Relationship between CATSPERB, NR5A2 gene polymorphisms and Peak Bone Mineral Density in College Students in China

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Abstract

Background: Peak bone mineral density (PBMD) is influenced by both genetic and environmental factors, genes explains most of variation. As the novel candidate genes *CATSPERB* and *NR5A2* may have been associated with spinal PBMD in adult. This study was to investigate the relationship among these two genes、 PBMD and the life style factors in young female.

Methods: The rs1298989 single nucleotide polymorphism (SNP) of the *CATSPERB* gene and the rs3762397 SNP of the *NR5A2* gene were genotyped using SNaPshot® in 359 students from Xinjiang. The prospective study included 203 Han and 156 Uyghur subjects. PBMD was measured using quantitative computed tomography (QCT). Calcium, phosphate and alkaline phosphatase were measured by ELISA method. Physical activity, dietary calcium and life styles were assessed by questionnaire.

Results: Both SNPs showed differences in genotype and allele frequencies ($P < 0.05$) between the Han and Uyghur subjects. Total calcium intake, energy intake, tea and milk intake were also significantly different between two groups($P < 0.05$). Multiple regression analysis showed an association between PBMD and vitamin D intake ($P = 0.000$), milk ($P = 0.000$), exercise ($P = 0.029$), rs1298989 ($P = 0.028$) , energy intake ($P = 0.043$).

Conclusion: This study demonstrated the polymorphisms of the rs1298989 and rs3762397 are associated with PBMD both in Han and Uyghur subjects. PBMD, in Xinjiang, appears to be associated with several known factors that are well described in the literature. While the genotypes of rs1298989 and rs3762397 do not appear have a strong effect on the PBMD.

Keywords: Peak bone mineral density (PBMD), Life styles, *CATSPERB*, rs1298989 *NR5A2*, rs3762397

Introduction

The Chinese population is aging, which has resulted in an increase in osteoporosis and fractures, particularly vertebral and hip fractures. A large-scale epidemiological survey of the Han population showed that in women over the age of 50 yr, the prevalence of osteoporosis was 30.8%, the incidence of vertebral osteoporosis was 27.3% and the prevalence of low bone mass was 64.6% (1). Women over the age of 50, with osteoporotic vertebral fractures, accounted for approximately 36–39% of all of osteoporotic fracture cases (2). Therefore, prevention of osteoporosis is compellingly important.

Rewrite: The fracture risk was influenced by both rate of bone loss and peak bone mineral density (PBMD) that will be higher in individuals with a low PBMD than normal even if the low rate of bone loss in the following years.

PBMD is a complex quantitative trait that is determined from early childhood and involves the in-

teraction of multiple genetic and environmental components (3). Environmental factors include calcium intake, diet, physical activity and other aspects of life style (4, 5). The measurement of PBMD is also affected by the site of evaluation. Assessing the bone mineral density (BMD) of spine, it has a heritability of approximately 40% (3).

Genetic factors play a major role as determinants of variation in BMD. This has been widely shown. There were many studies had been performed to identify the relationship between candidate gene polymorphisms and BMD in postmenopausal women with osteoporosis. Proteins such as estrogen receptors, osteoprotegerin, lipoprotein receptor-related protein and sclerostin had been assessed for polymorphisms (6-8). Recently, Koller et al. (9) investigated the effect of 50 single-nucleotide polymorphisms (SNPs) on BMD, an association was observed between BMD and SNPs that were identified in the sperm ion channel protein (*CATSPERB*) gene, on chromosome 14. The role of *CATSPERB* is poorly understood in bone, but its expression in the ovary indicates a potential role in hormonal regulation of bone metabolism (9, 10**)**.

The nuclear receptor subfamily 5 member A2 (*NR5A2*) gene is a member of the *NR5A* subfamily and is expressed in the preovulatory follicle and the corpus luteum.

Rewrite: *NR5A2* gene play a very important role in the process of regulation in the gonadal steroidogenic gene expression.

