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Polymorphisms in the Osteoprotegrin Gene with Risk of Osteoporosis and Urinary Calcium Level in a Chinese Population

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Abstract

Osteoporosis is an age-related disease caused by imbalanced calcium metabolism identified to be associated with genetic variations of multiple genes including osteoprotegrin (OPG). While bone mineral density (BMD) predicts the risk of osteoporotic fractures, the urinary calcium level (UCL) may reflect calcium metabolism, which thereby indicates osteoporotic trends. BMD of 1,206 local Chinese geriatrics in Shanghai was measured by dual X-ray absorptionmetry. UCL were examined in 728 fasting geriatric urine samples by photometry. Genotyping of the OPG SNPs rs1032128, rs334061 and rs3134063 in 481 subjects including healthy controls, osteopenia and osteoporosis patients was performed and the association between the OPG SNP variations and UCL was assessed among all comparative groups. Differences in age and BMD were statistically significant between males and females with either normal BMD or osteopenia, but were not between those with osteoporosis. Significant correlations were found between BMD and genotypes of rs1032128 in males, and between BMD and age in females. The genotypes of rs1032128 were significantly correlated with BMD in males, but were correlated with UCL in females. UCL was significantly correlated with BMD in males but was associated with rs1032128 genotypes in females. The AA type of rs1032128 was independently associated with risk of osteoporosis in males. The GG type of rs1032128 was negatively associated with UCL in males but was positively associated with UCL in females. Our data suggest that the genotypes of the OPG SNP rs1032128 may protect old males from osteoporosis development, and that UCL may be useful to predict osteoporosis if combined with the genotypes of the OPG SNP, at least in some local Chinese geriatrics.

Keywords: OPG; Osteoporosis; Urinary calcium level; Bone mineral density

Introduction

Osteoporosis is an age-related disease and confers substantial morbidity and mortality in aged people. Due to reduced bone mass and microarchitectural changes of bone tissue, the aged people are predisposed to fragile fractures at the hip, spine, wrist and other skeletal sites [1]. Bone mineral density (BMD) is usually used to predict osteoporotic fractures and also highly associated with familial factors approximately between 60% to 80% [2]. Besides genetic factors, BMD is also influenced by other factors such as calcium balance. Hence, osteoporosis is closely associated with both calcium metabolism and genetic variants of specific genes.

Calcium, the essential component of some organs, including bone and tooth, is one of the richest positive ions. Calcium maintains acid-base balance in cells and tissues, participates in many biochemical reactions [3,4]. Calcium deficiency may lead to a chaotic mechanism of protein, fat and carbonhydrate. Geriatrics lacking calcium for long time may have some symptoms, such as premature gomphiasis, teeth obscission, obvious hunchback, height decrease, pain in lumbar spine and cervical spine, constipation, hyposomnia [5,6]. Urinary calcium level (UCL) can reflect the calcium metabolism and bone status [7,8]. Many studies have reported that an increased intake of dietary calcium or a high-dairy diet may reduce inflammation, oxidative stress, particularly in over-weighted people, through promoting lipid metabolism and a loss of body fat [3,9]. It is well known that calcium supplementation can slow bone loss to prevent osteoporotic fractures through suppressing parathyroid hormone (PTH) [10,11]. Although the principal function of calcium as a versatile signaling molecule is well known [12,13], few studies have been done to clarify the associations of osteoporosis with UCL in geriatric populations.

In recent years, osteoprotegrin (OPG), the receptor activator of nuclear factor-B ligand (RANKL), and the receptor activator of nuclear factor-B (RANK) have been identified to be very important in regulation of bone turnover and bone mass [14]. In the OPG/RANKL/RANK axis, OPG acts as a decoy receptor for RANKL to inhibit osteoclast function and to maintain normal bone metabolism by preventing the interaction of RANKL with its receptor RANK. The gene TNFRSF11B coding for OPG has been considered as a candidate gene protecting bone from osteoporosis [15]. Numerous single nucleotide polymorphisms (SNPs) of TNFRSF11B including rs2073618, rs3134071, rs3102735, rs3134069, rs6993813, rs2073617, rs4876869 and rs4355801 have been reported to be associated with osteoporotic phenotypes [16-18]. However, the association of the SNP variants rs1032128, rs3134061 and rs3134063 with osteoporotic phenotypes has not been reported especially in Chinese geriatrics.

