Bone 76 (2015) 18–22

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Contributions of Caucasian-associated bone mass loci to the variation in bone mineral density in Vietnamese population

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ARTICLE INFO

Article history:
Received 21 September 2014
Revised 3 February 2015
Accepted 3 March 2015
Available online 11 March 2015

Edited by B. Langdahl

Keywords:
Osteoporosis
Bone mineral density
Single nucleotide polymorphism
MBL2/DKK1 gene
SP7 gene
ZBTB40 gene

ABSTRACT

Background and aim: Bone mineral density (BMD) is under strong genetic regulation, but it is not clear which genes are involved in the regulation, particularly in Asian populations. This study sought to determine the association between 29 genes discovered by Caucasian-based genome-wide association studies and BMD in a Vietnamese population.
Methods: The study involved 564 Vietnamese men and women aged 18 years and over (average age: 47 years) who were randomly sampled from the Ho Chi Minh City. BMD at the femoral neck, lumbar spine, total hip and whole body was measured by DXA (Hologic QDR4500, Bedford, MA, USA). Thirty-two single nucleotide polymorphisms (SNPs) in 29 genes were genotyped using Sequenom MassARRAY technology. The magnitude of association between SNPs and BMD was analyzed by the linear regression model. The Bayesian model average method was used to identify SNPs that are independently associated with BMD.
Results: The distribution of genotypes of all, but two, SNPs was consistent with the Hardy–Weinberg equilibrium law. After adjusting for age, gender and weight, 3 SNPs were associated with BMD: rs2016266 (SP7 gene), rs7543680 (ZBTB40 gene), and rs1373004 (MBL2/DKK1 gene). Among the three genetic variants, the SNP rs2016266 had the strongest association, with each minor allele being associated with ~0.02 g/cm^2 increase in BMD at the femoral neck and whole body. Each of these genetic variant explained about 0.2 to 1.1% variance of BMD. All other SNPs were not significantly associated with BMD.
Conclusion: These results suggest that genetic variants in the SP7, ZBTB40 and MBL2/DKK1 genes are associated with BMD in the Vietnamese population, and that the effect of these genes on BMD is likely to be modest.

Introduction

Osteoporosis is a skeletal disorder characterized by reduced bone strength and compromised bone microarchitecture, leading to an increased risk of fragility fracture [1]. Bone strength is largely determined by bone mineral density (BMD). Indeed, variation in BMD accounted for more than 70% of variation in bone strength [2]. Low BMD is also recognized as the most important risk factor for fracture, such that each standard deviation decrease in BMD measured at the femoral neck is associated with 2 to 3-fold increase in fracture risk [3]. This magnitude of association is equivalent to that of the association between blood pressure and the risk of cardiovascular diseases [3]. Therefore, femoral neck BMD has been used as a tool for the diagnosis of osteoporosis [4].

Numerous studies during the past three decades have consistently shown that BMD is under strong genetic effect. Twin studies showed that between 70 and 80% of variance in BMD is attributable to genetic factors [5–7], making BMD one of the most heritable traits in human. However, it is a considerable challenge to identify specific genes that are linked to BMD, because it is expected that there exists many genes that are involved in the genetic regulation. Genome-wide association study (GWAS) is a powerful hypothesis-free approach for identifying genes. Three meta-analyses of GWAS, mostly in European descents, showed that variants in the ZBTB40, ESR1, LRP5, TNFSF11, TNFRSF11A and TNFRSF11B genes were associated with BMD [8–10]. Moreover, results from a GWAS in the Korean population yielded two additional new loci (i.e. FAM3C and SFRP4) that are associated with BMD variation [11]. Other GWAS in the Chinese population reported that variants in the JAG1 and ALDH7A1 genes were associated with BMD [12,13].

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http://dx.doi.org/10.1016/j.bone.2015.03.003
8756-3282/

Genetic findings, even from GWAS, require independent validation. It is not clear whether the association between genetic variants and BMD in Caucasians is also present in Asian populations (or vice versa). Moreover, very few studies have identified genes influencing on BMD variation in populations other than Caucasian populations. Indeed, there have been no studies on the genetics of osteoporosis in the Vietnamese population who may have different geographic characteristics of ancestry [14–16]. Thus, in this study, we sought to investigate whether BMD-related genetic variants previously reported in GWAS on populations with European ancestry are associated with BMD in the Vietnamese population.