"It is thougt to play a key role in the regulation of gonadal steroidogenic gene expression" (11) and may also affect bone metabolism via hormonal regulation. Riancho et al. (12) reported that *NR5A2* is expressed at a high level in bone and modulates the expression of some osteoblastic genes. This implies that it plays a role in skeletal homeostasis. The rs3762397 SNP is a novel loci that affects BMD and is located in the *NR5A2* gene, at chromosome site 1q32 (11, 13).

Currently, the genetic research on osteoporosis were more concentrated in postmenopausal women (14), only a few studies had investigated the relationship between candidate gene polymor-phisms, which underlie PBMD variation, and environmental factors.

The objective of this study was to investigate the association between the rs1298989 SNP of the *CATSPERB* gene and rs3762397 SNP of the *NR5A2* gene with BMD in female Han and Uyghur college students. At the same time the relationships between BMD, gene polymorphisms and life styles also been analyzed.

Materials and Methods

Materials

A total of 370 female Xinjiang medical university students, aged between 19.75±1.21 and 21.46±1.18 yr, volunteered for the study between the years 2012 and 2013. They were recruited from grade four and included five classes of Han and four minority classes, of which Uyghur subjects accounted for 60%-70%. Genotypes were successfully obtained from 359 subjects. Samples from 11 individuals could not be amplified and genotyped due to the poor quality of DNA.

Female college students were excluded if they had any of the following: (A) chronic diseases (e.g. chronic liver disease, chronic renal disease), (B) an intake of drugs that could influence bone metabolism, (C) evidence of other metabolic or inherited bone diseases, (D) major gastrointestinal disease, (E) any disease, treatment or condition that could be a non-genetic cause of low bone mass. This study was conducted in accordance with the declaration of Helsinki.

This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from all participants.

Questionnaire

All participants answered a questionnaire on age, age at menarche, habitual tea consumption, current drinking habits, current smoking habits, habitual milk consumption, habitual coffee consumption, physical exercise (regular or not) and medical history, such as medications and fractures.

Measurement

Weight and height were measured in indoor clothes, without shoes, at the time of bone densitometry assessments. The body mass index (BMI) was calculated as body weight/(height^2) (kg/m^2).

Following on overnight fasting (at least 8 hour), venous blood samples were obtained for evaluation of bone metabolism. Bone biochemistry was evaluated by serum calcium (Ca), phosphorus, alkaline phosphatase, measured by a commercial ELISA kit.

The BMD of the lumbar spine (L2-L4) was measured by computed tomography (CT) (64 LightSpeed VCT, GE; Milwaukee, WI, USA). Mineral content was determined in the midspine of the L2-L4 vertebrae, with a 5 mm slice obtained at each level. Trabecular contours were defined in accordance with Hounsfield unit values. A reference phantom that contained calcium hydroxyapatite, at 200 mg/mL, and a water equivalent were used for control measurements. Representative volumes of trabeculae, at the midplane of the three vertebral bodies (L2-L4), were quantified and averaged. The results were expressed as mineral equivalents of calcium hydroxyapatite, in mg/cm^3. Based on multiple measurements of 10 subjects, 1.17% was taken as the precious for the lumbar spine.

Assessment of dietary calcium and vitamin D

The participants filled in a standardized food frequency questionnaire (FFQ), which included 12 questions that covered nine food items. All these foods contributed to 75% of the calcium intake and 90% of the vitamin D intake in the most dietary intake studies, performed in Denmark. The FFQ and calculations have been reported previously (15). The questionnaire in FFQ included nine predetermined potential frequencies (ranging from "less than one time per month" to "four to five times per day or more").

Genotyping test

Genomic DNA was extracted from blood using the salting-out method and DNA was stored at−20°C until use. The working concentration of DNA was 5-10ng/μl, the concentrations of primers is 0.8μM. The genotypes of the SNPs were determined using SNaPshot® (Applied Biosystems, Forster City, CA). This technology uses the polymerase chain reaction (PCR) and multiplexed single-base extension, followed by capillary electrophoresis. Primers for the rs1298989 SNP of *NR5A2* and the rs3762397 SNP of *CATSPERB* were designed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The sequences were as follows:rs1298989F: 5'-CCACAAAGTAG-TAAGGCCCAAAGTCA-**3'**;rs1298989R:**5'**-gaaAATTGTG-CCACCAGAAATGAA-**3'**;rs3762397F:**5'**-GAAGTTCAAA-TATCTTGTGGTCCATGT-**3'**;rs3762397R: **5'**-TTCCA-TTCCCCAACGTATCCA-**3'**.