In this study, we assessed BMD of lumbar spine (L1-4), total hip, proximal femur, wards region, and detected UCL in a group of aged Chinese population from local Shanghai. Then, we analyzed the

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relationship among BMD, UCL and the SNPs rs1032128, rs3134061 and rs3234063. We found that the genoptype GA of rs1032128 was statistically associated with either BMD or UCL in age-limited female population ($p<0.01$).

Material and Methods

Subjects

This study included 1206 geriatric people more than 45 years old diagnosed with osteoporosis or osteopenia, or without specific diseases, who were enrolled during the annual medical examination in The Fifth People's Hospital of Shanghai, Fudan University, between 2010 and 2014. This study was approved by the Institutional Medical Ethics Committee, and all patients and a healthy population were enrolled with a signed informed consent. Cases with any history of medicines interfering with bone and calcium metabolism, such as estrogen, calcitonin, bisphosphonates, vitamin D metabolites, were excluded. Those who had the specific diseases (including rheumatoid arthritis, systemic lupus erythematosus, Cushing's syndrome, hypogonadism, primary hyperparathyroidism, cirrhosis, malignancy) or other acute or chronic diseases (including kidney diseases, hepatopathy and endocrine diseases), or had scoliostros or ectopic calcifications known to affect bone and calcium metabolism, were also excluded in this study.

The information about the participants at the time of physical examination was recorded: participant's ID card number, name, gender, age, duration of osteoporosis, body mineral density (BMD) and UCL. All participants were asked not to eat for 12h prior to the test. UCL and BMD were detected or measured in both groups. They were divided into four groups by age: <60, 60-69, 70-79, and 80-89. UCL (N: 1.7-5.3 mmol/L) were measured by photometry (Modular, Roche and Ca R1, R2, Roche Diagnostics, Mannheim, Germany). Imprecision was 3-5% (CV, coefficient of variation).

Bone mineral density (BMD)

BMD was measured at lumbar spine (LS, L2-4), total hip (TH), proximal femur (PF) and wards region (WR) using dual-X-ray absorptiometry (DXA), on a Hologic Elite QDR 4500 instrument (Bedford, MA, USA) and analyzed according to World Health Organization (WHO) criteria. Densitometry was performed at baseline with an annual and 2-year follow-up. Osteopenia or osteoporosis were defined as bone density between 1 and 2.5 or more than 2.5 standard deviations below the mean value for young adult Chinese women at the LS (L2–L4), TH and femoral neck (FN) based on T scores. Patients with degenerative changes in the spine and vascular calcifications in the aorta were excluded from evaluation.

Genotyping

Peripheral blood samples were collected, and genomic DNA was extracted by centrifugal column method by standard procedures and quantified by UV method. The subjects(N=600) were genotyped for the OPG SNPs rs 1032128, rs3134061 and rs3134063 using a 7900HT Fast Real-Time PCR System Instrument by using allele-specific Taqman MGB probes labelled with fluorescent dyes FAM and VIC (Applied Biosystems), according to manufacturer's protocols. Allelic discrimination was performed with the ABI PRISM 7900HT SDS and the SDS 2.2.1 program (Applied Biosystems). The SNP genotyping success rate was >99.8% and error rate in 481 duplicate samples was <0.12%.

Statistical analysis

Data were analyzed using SPSS Software (Version 19.0, SPSS, Inc., Chicago, IL, USA). Distributions of demographic characteristics in terms of BMD, UCL and genotypes of the tested SNPs were analyzed by Pearson's $\chi 2$ or Fisher's exact test. The t test was used to compare BMD and UCL between males and females. Comparisons between different groups of BMD and UCL in terms of age or sex were performed using the analysis of variance (ANOVA) test for continuous data. Spearman's correlations between BMD and age, body mass index (BMI), UCL and the SNPs of the OPG gene were determined. Logistic regression analysis was performed to assess the possible risk factors. The odds ratio (OR) with a 95% confidence interval (CI) was calculated. Data were presented as mean ± standard deviation (SD). A p value of less than 0.05 was considered statistically significant.