Study design and methods

Study design and participants

The study was designed as a cross-sectional investigation, with the setting being Ho Chi Minh City, a major city of Vietnam. The recruitment procedure has been described previously [17]. Briefly, we approached community organizations, including church and temples, and obtained the list of members aged 18 years and above. In the next step, we used simple random sampling technique to identify potential participants. We sent a letter of invitation to the selected individuals. Approximately 5% did not respond to our letter of invitation, thus we contacted them via phone. The participants did not receive any financial incentive, but they received a free health check-up, and lipid analyses. Participants were excluded if they reported with diseases deemed to affect bone metabolism such as hyperthyroidism, hyperparathyroidism, renal failure, malabsorption syndrome, alcoholism, chronic colitis, multiple myeloma, leukemia and chronic arthritis.

The research protocol and study procedures were approved by the Medical Ethics Committees of the People's Hospital 115 and Pham Ngoc Thach University of Medicine. All volunteer participants were provided full information about the study's purpose and gave informed consent prior to participating in the study.

Measurements and data collection

Data collection was conducted by trained research doctors and nurses using a structured questionnaire. The questionnaire solicited information concerning anthropometry, lifestyle factors, dietary intakes, physical activity and clinical history. Anthropometric parameters including age, weight, and standing height were obtained. Body weight was measured on an electronic scale with indoor clothing without shoes. Height was determined without shoes on a portable stadiometer with mandible plane parallel to the floor. Body mass index (BMI) was calculated as weight in kg over height in meter squared.

Each participant was asked to provide information on current and past smoking habits. Alcohol intake in average numbers of standard drinks per day, at present as well as within the last 5 years, was obtained. Clinical data including blood pressure, pulse, reproductive history (i.e. parity, age of menarche and age of menopause), and medical history (i.e. previous fracture, previous and current use of pharmacological therapies) were also obtained.

Bone mineral density measurement

Areal BMD was measured at the lumbar spine, femoral neck, total hip and whole body using a Hologic QDR 4500 (Hologic Corp, Bedford, MA, USA). For the lumbar spine, we measured BMD from L2 to L4. The densitometer was standardized by phantom before each measurement. The measurement was done by a qualified radiology technologist. Based on 20 individuals, the coefficient of variation in BMD at our lab was 1.8% for the lumbar spine and 1.5% for the hip.

Marker selection and genotyping

Thirty-two single nucleotide polymorphisms (SNPs) were selected from previous studies which were identified to be associated with BMD in populations with European ancestry (Table S1). The SNPs were selected based on their strength of association with BMD, P-value, and allelic frequency. Multiplexed assays were designed for all SNPs using the Sequenom MassARRAY Assay Design software (version 4.0.0.2). SNPs were genotyped using iPLEX™ Gold chemistry and analyzed using a Sequenom MassARRAY Compact Mass Spectrometer (Sequenom Inc., San Diego, CA, USA). All reactions were carried out using standard conditions. The post-PCR products were spotted on a Sequenom SpectroChip 2, and the data was processed and analyzed using Sequenom MassARRAY TYPER 4.0.20.65 software.

Sample size and statistical analysis

Sample size determination was formally determined prior to the study. Based on results of previous meta-analysis of GWAS [8–10], we assumed that the effect size (i.e. difference in BMD between genotypes in relation to its standard deviation) is varied between 0.03 and 0.05 which is considered clinically relevant. We estimated that a sample size of between 350 and 550 should have at least 80% chance to detect the effect size at the significance level of 5%.

The allelic frequency distribution of each SNP was tested for deviation from the expected frequency by the Hardy–Weinberg equilibrium (HWE) law using the "HardyWeinberg" package in R [18]. Two SNPs were excluded from the analysis because the P-value from the test was less than 0.05. We reported minor allele frequency (MAF) for each study SNP to enable the comparison with its frequency in European or Chinese descents (available at http://www.ncbi.nlm.nih.gov).

The linear regression model was used to analyze the association between each SNP and BMD. The SNP's genotypes were coded as the number of minor alleles (e.g., 0, 1, and 2). Therefore, the regression coefficient associated with each SNP can be interpreted as the effect size. We performed the analysis separately for BMD measured at the lumbar spine, femoral neck, total hip and whole body. Age, sex and weight, which are known to be strong determinants of BMD, were included in the model as covariates in the multivariate linear regression model.