The PCR premix contained 20 μl of 10× Buffer I (ABI Co., Ltd., Foster City, USA), 3.0 mM MgCl$_2$, 0.3 mM dNTPs, 1 unit of HotStarTaq DNA polymerase (QIAGEN Co., Ltd., Valencia, Spain), 1 μl of DNA and 2 μl of multi-PCR primer. The PCR thermocycling conditions were: 95 °C for 2 min; 11 cycles of 94 °C for 20 s, 65 °C (reduced by 0.5 °C each cycle) for 40 s, 72 °C for 90 s; followed by 24 cycles of 94 °C for 20 s, 59 °C for 30 s, 72 °C for 90 s, and a final extension at 72 °C for 2 min. All PCR products were pooled and removed unincorporated primers. Multiplex single-base primer extension reactions were performed using the SNaPshot Multiplex Kit (ABI Co., Ltd., Foster City, USA). The reactions were then treated with one unit of calf intestinal phosphatase (NEW ENGLAND Biolabs, MA, USA), at 37 °C for 60 min, followed by 15 min at 75 °C.Labelled extension products were resolved by capillary electrophoresis, on an DNA Analyzer (ABI Co., Ltd., Foster City, USA), and data analysis was performed using GeneMapper Analysis Software, version 4.0 (Biosystems Co., Ltd., USA)

Statistical analysis

Participants were divided into two groups in accordance with the ethnicity given in the baseline survey: (A) Han nationality (B) Uyghur nationality. Data for all variables were normally distributed, which allowed parametric tests of significance to be performed. Data were presented as means and standard errors. Comparisons of the basic characteristics, BMD and serum biology, between the

groups, were calculated through *t*-tests or *t* '-tests. To determine whether the allele distributions of the SNPs satisfied the Hardy–Weinberg equilibrium, a chi-squared test was used to compare the observed and expected allele frequencies. The association between each SNP and physical characteristics (age, weight and height), in addition to dietary calcium intake, energy intake, vitamin D intake and BMD, was assessed by an analysis of variance (ANOVA). Differences in BMD and SNP alleles, in relation to ethnicity, were assessed by *t*-tests.

Regression analysis was used to determine the factors that predict BMD in the lumbar spine, evaluate their significance and assess the proportion of variance that these factors can explain. The regression model analyzed the following variables: ethnic group, rs3762397 genotype, rs1298989 genotype, age, BMI, age at menarche, total calcium intake, energy intake, vitamin D intake, regular exercise, current smoking habits, current drinking habits, habitual tea consumption and habitual milk consumption.

The limit of significance was set at *P* < 0.05 for all analyses. All statistical analyses were carried out using SPSS for Windows, Version 13.0 (SPSS, Chicago, IL, USA).

Results

Comparison the basic characteristics, bone mineral densities and lifestyle factors

Data from the 359 baseline study participants are presented in Table 1. There were no differences in age, weight, height, BMI index, menarche and serum biology, between the Han and Uyghur groups. In contrast, total calcium intake, energy intake, habitual tea consumption and habitual milk consumption were significantly different between the two groups.

Table 1: Basic characteristics, bone mineral densities and lifestyle factors of Han and Uyghur female college students