Results

Distributions of BMD, UCL and the OPG gene SNPs in the study population

Of 1,206 subjects who were completed the examination of BMD, 728 were detected with UCL. Following the exclusion criteria, 481sujetcs were included in the final analysis. Analysis of BMD revealed that subjects with normal BMD, osteopenia, and osteoporosis were 33% (399/1206), 49% (590/1206), and 18% (217/1206), respectively, whereas differences in age and BMD of all tests were statistically significant between males and females with either normal BMD or osteopenia ($p<0.05$), but were not between those with osteoporosis ($p>0.05$) (Table 1A). These results suggest that osteoporosis may not be significantly associated with sex. Of 728 geriatrics, 180 subjects had low UCL with 29.7% in males (79/266, mean age 69.38 ± 5.72) and 22.08% in females (102/462, mean age at 68.38 ± 5.82), while 86 had high UCL with 10.53% in males (28/266, mean age 66.67 ± 6.13) and 12.55% in females (58/462, mean age 65.29 ± 6.42) (Table 1B). Statistical significance was found between male and female subjects with normal UCL ($p=0.032$) and high UCL ($p=0.013$), and between males and females tested with BMD ($p<0.001$) (Table 1B). Of 481 geriatrics, the genotypes of rs1032128 were 36.42% (AA), 51.45% (AG) and 12.14% (GG) in males and 39.29% (AA), 46.75% (AG) and 13.96% (GG) in females (Table 2A). Age difference was found between males and females with AA ($p=0.015$) or AG ($p=0.014$), while UCL difference was found between males and females with GG ($p=0.017$), and BMD of all tested bones were significantly different between males and females with either genotype ($p<0.05$) (Table 2A). The genotypes of rs3134061 were 2.31% (AA), 24.86% (AT), 73.41% (TT) in males and 3.57% (AA), 18.83% (AT), 77.27% (TT) in females (Table 2B). Similarly, differences in age and BMD of tested bones were significantly found between males and females with AA ($p=0.040$) or TT ($p=0.005$), and with AT or TT ($p<0.05$) (except AA for BMD of LS, TH, FN, and WR) (Table 2B). The genotypes of rs3134063 were 15.27% (CC), 49.75% (CT), 34.98% (TT) in males and 13.64% (CC), 43.94% (CT), 42.42% (TT) in females (Table 2C). Also, age difference and BMD difference of tested bones were significantly associated with CC ($p=0.034$) or TT ($p=0.005$), and with AT or TT ($p<0.05$) between males and females (Table 2C).

Statistical correlation among BMD, UCL, and genotypes of rs1032128, rs3134061 and rs3134063

Spearman's correlations were analyzed among age, BMI, genotypes of rs1032128, rs3134061, rs3134063, UCL, and BMD distributions. Significant correlations were found between BMD and age ($p<0.01$), sex ($p<0.001$), or BMI ($P<0.001$), but no correlations were found between genotypes of rs1032128 ($P=0.053$), rs3134061 ($P=0.529$), rs3134063 ($P=0.828$), and UCL ($P=0.291$) in all subjects (Table 3). In males,

