the site examined [3]. Nevertheless, environmental factors including hormonal, nutritional, physical activity, and lifestyle factors have been shown to affect BMD and inversely associated with osteoporosis risk [4,5]. In most studied, low BMD is due to the combined effects of

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several different genes and their interaction with environmental influences, but it can occasionally occur as the result of mutation in a single gene [6]. Genes that have been implicated in the regulation of bone mass in humans include the sex hormones and their receptors, cytokines and their receptors, calcitrophic hormones, growth factors, and collagen genes [7–17]. To determine the impact of genetic and lifestyle factors on BMD in Asian populations, we studied a cohort of community dwelling southern Chinese women, focusing on twelve polymorphic loci of six candidate genes, including estrogen receptor α (ESR1) and β (ESR2), calcium sensing receptor (CASR), vitamin D receptor (VDR), the collagen type Iα1 (COLIA1), and the LDL receptor-related protein 5 (LRP5) gene.

Materials and methods

Subjects

Community dwelling southern Chinese women in Hong Kong were enrolled in the study to determine the genetic and environmental risk factors for osteoporosis in our population. These subjects were recruited from the community when they passed by road shows and health talks on osteoporosis held in various districts of Hong Kong from November 1998 to October 2002. Individuals with disease known to affect bone metabolism, premature menopause (age <40), bilateral oophorectomy or drug use that could affect bone turnover and BMD were excluded. Subjects with vitamin D deficiency as defined by 25(OH)D level below 12 ng/ml were also excluded. A total of 531 (262 premenopausal and 269 postmenopausal) women were recruited for the study. All subjects underwent a physical examination and were interviewed by a trained research assistant using a structured questionnaire. Data were collected on anthropometric measurements, ethnicity, socio-economic status, education level, medical and reproductive history, drug history, dietary and lifestyle factors, smoking and drinking history, exercise habits, self and family history of osteoporosis. Dietary calcium and phytoestrogen intake was determined by a semi-quantitative food frequency questionnaire [18]. Physical activity included all weight-bearing exercise as well as walking. The time spent on all weight-bearing physical activity was added and scored 1 to 4 (1 = 0 min/day, 2 = 0–30 min/day, 3 = 30–60 min/day, 4 ≥ 60 min/day). All participants gave informed consent and the study was approved by the Ethics Committee of the University of Hong Kong and conducted according to the Declaration of Helsinki.

Measurement of bone size, mass and density

Bone size, BMC (g), and bone mineral density (BMD, g/cm^2) were measured at the L1–4 lumbar spine, total hip, femoral neck, trochanter and Ward's triangle using dual-energy X-ray absortiometry (DEXA; Hologic QDR 4500 plus, Hologic Waltham, MA, USA). The in vivo precision of the machine for lumbar spine, femoral neck, Ward's triangle and total hip region was 1.2%, 1.5%, 2.2%, and 1.5%, respectively [7].

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by a standard procedure for PCR-based genotyping. Genotypes at 12 genetic loci of six candidate genes were determined for each participant. The genes studied include ESR1 and 2, CASR, VDR, COLIA1, and LRP5 (Table 1). In view of previous observation that the polymorphic allele at the Sp1 binding site of the COLIA1 gene is not found in Chinese [8], we tried to determine the single nucleotide polymorphisms (SNPs) of the promoter region by sequencing 3000 base pairs of the upstream region of the COL1A1 gene in 50 normal controls. Two polymorphisms were detected at nucleotide (nt) -1363C/G and -1997G/T. Subsequent genotyping of -1363C/G of the studied cohort was done by using the ABI PRISM 7700 Sequence Detection System with TaqMan Universal PCR Master Mix and Assays-on-Demand Gene expression probes (Applied Biosystems, Foster City, CA, USA). Genotyping of -1997G/T polymorphism was done by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based methods as previously described [9]. As SNPs of the LRP5 gene in Chinese have not been described, we sequenced 1500 bp of the 5′ region, all exons and their adjacent intronic region. The association of the SNPs between BMD and LRP5 gene was analyzed at three polymorphic sites: nt 266A/G, 2220C/T and

Table 1
Candidate gene

Candidate gene	Variation	References
Estrogen receptor α (ESR1)	Intron 1-397 T/C (*Pvu*II RFLP, PP = CC, Pp = CT, pp = TT)	[10]
	Intron1-351G/A (*Xba*I RFLP, XX = GG, Xx = AG, xx = AA)	
Estrogen receptor β (ESR2)	1082A/G (*Rsa*I RFLP, RR = AA, Rr = AG, rr = GG)	[19]
	1730G/A (*Alu*I RFLP, AA = AA, Aa = AG, aa = GG)	
	CA repeat	[12,13]
Calcium sensing receptor (CASR)	CA repeat	[15]
Collagen typeIα1 (COLIA1)	-1363C/G (Assay on demand)	
	-1997T/G (*Eco31*I RFLP, EE = GG, Ee = GT, ee = TT)	[9]
LDL receptor-related protein 5 (LRP5)	266A/G (*Ava*II RFLP, AA = AA, Aa = AG, aa = GG)	[20]
	2220C/T (Assay by design)	
	3989T/C (*Dra*III RFLP, DD = CC, Dd = CT, dd = TT)	[20]
Vitamin D receptor (VDR)	*Fok*I RFLP (2T/C, FF = TT, Ff = TC, ff = CC)	[14]