Parameter	Total (n=359) Mean (SD)/n(%)	Han nationality (n=203) Mean (SD)/n(%)	Uyghur nationality (n=156) Mean (SD)/n(%)	*P* value
Age (yr)	21.24 (1.38)	21.30 (1.39)	21.15 (1.38)	0.32
Height (cm)	161.46 (4.39)	161.33 (4.70)	161.63 (3.97)	0.525
Weight (kg)	55.50 (5.37)	55.51 (5.42)	55.48 (5.32)	0.956
BMI (kg/m²)	21.29 (1.91)	21.40 (2.08)	21.14 (1.66)	0.185
Age at menarche (years)	13.75 (3.52)	13.85 (3.50)	13.68 (3.54)	0.645
Total calcium intake (mg/day)	416.68 (159.36)	392.00 (169.43)	448.79 (139.32)	0.001
energy intake (kcal /day)	2041.31 (324.33)	2008.87 (300.97)	2083.53 (348.90)	0.03
Vitamin D intake (μg/day)	5.35 (.82)	5.4228 (0.84)	5.26 (0.80)	0.061
L2-4BMD (g/cm³)	177.26 (28.50)	175.89 (28.83)	179.04 (28.05)	0.299
L2 (g/cm³)	178.77 (26.86)	177.36 (27.33)	180.62 (26.22)	0.255
L3 (g/cm³)	171.97 (26.69)	169.15 (27.06)	175.64 (25.84)	0.222
L4 (g/cm³)	175.82 (27.03)	173.56 (27.98)	178.77 (25.55)	0.07
T-score	−0.20 (0.83)	−0.25 (0.79)	−0.13 (0.86)	0.162
Z-score	−0.03 (0.83)	−0.04 (.8375)	−0.01 (0.83)	0.727
Ca (mmol/L)	1.98 (0.15)	1.98 (0.15)	1.99 (.150)	0.345
P (mmol/L)	1.26 (0.14)	1.26 (0.14)	1.26 (.140)	0.75
ALP (IU/L)	52.54 (10.00)	52.68 (9.48)	52.35 (10.67)	0.751
Regular exercise[1] (yes/no),%	98 (27.30)	52 (25.62)	46 (29.49)	0.414
Current smoker (yes/no), %	8 (2.22)	3 (1.48)	5 (3.21)	0.302
Current drink	12 (3.34)	9 (4.43)	3 (1.92)	0.243
Habitual tea consumption[2],%	116 (32.31)	36 (17.73)	80 (51.28)	0
Habitual coffee consumption[2],%	6 (1.68)	2 (0.99)	4 (2.56)	0.41
Habitual milk consumption[2],%	200 (55.71)	94 (46.31)	106 (67.95)	0

[1]Regular exercisers are defined as participating in regular physical activities two or more times per week.
[2]Habitual tea consumption, habitual milk consumption and habitual coffee consumption are defined as drinking the beverage more than three to four times per week

Comparison of the distribution of genotypes and allele frequencies for rs3762397 and rs1298989

Analysis of the rs3762397 SNP identified three possible genotypes; CC, CT and TT. Of the 203 Han participants, 62.56% had the CC genotype, 33.99% had the CT genotype and 3.45% had the TT genotype. Allele frequencies were 0.796 for the C allele and 0.204 for the T allele, which were consistent with the Hardy–Weinberg equilibrium (χ^2=0.410, P = 0.522). Of the 156 Uyghur participants, 36.54% had the CC genotype, 53.21% had the CT genotype and 10.26% had the TT genotype. Allele frequencies were 0.631 for the C allele and 0.369 for the T allele, which were consistent with the Hardy–Weinberg equilibrium (χ^2=3.193, P = 0.074).Significant differences were observed between the Han and Uyghur genotype and allele frequencies (P = 0.000, P = 0.000) (Table 2).

The observed rs1298989 genotypes were GA and GG, AA was absent. Of the 203 Han participants, 4.93% had the GA genotype, whilst 95.07% had the GG genotype. Allele frequencies were 0.975 for the G allele and 0.025 for the A allele, which were consistent with the Hardy-Weinberg equilibrium (χ^2=0.128, P = 0.279). Of the 156 Uyghur participants, 23.72% had the GA genotype, whilst 76.28% had the GG genotype. Allele frequencies were 0.881 for the G allele and 0.119 for the A allele, which satisfied the Hardy–Weinberg equilibrium (χ^2=2.808, P = 0.906). Significant differences were observed between Han and Uyghur genotype and allele frequencies (P = 0.000, P = 0.000) (Table 3).