A. **Demographic characteristics of BMD measured subjects**									
Variables	**Subjects (n = 1,206)**								
	Normal BMD (n = 399)			**Osteopenia (n = 590)**			**Osteoporosis (n = 217)**		
	M (218)	**F (181)**	***p***	**M (179)**	**F (411)**	***p***	**M (17)**	**F (200)**	***p***
Age	69.63 ± 6.76	64.13 ± 7.19	< 0.001	71.16 ± 6.69	67.31 ± 6.82	< 0.001	70.65 ± 6.09	70.89 ± 7.49	0.89
BMI (kg/m^2)	25.13 ± 2.61	25.22 ± 3.08	0.823	23.66 ± 2.68	24.02 ± 3.26	0.298	20.53 ± 2.22	22.64 ± 3.14	0.054
BMD of LS	1.31 ± 0.19	1.20 ± 0.13	< 0.001	1.11 ± 0.18	1.00 ± 0.12	< 0.001	0.87 ± 0.11	0.83 ± 0.15	1
BMD of TH	1.06 ± 0.10	1.00 ± 0.10	< 0.001	0.87 ± 0.08	0.83 ± 0.08	< 0.001	0.69 ± 0.05	0.72 ± 0.10	0.344
BMD of FN	0.97 ± 0.09	0.92 ± 0.09	< 0.001	0.80 ± 0.06	0.76 ± 0.07	< 0.001	0.61 ± 0.04	0.66 ± 0.09	0.068
BMD of WR	0.76 ± 0.11	0.74 ± 0.11	0.041	0.60 ± 0.07	0.57 ± 0.09	0.005	0.41 ± 0.03	0.46 ± 0.09	0.091
Percentage	52.66	22.85	< 0.001	43.24	51.89	0.051	4.11	25.25	< 0.001
B. **Demographic characteristics of UCaL measured subjects**									
Variables	**Subjects (n = 728)**								
	Normal UCL (n = 461)			**Low UCL (n =180)**			**High UCL (n = 86)**		
	M (159)	**F (302)**	***p***	**M (79)**	**F (102)**	***p***	**M (28)**	**F (58)**	***p***
Age	69.00 ± 6.20	66.96 ± 6.06	0.117	69.38 ± 5.72	68.38 ± 5.82	0.243	66.67 ± 6.13	65.29 ± 6.42	0.113
BMI (kg/m^2)	24.49 ± 2.80	24.03 ± 3.06	0.133	24.44 ± 4.90	23.24 ± 3.67	0.011	23.57 ± 2.45	24.35 ± 3.74	0.274
UCL	3.33 ± 1.03	3.11 ± 0.96	0.032	1.17 ± 0.35	1.13 ± 0.41	0.804	6.62 ± 1.36	7.20 ± 2.01	0.013
BMD of LS	1.19 ± 0.21	1.00 ± 0.19	< 0.001	1.27 ± 0.23	1.00 ± 0.17	< 0.001	1.20 ± 0.19	0.96 ± 0.17	< 0.001
BMD of TH	0.97 ± 0.13	0.84 ± 0.14	< 0.001	0.98 ± 0.14	0.83 ± 0.13	< 0.001	0.95 ± 0.13	0.84 ± 0.11	< 0.001
BMD of FN	0.88 ± 0.13	0.77 ± 0.12	< 0.001	0.89 ± 0.13	0.77 ± 0.12	< 0.001	0.88 ± 0.12	0.76 ± 0.12	< 0.001
BMD of WR	0.68 ± 0.13	0.60 ± 0.13	< 0.001	0.69 ± 0.14	0.59 ± 0.14	< 0.001	0.68 ± 0.10	0.58 ± 0.10	< 0.001
Percentage	59.77	65.37	0.039	29.7	22.08	0.199	10.53	12.55	0.118

Data are shown as number/percentage/mean ± SD, one-way ANOVA test; M: male; F: female; LS: lumbar spine (L_{2-4}); TH: total hip; FN: femoral neck; WR: wards region.

Table 1: Demographic and clinical characteristics of subjects.

