行政院國家科學委員會專題研究計畫　成果報告

骨骼代謝相關基因單核苷酸多型性與骨骼代謝生化指標之相關性

計畫類別：個別型計畫
計畫編號：NSC94-2320-B-028-001-
執行期間：94 年 08 月 01 日至 95 年 07 月 31 日
執行單位：國立臺灣體育學院運動管理學系

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報告類型：精簡報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 95 年 10 月 25 日

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Abstract

Many polymorphisms of genes were reported to associate with osteoporosis, yet few studies have tested its combined effects for association with bone mineral density (BMD). Six polymorphisms were chosen for this osteoporosis study, including TNFα-857 (rs1799724), TNFα-308 (rs1800469), TGFβ1-509 (rs1800247), osteocalcin (rs1800629), PTH (rs6254), PTH (rs6256), The relation between several combined polymorphisms in different genomic regions and BMD variation was addressed.
For all women combined, the proportion of low BMD among low, middle, and high BMI groups was significantly different. The combined genotypes with the largest difference in occurrence of low BMD were determined for different numbers of combined SNPs. Our data suggest that several polymorphisms of genes involved in osteoporosis pathway, that located on different chromosome and formed statistic-linked SNPs, together account for a higher occurrence of the low BMD.

Key words: Statistic-linked SNP, BMD, BMI, association, osteoporosis

Introduction

Osteoporosis is defined by World Health Organization (WHO) as a skeletal disorder characterized by compromised bone strength predisposing to an increase risk of fracture (1). Bone strength depends on the interaction of environmental and genetic factors (2). Environmental factors including aging (3-5), menopause (5,6) and body mass index (BMI) (4,5,7,8) had been reported. As genetic determinants of osteoporosis, several candidate genes including vitamin D receptor (VDR), estrogen receptor (ESR), parathyroid hormone (PTH) (9), glucocorticoid receptor, calcitonin receptor (CTR) (10-12), insulin-like growth factor-1, collagen 1-α-1 (COL1A1), interleukin-6, transforming growth factor-beta1 (TGF-β1) (13), and APOE had been reviewed (14,15). Recently, some other gene candidates were reported to be associated with osteoporosis. For examples, TNFα is a proinflammatory cytokine that promotes osteoclastic bone resorption. Single nucleotide polymorphisms (SNPs) in promoter -857 and -308 for TNFα gene were found to be associated with the osteoporosis in post-menopausal Japanese women (16,17). Osteocalcin (also known as BGP, for bone Gla protein) gene polymorphism, detected with the Hind III genotype, was suggested to influence bone density and was a possible genetic marker for bone metabolism determined in post-menopausal Japanese women (18).

Noncarriage of the 240-base pair allele of the interleukin 1 receptor antagonist (IL-1ra) gene was reported to be associated with increased bone loss, while the genotypes for heat shock protein 70-2 (hsp 70-2) and heat shock protein 70-hom (hsp hom) were not associated with degree of bone loss (19). The polymorphism of human bone morphogenetic protein-4 (BMP4) gene affecting amino acid sequence was found to be associated with hip bone density in

post-menopausal women (20). Obviously, the genetics of osteoporosis are polygenic in nature.

Methods

Subjects

The study was approved by the Institutional Review Board of Kaohsiung Medical University, Kaohsiung, Taiwan. All subjects signed the informed consent. No individual was receiving or had previously received hormone replacement therapy. Women with surgical menopause were excluded. Clinical data, including body mass index, smoking history, and blood pressure, were collected. Women were randomly recruited from general health inspection in Center of Health Examination, Department of Preventive Medicine, Kaohsiung Medical University. Fifty pre-menopausal and 257 post-menopausal women with mean age at 43 and 59 years were included, respectively. Post-menopausal women were defined by the absence of menstruation for > 6 months or having attained an age≧50 years. The subjects were categorized into underweight (BMI≦18.5 kg/m^2), standard weight (BMI 18.51~22.99 kg/m^2) and overweight (BMI≧ 23 kg/m^2) according to the re-defined WHO criterion for obesity in Asia Pacific Region (21). Blood samples were collected and stored at -70^{o}C for further analysis.

Measurements of BMD and definition of low and high bone density groups

Body height and weight were measured at the initial examination with the subjects in a standing position without shoes. Bone mineral density (BMD, in grams per square centimeter) was determined by dual-energy x-ray absorptiometry (XR36, Norland Corp., Fort Atkinson, WI) at the lumbar spine (vertebrae L2, L3, and L4) in a posteroanterior projection. T-score were calculated according to the WHO classification using a locally derived reference range provided by the manufacturer. The subjects were divided into two BMD groups according to their T-score (22-24). The high BMD group was defined as T-score >-1 and low BMD group was defined as T-score ≦-1.