Table 2: Comparison of the distribution of genotypes and allele frequencies for rs3762397 between Han and Uyghur female college students

Group	rs3762397			Allele	
	CC	**CT**	**TT**	**C**	**T**
Uyghur	57	83	16	197 (0.631)	115(0.369)
Han	127	69	7	323 (0.796)	83 (0.204)
χ^2 value		25.729		23.804	
p value		0.000		0.000	

Table 3: Comparison of the distribution of genotypes and allele frequencies for rs1298989 between Han and Uyghur female college students

Group n	rs1298989		Allele	
	GA	**GG**	**G**	**A**
Uyghur	37	119	275 (0.881)	37 (0.119)
Han	10	193	396 (0.975)	10 (0.025)
χ^2 value	27.378		25.460	
p value	0.000		0.000	

Comparison of demographic features, clinical characteristics, daily energy and nutrient intake and BMD values

Although age, weight, height, menarche, total calcium intake and vitamin D intake were similar across all rs3762397 and rs1298989 genotypes, in both the Han and Uyghur groups, the L2-4 BMD association with rs3762397 genotypes was different between the two groups. The BMD values in Han subjects with the TT genotype were significantly (P < 0.05) higher than in those with the CC genotype. The BMD values in Uyghur subjects with the CC or TT genotype were significantly (P < 0.05) higher than in those with the CT genotype. Significant differences were observed in the L2-4 BMD values of the Uyghur group, between the rs1298989 genotypes. The Uyghur subjects with the GG genotype had significantly higher BMD values, compared with subjects with the GA genotype (Tables 4 and 5).

Table 4: Comparison of demographic features, clinical characteristics, daily energy and nutrient intake and BMD values in the Han and Uyghur subjects, categorized by rs3762397 genotype

Genotype	Han					Uyghur				
	CC	CT	TT	F	p value	CC	CT	TT	F	P value
Total (n)	127	69	7			57	83	16		
Age (year)	21.39 (1.46)	21.20 (1.26)	20.71 (1.11)	1.037	0.357	21.18 (1.35)	21.10 (1.39)	21.38 (1.45)	0.283	0.754
Height (cm)	161.39 (5.17)	161.46 (4.36)	159.86 (4.14)	0.351	0.705	161.18 (4.11)	161.14 (4.25)	161.38 (3.73)	0.021	0.98
Weight (kg)	56.66 (7.84)	56.87 (7.12)	56.00 (5.13)	0.049	0.952	54.04 (6.33)	55.55 (7.08)	53.94 (8.17)	0.96	0.385
Menarche (year)	13.85 (3.60)	13.49 (3.58)	12.43 (0.98)	0.68	0.508	14.02 (4.98)	13.77 (2.22)	13.58 (.99)	0.211	0.81
Total calcium intake (mg/day)	379.83 (164.76)	407.09 (170.87)	464.29 (231.52)	1.241	0.291	430.11 (158.479)	457.63 (129.07)	469.56 (116.267)	0.856	0.427
Energy intake (cal/day)	2004.71 (335.29)	2013.32 (228.22)	2040.43 (311.06)	0.058	0.944	1973.81 (259.11)	2167.96 (390.40)	2036.44 (294.11)	5.724	0.004
Vitamin D intake (µg/day)	5.3853 (0.92)	5.4880 (0.71)	5.46 (0.39)	0.344	0.71	5.19 (0.77)	5.32 (0.82)	5.26 (0.84)	0.517	0.598
L2-4BMD (g/cm³)	171.99 (29.73)	182.10 (26.94)	185.19 (18.37)	3.193	0.043	186.56 (33.28)	172.45 (23.33)	186.43 (22.48)	5.153	0.007

Table 5: Comparison of demographic features, clinical characteristics, daily energy and nutrient intake and BMD values in the Han and Uyghur subjects, categorized by rs1298989 genotype