In the presence of multiple genetic variants, the selection of relevant variants is a challenge, because there are many competing models. For 30 SNPs, the number of possible models can be more than 1 billion (e.g. 2^{30}). In this study, we used the Bayesian model average (BMA) approach [19] to identify genetic variants that were associated with BMD. This approach has been shown to have superior performance compared to "traditional" approaches such as stepwise regression [20,21]. If $M = (M_1, M_2, M_3, \ldots, M_k)$ denotes the set of all possible models considered, the idea is to find the "optimal" models in that space. In the Bayesian approach, the idea is to find the posterior probability distribution of a model M_k or a set of models that optimally explains the data. This posterior probability is actually a function of a prior distribution of M_k and marginal distribution of actual data. Prior probability distribution is assigned to the model parameters which include β and σ^2, and the model M_k. We assume that the model M_k has a prior probability of $P(M_k)$, and that the vector of model parameters is generated from the conditional distribution of $\sigma^2|M_k \sim P(\sigma^2|M_k)$ and $\beta_w|\sigma^2, M_k \sim P(\beta_w|M_k, \sigma^2)$, where $w_1, w_2, w_3, \ldots, w_p$ is a vector of 1 and 0 indicating the inclusion or exclusion of SNPs in model M_k. The posterior probability of model M_k can be written as: $P(M_k|D) = \frac{P(D|M_k)\times P(M_k)}{\sum_{k=0}^{p} P(D|M_k) \times P(M_k)}$, where $P(D|M_k)$ is the marginal distribution of the data D under model M_k. In this study, given the large number of genetic variant and there is little information available for eliciting prior distributions, we used

the "uninformative" prior distributions, that a priori, make all models and parameters equally likely are appealing. BMA produces a posterior probability of each possible model and posterior probability for regression coefficient associated with each genetic variant. The "best" model was identified if it has the posterior probability of at least 1/C, where C was set to 20 [22]. The analysis was done with the R statistical environment [23] and the BMA package [24].

Results

The study involved 564 individuals; however, 3 individuals were excluded from the analysis because they had more than 8 SNPs (25% of total SNPs) with missing data. Ultimately, data from 180 men and 381 women were used in the analysis. Baseline characteristics of all participants stratified by gender are shown in Table 1. Sixty-eight percent of participants were women. The average age of all participants was 47 years (SD 17). As expected, the proportion of current smokers in men (54%) was higher than in women (0.5%). Also, more men reported to be alcohol users than women (54% vs 5%). There was no significant difference in BMI between genders. However, men had significantly greater BMD (by between 5 and 10%) than women in all skeletal sites.

The distribution of genotypes of all SNPs, except for rs2010281 (MARK3) and rs87938 (CTNNB1), was consistent with the Hardy–Weinberg (HW) equilibrium law ($P > 0.05$). The two SNPs deviated from the HW law were excluded from subsequent analyses. A list of SNPs and their genotypic frequency distribution is shown in Table S1.

Table 2 presents the two "best" models identified by the BMA analysis for each BMD outcome. After adjusting for age, gender, and body weight, we identified 3 genetic variants that were associated with BMD: rs2016266 (SP7 gene), rs7543680 (ZBTB40 gene), and rs1373004 (MBL2/DKK1 gene). However, not all three genetic variants were associated with all BMDs, but there were BMD-specific variants. For example, for whole body BMD, the best genetic variant was rs2016266, with each minor allele being associated with a 0.025 (SE 0.007) g/cm^2 in BMD; the second most parsimonious model included both rs2016266 and rs7543680 variants. For total hip and femoral neck BMDs, rs2016266 or rs1373004 was found to be associated with BMD. For lumbar spine BMD, a model with rs7543680 and a model with rs2016266 were found to have equal contribution to the variation in BMD. Each of the three SNP accounted for between 0.2 and 1.8% total variance in BMD.

Discussion

Bone mineral density is the most important predictor of fracture, and is used as a surrogate to define "osteoporosis". Although it has long been known that genetic factors play an important role in the determination of bone density, it is a challenge to identify specific loci or genes that are linked to variation in this phenotype. A number of GWAS have identified several genetic variants that were associated with variation in bone density in Caucasian populations [25,26]. In this study, we analyzed the association between those genetic variants and BMD in a cohort of Vietnamese adults, and found that only 3 variants (i.e. rs2016266, rs7543680, and rs1373004) were significantly associated with BMD. The association was independent of age, gender and weight.