DNA preparation and SNP genotyping by PCR-RFLP

Genomic DNA of the subjects was purified from peripheral leukocytes by QIAamp DNA Blood Kit (Qiagen, Valencia, CA) (25). In Table 1, eleven SNP candidates are chosen for the association studies, and PCR primers and their annealing temperature and restriction enzymes as well as genotype numbering are provided. PCR reaction mixture (10 μl) containing 1 μl of 10x PCR buffer, 0.3 μl of 50 mM $MgCl_2$, 0.2 μl of 10 mM dNTP each, 0.6 μl DMSO, 0.14 μl of Taq enzyme, 0.12μl of 350 μg/ml primers mix (1:1), and 7.64 μl of DNA in water was performed as described (25,26). PCR was performed in a single step with the following protocol: 94°C (3 min); 40 cycles of 94°C (30 s), 68°C (30 s), 72°C (10 s); 72°C (7 min); and 25°C (end). The RFLP available restriction enzymes are retrieval from our established web-tool, SNP-RFLPing (27). After digesting with corresponding restriction enzymes (New England Biolabs, UK) overnight, DNA was electrophoresed for genotype determination.

Results

Characteristics of the study population

T-score for bone marrow density (BMD), age, and BMI of subjects in this study, separately by menopausal status, were presented in Table 2. The average T-score, age, and BMI between high and low BMD subjects were significantly different for pre-menopausal as well as post-menopausal women. For all women combined, the proportions of low BMD among low (≦ 18.50), middle (18.51-22.99), and high (≧ 23.00) BMI groups were 90.9%, 65.9%, and 57.3%, respectively ($p<0.05$).

Association between combined polymorphisms and BMD

Table 3 showed the proportion of low BMD among women with specific SNP combination and other combinations,

separately by menopausal status. Among pre-menopausal women, the proportion of low BMD was not significantly different between subjects with specific SNP combination and other combinations. Among post-menopausal women, proportions of low BMD among subjects with specific SNP combinations were significantly higher than among those with other combinations. For example, the proportion of low BMD among post-menopausal women with a 3, 3 (CC-GG) genotype combination in 1, 5 SNPs (rs1799724-rs6254) was 82.61%, comparing to 57.75% among those with other combination (Chi-square=18.32, $p<0.01$). The proportions of low BMD among post-menopausal with specific SNP combinations (two SNPs to seven SNPs) were 20%-25% higher than those with other combinations.

Discussion

The importance of the relationship between multigene polymorphism combinations, environmental factors, and multifactorial disease risk had been reviewed (28). In this study, we introduced this idea to test the important role of phenotype and genotype factors in osteoporosis. BMD was reported to negatively relating to age and positively to body size (3,29). In this study, all of the underweight (BMI$\leqq$18.5) post-menopausal women had low BMD (n = 9), while among overweight & obesity (BMI $\geqq$23) post-menopausal women, only 65.1% of them had low BMD.

The results of this study suggested that specific SNP combination may be a risk factor for post-menopausal osteoporosis in Taiwanese. These results indicated that the specific SNP combination, BMI, and age were independently associated with BMD in post-menopausal Taiwanese women. The study methods reported here may enable to study multiple low-penetrance genetic factors that together determine phenotypic traits like osteoporosis.

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Table 1. T-score for bone marrow density (BMD), age, and BMI distribution among pre- and post-menopausal women in this study

	Pre-menopausal (n=50)		Post-menopausal (n=257)		Combined (n=307)	
	BMD (T-score)		BMD (T-score)		BMD (T-score)	
	High (T>-1) n=37	Low (T≦ -1) n=13	High (T>-1) n=80	Low (T≦ -1) n=177	High (T>-1) n=117	Low (T≦ -1) n=190
Average T-score (S.D.)[a]	1.59 (1.71)	-2.31 (1.23) [c]	0.91 (2.35)	-3.28 (1.28) [c]	1.13 (2.18)	-3.21 (1.30) [c]
Average age (S.D.) [a]	43.43 (5.80)	41.92 (6.37) [c]	53.78 (8.63)	61.52 (7.92) [d]	50.50 (9.19)	60.18 (9.26) [c]
Average BMI (S.D.) [a]	23.26 (2.83)	21.25 (1.97) [c]	24.21 (2.73)	23.52 (2.93) [c]	23.94 (2.78)	23.36 (2.90) [d]
BMI groups[b]						
≦18.50	1 (50.0%)	1 (50.0%)	0 (0.0%)	9 (100.0%)	1 (9.1%)	10 (90.9%) [d]
18.51-22.99	14 (60.9%)	9 (39.1%)	28 (28.0%)	72 (72.0%)	42 (34.1%)	81 (65.9%)
≧23	22 (88.0%)	3 (12.0%)	51 (34.9%)	95 (65.1%)	73 (42.7%)	98 (57.3%)

a. S.D.: Standard deviation

b. Two post-menopausal women did not have BMI data

c. P<0.01 between high and low BMD groups

d. P<0.05 between high and low BMD groups