Genotype	Han				Uyghur			
	GG	GA	t value	p value	GG	GA	t value	P value
Total (n)	193	9			119	37		
Age (year)	21.33 (1.397)	20.78 (1.09)	1.172	0.243	21.09 (1.38)	21.35 (1.36)	−0.998	0.320
Height (cm)	161.52 (4.86)	158.33 (4.21)	1.934	0.055	161.35 (4.19)	160.62 (3.90)	0.941	0.348
Weight (kg)	56.68 (7.48)	56.78 (8.38)	−.039	0.969	54.75 (7.13)	55.97 (6.27)	−0.938	0.350
Menarche (year)	13.73 (3.61)	12.78 (1.20)	0.787	0.432	13.93 (3.94)	13.59 (1.38)	0.511	0.610
Total calcium intake (mg/day)	389.41 (169.107)	428.33 (181.80)	−0.673	0.502	441.73 (138.76)	471.51 (140.57)	−1.137	0.257
Energy intake (cal/day)	2012.31 (306.029)	1977.89 (139.97)	0.335	0.738	2082.13 (354.10)	2088.03 (336.30)	−0.089	0.929
Vitamin D intake (µg/day)	5.4121 (0.845)	5.601 (.636)	−0.662	0.509	5.25 (.84)	5.27 (0.68)	−0.139	0.890
L2-4BMD (g/cm³)	175.565 (28.53)	180.522 (37.03)	−0.503	0.616	176.32 (27.82)	187.79 (27.32)	−2.198	0.029

There was no association between rs1298989 genotype and BMD in the Han group ($P > 0.05$)
The allelic association with BMD was different between the two groups for the rs3762397 SNP. A high BMD was associated with the CC genotype in Uyghur subjects ($P = 0.003$), while it was associated with the CT genotype in Han subjects ($P = 0.019$) (Table 6). No differences were observed in allelic association with BMD for the rs1298989 SNP.

Variables associated with BMD

We also analyzed the correlation between BMD and risk factors using a multivariate linear statistical method. The relationships are listed in Table 7. Five factors were found to correlate with BMD in the Uyghur and Han participants. The regression equation was: y(BMD) = 76.208 + 8.465×Vitamin D intake + 11.904×habitual milk consumption+ 9.18×rs1298989 + 6.969×regular exercise + 0.009×energy intake.

The variables explained 13.4% of the variance in BMD. The greatest impact on BMD was observed for vitamin D intake. The second variable, in terms of the size of impact, was habitual milk consumption.

Table 6: BMD of rs3762397 and rs1298989 genotypes in Han and Uyghur female college students

Gene	Genotype	n	Han	Uyghur	*p* value
rs3762397	CC	184	171.99 (29.73)	186.55 (33.28)	0.003
	CT	152	182.10 (26.94)	172.45 (23.33)	0.019
	TT	23	185.19 (18.37)	186.43 (22.48)	0.899
rs1298989	GG	312	175.57 (28.53)	176.32 (27.82)	0.819
	GA	47	182.06 (35.25)	187.79 (27.32)	0.583

Table 7: Variables associated with BMD in Han and Uyghur female college students, with increased BMD of the lumbar spine evaluated by multiple regression analysis

Predictors of BMD	Unstandardized Coefficients		Standardized Coefficients		
	B	SE	Beta	*t* value	*p* value
(Constant)	76.208	14.871		5.125	0.000
Vitamin D intake	8.465	1.701	0.245	4.976	0.000
Habitual milk consumption	11.904	2.856	0.208	4.168	0.000
rs1298989	9.186	4.173	0.109	2.201	0.028
Regular exercise	6.969	3.177	0.109	2.194	0.029
Energy intake	0.009	0.004	0.100	2.027	0.043

F=12.112, *p*<0.001, adjusted R^2 = 13.4%.

Discussion

In this study, we observed differences in genotype and allele frequencies of the rs1298989 SNP in the *CATSPERB* gene and rs3762397 SNP in the *NR5A2* gene, between female Han and Uyghur college students. Differences in genotype distributions were observed, between the Han and Uyghur participants, for both SNPs. This was consistent with other studies that have shown population differences in the distribution of genotypes (16, 17), which were consistent with the Hardy-Weinberg equilibrium within a population. For example, the AA genotype of the *Apa I* SNP in the vitamin D receptor (*VDR*) gene was found in 7.8% of Chinese Han women (18), 15.1% of Japanese women (19), 36% of an Indian population (20). Anthropological research has demonstrated that the gene structure of Uyghur subjects contains Caucasian and Mongolian ancestry (21, 22). These gene fusion characteristics may explain the differences observed in the genotype distributions and BMD phenotype, when compared with the Han Chinese.