Genetic susceptibility to BMD is complex with only few loci have been replicated in GWAS. A meta-analysis pooling five GWAS confirmed the association between genetic variants in SP7, ZBTB40, ESR1, LRP5, RANKL and RANK genes and BMD [9]. Another meta-analysis found similar relationship with ESR1, LRP5, RANKL and RANK but not for other genes [8]. Different findings between large-scale GWAS and the subsequent meta-analyses could be due to the effect of population stratification or heterogeneity in phenotypes. In this study, out of 32 SNPs validated, only 3 SNPs were significantly associated with BMD in the Vietnamese population. It is interesting to note that two recent validation studies also found that the rate of replication of Caucasian associated SNPs in Korean populations was low [27–29]. The lack of association for the majority of tested SNPs could likely be due to inadequate power (i.e., modest sample size) in the present study. Indeed, most of the observed effect sizes were lower than the effect size that we assumed in the determination of sample size. Based on our results, the real effect sizes are likely between 0.01 and 0.02 g/cm^2 per minor allele. This effect size should serve as a referent point for future validation studies.

Of the three genes found to be associated with BMD in this population, the SP7 gene was the most robust predictor. The SP7 gene, also known as Osterix gene (on 12q13) is involved in the regulation of osteoblast differentiation [30]. The gene was originally found to be associated with lumbar spine BMD [31] and whole body BMD [32] in Caucasian populations. The direction and magnitude of association in this study are concordant with those observed in Korean populations.

The rs1373004 is located in the downstream of the MBL2 gene (mannose-binding lectin 2 gene) and the DKK1 gene (dickkopf WNT signaling pathway inhibitor 1). MBL2 and DKK1 genes are relatively "new" in osteoporosis research in the sense that they were only identified a few years ago. While there is no published data concerning the function of MBL2 gene on bone metabolism, the gene has been reported as a gene encoding protein associated with infectious conditions [33]. Because the pathogenesis of osteoporosis is involved with inflammatory characteristics [34] and MBL protein is considered a modifier of inflammatory response, there may be a biological reason for the relationship between heterogeneity in the MBL2 gene which could alter its serum concentration and osteoporosis [35]. This is, of course, a hypothesis that remains to be tested in future studies.

Nevertheless, the mechanism for the association between DKK1 gene and BMD is largely unknown. It is known that LRP5 is a critical regulator of bone mass, and that DKK proteins are involved in the regulation. In mice, it has been shown that DKK1 protein is a negative regulator of osteoblasts, and that DKK1 expression can induce significant increase in bone mass [36]. In a study on patients with rheumatoid arthritis, it was noted that up-regulated gene expression pattern of the DKK1 gene was associated with bone fragility [37]. Thus, although the mechanism of the association between MBL2/DKK1 gene and BMD is not clear, the association appears to have biological basis.

The direction of association between MBL2 gene and BMD in this cohort is in contrast with a previous GWAS study. In this cohort, we found that the minor allele of the SNP rs1373004 was associated with greater BMD, and this was in contrast with a previous meta-analysis which found that the minor allele of the gene was associated with lower BMD and increased risk of low-trauma fracture [10]. Another

Table 1
Characteristics of participants.

	Men (N = 180)	Women (N = 381)	P value
Age (year)	45.4 (18.3)	47.9 (16.7)	0.10
Weight (kg)	61.1 (9.5)	52.4 (9.4)	<0.0001
Height (cm)	164.4 (6.8)	153.5 (5.4)	<0.0001
BMI (kg/m^2)	22.6 (3.3)	22.2 (4.0)	0.24
Femoral neck BMD (g/cm^2)	0.73 (0.15)	0.67 (0.13)	<0.0001
Total hip BMD (g/cm^2)	0.91 (0.15)	0.82 (0.13)	<0.0001
Lumbar spine BMD (g/cm^2)	0.91 (0.15)	0.87 (0.16)	0.001
Whole body BMD (g/cm^2)	1.05 (0.10)	0.98 (0.11)	<0.0001
Current smoker, n (%)			
No	81 (45.8)	379 (99.5)	
Yes	96 (54.2)	2 (0.5)	<0.0001
Alcohol use, n (%)			
No	81 (45.8)	364 (95.5)	
Yes	96 (54.2)	17 (4.5)	<0.0001

Notes: Values were mean (standard deviation) or n (%) as specified. BMD: bone mineral density; BMI: body mass index.