A. **Demographic characteristics of different genotypes in OPG rs1032128 genotyped subjects**									
Variables	**Subjects (n = 481)**								
	AA (n = 184)			**AG (n = 233)**			**GG (n = 64)**		
	M (63)	**F (121)**	***p***	**M (89)**	**F (144)**	***p***	**M (21)**	**F (43)**	***p***
Age	69.86 ± 5.89	67.45 ± 6.66	0.015	69.53 ± 6.25	67.40 ± 6.35	0.014	68.62 ± 5.91	66.35 ± 6.76	0.181
BMI (kg/m^2)	24.12 ± 2.64	24.01 ± 3.32	0.82	24.48 ± 2.64	23.85 ± 3.08	0.122	24.68 ± 3.23	23.86 ± 2.99	0.305
UCL	3.04 ± 1.80	3.15 ± 1.65	0.689	2.93 ± 1.83	2.95 ± 1.69	0.949	3.54 ± 1.78	2.44 ± 1.77	0.017
BMD of LS	1.20 ± 0.21	0.99 ± 0.21	< 0.001	1.24 ± 0.21	0.99 ± 0.18	< 0.001	1.25 ± 0.22	1.01 ± 0.19	< 0.001
BMD of TH	0.94 ± 0.13	0.82 ± 0.13	< 0.001	1.00 ± 0.14	0.83 ± 0.14	< 0.001	0.99 ± 0.14	0.86 ± 0.14	0.001
BMD of FN	0.86 ± 0.12	0.75 ± 0.11	< 0.001	0.92 ± 0.12	0.77 ± 0.13	< 0.001	0.92 ± 0.15	0.80 ± 0.12	< 0.001
BMD of WR	0.66 ± 0.13	0.56 ± 0.13	< 0.001	0.71 ± 0.14	0.57 ± 0.14	< 0.001	0.69 ± 0.13	0.61 ± 0.13	0.015
Percentage	36.42	39.29	0.374	51.45	46.75	0.309	12.14	13.96	0.364
B. **Demographic characteristics of different genotypes in OPG rs3134061 genotyped subjects**									
Variables	Subjects (n=481)								
	AA (n=15)			AT (n=101)			TT (n=365)		
	M (4)	F (11)	*p*	M (43)	F (58)	*p*	M (127)	F (238)	*p*
Age	76.33 ± 6.25	69.31 ± 6.87	0.04	69.04 ± 6.62	67.51 ± 7.52	0.224	69.40 ± 6.53	67.45 ± 7.04	0.005
BMI (kg/m^2)	26.83 ± 3.00	24.41 ± 2.44	0.173	23.66 ± 2.81	23.77 ± 3.19	0.86	24.43 ± 2.70	23.94 ± 3.22	0.142
UCL	2.45 ± 1.54	2.83 ± 1.28	0.688	2.92 ± 1.74	2.87 ± 1.59	0.877	3.05 ± 1.76	2.90 ± 1.60	0.413
BMD of LS	1.20 ± 0.11	0.94 ± 0.13	0.024	1.19 ± 0.23	1.00 ± 0.16	< 0.001	1.24 ± 0.21	0.99 ± 0.20	< 0.001
BMD of TH	0.99 ± 0.03	0.77 ± 0.09	0.005	0.93 ± 0.15	0.82 ± 0.13	< 0.001	0.99 ± 0.13	0.84 ± 0.14	< 0.001
BMD of FN	0.86 ± 0.06	0.72 ± 0.14	0.058	0.86 ± 0.14	0.76 ± 0.11	< 0.001	0.91 ± 0.12	0.77 ± 0.13	< 0.001
BMD of WR	0.61 ± 0.06	0.51 ± 0.13	0.204	0.65 ± 0.14	0.57 ± 0.11	0.002	0.71 ± 0.13	0.57 ± 0.14	< 0.001
Percentage	2.31	3.57	0.328	24.86	18.83	0.128	73.41	77.27	0.39
C. **Demographic characteristics of different genotypes in OPG rs3134063 genotyped subjects**									
Variables	**Subjects (n=481)**								
	CC (n = 184)			**CT (n = 233)**			**TT (n = 64)**		
	M (23)	**F (47)**	***p***	**M (87)**	**F (132)**	***p***	**M (63)**	**F (129)**	***P***
Age	71.57 ± 5.41	68.13 ± 6.95	0.034	68.78 ± 5.73	67.14 ± 6.20	0.061	69.84 ± 6.59	67.10 ± 6.69	0.005
BMI (kg/m^2)	24.89 ± 2.80	23.53 ± 2.97	0.077	24.21 ± 3.02	23.95 ± 3.08	0.529	24.41 ± 2.18	24.02 ± 3.32	0.403
UCL	3.42 ± 2.02	2.60 ± 1.62	0.063	3.08 ± 1.83	2.81 ± 1.60	0.264	2.87 ± 1.70	3.24 ± 1.78	0.167
BMD of LS	1.22 ± 0.23	0.96 ± 0.16	<0.001	1.22 ± 0.21	1.01 ± 0.19	<0.001	1.24 ± 0.21	0.98 ± 0.21	< 0.001
BMD of TH	0.95 ± 0.12	0.81 ± 0.13	< 0.001	0.98 ± 0.14	0.84 ± 0.15	< 0.001	0.99 ± 0.13	0.83 ± 0.13	< 0.001

BMD of FN	0.86 ± 0.13	0.76 ± 0.13	0.001	0.90 ± 0.13	0.77 ± 0.13	< 0.001	0.90 ± 0.13	0.76 ± 0.11	< 0.001
BMD of WR	0.64 ± 0.11	0.55 ± 0.12	0.012	0.69 ± 0.14	0.58 ± 0.14	< 0.001	0.71 ± 0.14	0.57 ± 0.13	< 0.001
Percentage	13.29	15.26	0.357	50.29	42.86	0.192	36.42	41.88	0.247

Data are shown as number/percentage/mean ± SD, one-way ANOVA test; M: male; F: female; LS: lumbar spine (L_{2-4}); TH: total hip; FN: femoral neck; WR: wards region.

Table 2: Demographic characteristics of OPG gene genotype subjects.