BMD is related to hormonal balance, aging, environmental factors, life style and genetic predisposition. These causes account for 50–80% of individual variability in bone mass. Familial and twin studies have estimated that 60–80% of the variance in PBMD is due to genetic factors (23). Our investigation showed that adequate vitamin D intake, drinking milk, rs1298989 genotype, regular exercise and adequate energy intake were factors that can be used to predict increased BMD in the lumbar spine, in female college students. Although the genotype of rs1298989 and rs3762397 affected the PBMD, they did not show a large effect. It may be due to existence of multiple genes and their interactions effect on PBMD (3, 24).

Our research showed that the factor with the greatest impact on BMD in the lumbar spine was vitamin D intake. Vitamin D is a secosteroid hormone that is essential for calcium absorption and bone mineralization, which are positively associated with BMD. A number of studies have investigated the relationship between adequate calcium intake and BMD (25, 26). Vitamin D deficiency leads to decreased calcium absorption and ultimately the release of calcium from the bones, in order to maintain circulating calcium concentrations (27).

Xinjiang is located in Northwest China, which is a livestock area. Uyghur is the main minority group and the ethnic culture, religion, lifestyle and dietary customs are different from those of the Han people. The Uyghur diet consists mainly of meat (lamb and beef), milk, cooked wheaten food and dairy products. It is high in calories, fat, spices, strong flavors, strong and milky tea (milky tea is the conventional drink of the Uyghur). In contrast, the Han Chinese population in the same region eats mainly vegetables, fish, pork, eggs and rice. This study showed that total calcium intake, energy intake, habitual tea consumption and habitual milk consumption were significantly different between the Han and Uyghur populations.

Our data showed that the second variable, in terms of impact on BMD in the lumbar spine, was the habit of drinking milk. The presence of minerals and macronutrients in milk, in addition to calcium, may promote bone mineralization in adoles-

cent girls or young women. Studies have shown that adequate calcium intake during childhood and adolescence is vital for BMD (28, 29). Esterle et al. (28) investigated 192 unrelated, healthy adolescent girls and young women and found that milk consumption, in preference to other calcium sources, is associated with lumbar bone mineral content (BMC) and BMD.

Exercise that involves the loading of bone also has a major impact on bone mass (30, 31). Nilsson et al. (32) showed that increased physical activity is related to an advantageous development of BMD and cortical bone size, which indicated that exercise is important for the optimization of PBMD in young men. However, there was no statistical difference in the levels of regular exercise between Han and Uyghur subjects. The natural instinct of the Uyghur is to exercise; mainly running and dancing. This caused the Uyghur subjects to use more energy than the Han subjects, who are relatively quiet, introverted and like to play badminton, walk and do yoga.

There were some limitations in this study. Firstly, there are more daylight hours in Xinjiang, than hours of darkness, and cold, dry conditions are the main climatic features that differ from inland areas. Vitamin D plays an important role in skeletal development and maintenance, and it is generally accepted that serum 25(OH) D reflects vitamin D status. Healthy adults obtain most of their vitamin D through exposure of skin to sunlight, with natural dietary sources being limited (33, 34). The levels of sunshine and 25(OH) D levels were not measured in this study. These environmental factors may affect the PBMD. Secondly, BMD is correlated with genetic pedigree. We did not collect familial information or analyze the relevant factors, especially the information of their mothers, it should be estimated in the further study. In addition, not all potential confounding factors, such as estrogen hormone, were assessed and measured.

Conclusion

This study showed that the rs1298989 polymorphism of the *CATSPERB* gene and rs3762397

polymorphism of the *NR5A2* gene were associated with PBMD in Han and Uyghur populations. It also demonstrated the correlations between BMD and variables such as adequate vitamin D intake, drinking milk, genotype, regular exercise and adequate energy intake. These variables were the main predictors of increased BMD in the lumbar spine of female college students. Genotypes of rs1298989 and rs3762397 did not have a strong effect on BMD.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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