Table 2
Association between genetic variants at the SP7, ZBTB40 and MBL2 genes, and measures of BMD.

Outcome	Model	SNP (gene)	Regression coefficient (SE) (g/cm^2)	P-value	Coefficient of determination (%)
Femoral neck BMD	I	rs1373004 (MBL2)	0.015 (0.006)	0.029	1.8
	II	rs2016266 (SP7)	0.015 (0.007)	0.034	0.2
Total hip BMD	I	rs2016266 (SP7)	0.020 (0.008)	0.022	0.4
	II	rs1373004 (MBL2)	0.017 (0.008)	0.024	1.7
Lumbar spine BMD	I	rs2016266 (SP7)	0.024 (0.009)	0.015	0.4
	II	rs7543680 (ZBTB40)	−0.018 (0.009)	0.042	0.1
		rs2016266 (SP7)	0.024 (0.009)	0.015	0.4
Whole body BMD	I	rs2016266 (SP7)	0.025 (0.007)	0.0005	1.1
	II	rs2016266 (SP7)	0.025 (0.007)	0.0004	1.1
		rs7543680 (ZBTB40)	−0.015 (0.006)	0.026	0.2

study on the Southern Chinese population also found that the minor allele of rs1373004 (frequency 19%) was associated with lower BMD [38]. While it is not clear why there was difference between our finding and the previous finding, such a difference could reflect a gene–ethnicity interaction effect. The meta-analysis was largely based on Caucasian populations, among whom the minor allele frequency of rs1373004 was about 12% [10]. In our population, the minor allele frequency of rs1373004 was 23%. In other words, the effect of the gene on BMD could be dependent on ethnicity.

We also observed a significant association between rs7543680 (ZBTB40 gene) and BMD, and to our knowledge, this is the second finding in an Asian population. A recent validation study in a Korean population [29] also found that the minor allele of this gene was significantly associated with lower BMD at the spine and femoral neck. This association was first observed in Caucasian populations [25] and validated in another study [39], but the underlying mechanism of this association is still unknown.

It is increasingly clear that the variation in BMD is determined by multiple genes, but it is not known how many genes are involved. However, all SNPs identified so far explained less than 10% of total variation in BMD in Caucasian populations [10], raising the issue of "missing heritability". It should be noted that GWAS assumes that multiple genetic variants, each will small effect size, are associated with BMD. There is a possibility that rare variants with large effect sizes contribute to the variation in BMD. In the present study, a single genetic variant rs1373004 explained ~2% of variation in BMD. Although this effect size is very modest, it suggests that there are other genes, including rare genes, remained to be identified in this population. Current technology (such as next generation sequencing technology) can help unravel the putative BMD-associated genes in this population.

The present study's finding should be considered within the context of strengths and weaknesses. We used candidate gene approach to directly test the variation in genes identified to be involved in the pathogenesis of osteoporosis which might decrease the possibility of chance findings. Traditionally, the selection of "optimal" models are done with the stepwise regression method, in which SNPs are added one at a time (i.e. forward elimination), or SNPs are removed one at a time (i.e. backward elimination), and the final result is a single "best" model. However, stepwise regression method has been demonstrated to yield misleading and/or false positive results [40]. In this study, we used the Bayesian model average approach which has been shown to have better performance than the traditional stepwise method [20,21] in terms of identification of relevant variables in the regression model. The Bayesian approach produces a posterior distribution for a model and parameters of the model, and there is no need for adjustment for multiple tests of hypothesis.

In summary, the present study suggests that there is a modest association between genetic variants in the SP7, MBL2, and ZBTB40 genes and BMD in the Vietnamese population. These results suggest that Caucasian and Vietnamese populations have some common genetic risk factors for osteoporosis.

Author contributions

Conceived and designed the experiments: LTH-P, TVN. Performed the experiments: LTH-P, SCN, BT, TVN. Analyzed the data: BT, TVN, LTH-P. Contributed reagents/materials/analysis tools: BT, SCN. Wrote the paper: LTH-P, BT, TVN.