Factors	Correlation coefficient	*p*
Age	0.137**	0.003
Sex	0.422**	< 0.001
BMI	-0.355**	< 0.001
rs 1032128	-0.088	0.053
rs 3134061	-0.026	0.529
rs 3134063	-0.009	0.828
UCL	0.048	0.291

Table 3: Spearman's correlation of BMD with age, sex, BMI genotypes of the tested SNPs, and UCL.

significant correlation between BMD and BMI (P<0.001) or genotypes of rs1032128 (P=0.044) was found, but no correlation between BMD and age (P=0.854), genotypes of rs3134061 (P=0.051) and rs3134063 (P=0.202), or UCL (P=0.218) was found. In females, we found that age (P<0.001) and BMI (P<0.001) were significantly correlated with BMD, but that BMD was not correlated with genotypes of all SNPs or UCL (p>0.05) (Table 4A). Since the genotypes of rs1032128 were only correlated with male BMD, we further analyzed whether the genotypes of rs1032128 were correlated with age, BMI, UCL, and BMD tested in different bones. As shown in Table 4B, genotypes of rs1032128 were significantly correlated with BMD of FN (P=0.003) and TH (P=0.031) in males, but were correlated with UCL (P=0.025) and BMD of FN (P=0.038) in females. The correlation analysis of UCL with age, BMI, rs1032128 genotypes, and BMD revealed that UCL was significantly correlated with BMD of LS (P=0.038) in males but was associated with rs1032128 genotypes in females (P=0.025) (Table 4C). These results suggest that difference of sex may have some profound difference in terms of the tested rs1032128 genotypes and UCL, which may be associated with osteoporosis prevalence between aged man and woman.

A. Spearman's correlation of BMD with age, BMI, genotypes of the OPG SNPs, and UCL in males and females

Factors	Male		Female	
	Correlation coefficient	P	Correlation coefficient	P
Age	0.011	0.854	0.236**	< 0.001**
BMI	-0.317**	< 0.001**	-0.313**	< 0.001**
rs 1032128	-0.154*	0.044*	-0.075	0.135
rs 3134061	-0.137	0.051	-0.001	0.985
rs 3134063	-0.09	0.202	0.013	0.804
UCL	0.076	0.218	0.096	0.093

B. Spearman's correlation of rs1032128 genotypes with age, BMI, UCL, and BMD

Factors	Male		Female	
	Correlation coefficient	P	Correlation coefficient	P
Age	-0.067	0.38	0.044	0.439
BMI	0.006	0.92	-0.021	0.708
UCL	0.051	0.506	-0.128*	0.025*
BMD of FN	0.223**	0.003**	0.118*	0.038*
BMD of TH	0.164*	0.031*	0.088	0.124
BMD of LS	0.084	0.276	0.043	0.452
BMD of WR	0.136	0.075	0.107	0.06

C. Spearman's correlation of UCL with age, BMI, rs1032128 genotypes, and BMD

Factors	Male		Female	
	Correlation coefficient	P	Correlation coefficient	P
Age	-0.073	0.345	-0.042	0.46
BMI	-0.017	0.829	0.069	0.226
rs 1032128	-0.062	0.174	-0.128*	0.025*
BMD of FN	-0.072	0.24	-0.029	0.607
BMD of TH	-0.116	0.06	-0.021	0.712
BMD of LS	-0.127*	0.038*	-0.089	0.117
BMD of WR	-0.078	0.174	-0.036	0.532

M: male; F: female; LS: lumbar spine (L_{2-4}); TH: total hip; FN: femoral neck; WR: wards region

Table 4: Association of BMD, age, BMI, genotypes of the OPG SNPs, and UCL in males and females population respectively.

Risk factors for osteoporosis

To find independent risk factors for osteoporosis, we analyzed sex, BMI, age, genotypes of rs1032128, and low or high UCL with multivariable logistic regression. The results showed that females (P<0.001) and BMI ≤ 23 (P<0.001) were independently associated with risk of osteoporosis (Table 5A). Ages older than 60 was an independently risk factor for osteoporosis in females (P<0.001) (Table 5B). The AA type of rs1032128 was independently associated with osteoporosis in males (P=0.050) (Table 5B). The GG type of rs1032128 was negatively associated with UCL in males (P=0.016) but was positively associated with UCL in females (P=0.022) (Table 6).

Discussion

Calcium in human body is mainly taken from dietary. The normal urinary calcium level (UCL) accounts for 20% amount of daily intake. The excreted amount of calcium in urinary is positively correlated with the intestinal calcium absorption, and has an exponential relationship with the calcium intake [9,19]. So using of UCL to evaluate the index of calcium nutrition and bone absorption status may be an important tool in judging the calcium deficiency and BMD of geriatrics [6,20].

In this study, 1206 geriatric people with ages ranging from 45 to 90 in local Minhang District of Shanghai were enrolled, and their BMD was investigated during annual medical examination from 2010 to 2014. We measured UCL in 728 of the geriatric subjects and detected the genotypes of rs1032128, rs3134061, and rs3134063 in 481 subjects from those with UCL data, and found that rs1032128 was only associated with risk of osteoporosis in males. The osteoporosis prevalence with rs1032128 was 4.11% in males, but was 25.26% in the females in this study population. Moreover, we found that the osteoporosis was mainly affected by body mass index (BMI) and genetics in males but by BMI and age in females.

The most interesting result was that the low UCL was inversely associated with the GG genotype of rs1032128 in males but was positively associated with the GG genotype in females, and that the AA genotype of rs1032128 was positively associated with osteoporosis prevalence in males but did not have any relationship with all factors in females. Thus, the genotypes of rs1032128 may play a key role during osteoporosis development besides the body mass index and aging. The

A. Odds ratio of Sex and BMI based on osteoporosis prevalence				
Variables		**OR**	**95%CI**	***P***
Sex	**Male (n = 414)**	**1**		
(n =1206)	Female (n =792)	14.17**	8.316-24.144	< 0.001
BMI	BMI1 (obesity, n =75)	1		
(n =728)	BMI2(overweight,n=394)	1.052	0.391-2.829	0.571
	BMI3 (normal, n =247)	6.583**	2.535-17.094	< 0.001
	BMI4(low-weight,n =12)	30.3333**	3.116-295.249	< 0.001

B. Odds ratio of age and rs1032128 genotypes in male and female subjects with osteoporosis						
Variables	**M**			**F**		
	OR	**95%CI**	***p***	**OR**	**95%CI**	***p***
Age (<60, n =61)	1			1		
Age(60-69,n=411)	1.071	1.021-1.124	0.627	4.343**	2.005-9.408	< 0.001
Age(70-79,n =227)	1.085	1.021-1.153	0.578	12.533**	5.548-28.312	< 0.001
Age (> 80, n =28)	1.118	0.958-1.304	0.526	36.556**	10.293-129.822	< 0.001
AG (n =233)	1			1		
GG (n =64)	2.179	0.184-25.749	0.478	0.65	0.267-2.578	0.232
AA (n =184)	4.766*	0.875-25.945	0.050*	1.261	0.659-2.413	0.297

Table 5: Odds ratio of sex, BMI and genotypes of rs1032128 based on osteoporosis prevalence in geriatrics.

Variables		**M**			**F**		
		OR	**95%CI**	***p***	**OR**	**95%CI**	***p***
Low UCL (n = 128)	**AG(n= 67)**	**1**			**1**		
	GG(n=20)	0.188*	0.040-0.872	0.016	2.032*	1.099-4.823	0.022
	AA(n=41)	0.814	0.398-1.665	0.352	0.695	0.378-1.278	0.154
High UCL	AG(n=24)	1			1		
(n = 53)	GG(n=7)	1	0.241-4.153	0.655	1.194	2.164-3.963	0.492
	AA(n=22)	1.219	0.428-3.474	0.456	1.032	0.471-2.263	0.547

Table 6: Odds ratio of UCL in male and female subjects with different genotypes of rs 1032128.

significant difference in osteoporosis rate between male and female geriatrics may be mainly associated with the function of bidirectional regulating calcium balance from the OPG gene. The GG genotype of rs1032128 in the OPG gene may induce high UCL in males but low UCL in females, likely due to different levels of hormones. Therefore, to prevent menopausal osteoporosis through oral administration, the use of estrogen to balance the body calcium level may be effective females. Our data also suggest that taking steps to keep the BMI over than 23, and anti-aging may also reduce the risk of osteoporosis in females.

Overall, our study suggests that the genotypes of rs1032128 in the OPG gene may play an important role in osteoporosis development of males and influence the calcium balance in all geriatrics, and that UCL may be useful to predict osteoporosis if combined with the genotypes of the OPG SNP rs1032128, at least in some local Chinese geriatrics in Shanghai.

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Conflict of Interest

LI Yinghua, WU Yougen, LU Tong, YUAN Meng, CUI Yunqing, ZHOU Yunjiao, YANG Gong and HONG Yang declare that they have no conflict of interest.

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