Funding

The study was partially supported by a grant from the Department of Science and Technology, Ho Chi Minh City (grant number 211/TB-SKHCN), and a grant from the University Commission for Development (CUD) program, Belgium. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgments

We thank the following friends and colleagues for their support and help in the recruitment and providing logistic support for the study: Fr. Pham Ba Lam, Fr. Vu Minh Danh, Mr. Pham Doan Phong, Mr. Luong Thang Phat, Mr. Nguyen Cong Phu, and Mr. Tien Ngoc Tuan. We thank Dr. Le Thi Ngoc Linh, Dr. Pham Ngoc Khanh of the People's Hospital 115; and our medical students Nguyen Thi Thanh Mai, Nguyen Hai Dang, Vo Thi Thuy An, Nguyen Thi Thanh Thao, Mai Duy Linh, Nguyen Vu Dat, Diem Dang Khoa, and Tran Hong Bao for their assistance in the interview of participants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2015.03.003.

References

[1] NIH consensus development panel on osteoporosis. Osteoporosis prevention, diagnosis, and therapy. JAMA Feb 14 2001;285(6):785–95.
[2] Wachter NJ, Krischak GD, Mentzel M, et al. Correlation of bone mineral density with strength and microstructural parameters of cortical bone in vitro. Bone Jul 2002; 31(1):90–5.
[3] Partington GA, Fuller K, Chambers TJ, Pondel M. Mitf–PU.1 interactions with the tartrate-resistant acid phosphatase gene promoter during osteoclast differentiation. Bone Feb 2004;34(2):237–45.
[4] Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet Jun 1 2002;359(9321):1929–36.
[5] Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest Sep 1987;80(3):706–10.
[6] Arden NK, Baker J, Hogg C, Baan K, Spector TD. The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. J Bone Miner Res Apr 1996;11(4):530–4.

[7] Young D, Hopper JL, Nowson CA, et al. Determinants of bone mass in 10- to 26-year-old females: a twin study. J Bone Miner Res Apr 1995;10(4):558–67.
[8] Richards JB, Kavvoura FK, Rivadeneira F, et al. Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture. Ann Intern Med Oct 20 2009;151(8):528–37.
[9] Rivadeneira F, Styrkarsdottir U, Estrada K, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet Nov 2009;41(11):1199–206.
[10] Estrada K, Styrkarsdottir U, Evangelou E, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet May 2012;44(5):491–501.
[11] Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet May 2009;41(5):527–34.
[12] Kung AW, Xiao SM, Cherny S, et al. Association of JAG1 with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies. Am J Hum Genet Feb 12 2010;86(2):229–39.
[13] Guo Y, Tan LJ, Lei SF, et al. Genome-wide association study identifies ALDH7A1 as a novel susceptibility gene for osteoporosis. PLoS Genet Jan 2010;6(1):e1000806.
[14] Chu JY, Huang W, Kuang SQ, et al. Genetic relationship of populations in China. Proc Natl Acad Sci U S A Sep 29 1998;95(20):11763–8.
[15] Ivanova R, Astrinidis A, Lepage V, et al. Mitochondrial DNA polymorphism in the Vietnamese population. Eur J Immunogenet Dec 1999;26(6):417–22.
[16] Ballinger SW, Schurr TG, Torroni A, et al. Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient mongoloid migrations. Genetics Jan 1992;130(1):139–52.
[17] Ho-Pham LT, Nguyen UD, Pham HN, Nguyen ND, Nguyen TV. Reference ranges for bone mineral density and prevalence of osteoporosis in Vietnamese men and women. BMC Musculoskelet Disord 2011;12:182.
[18] Graffelman J. HardyWeinberg: graphical tests for Hardy–Weinberg equilibrium. R package version 1.5.2; 2013.
[19] Hoeting JA, Madigan D, Raftery AE, Volinsky CT. Bayesian model averaging: a tutorial. Statist Sci 1999;14(4):382–417.
[20] Wang D, Zhang W, Bakhai A. Comparison of Bayesian model averaging and stepwise methods for model selection in logistic regression. Stat Med Nov 30 2004;23(22):3451–67.
[21] Genell A, Nemes S, Steineck G, Dickman PW. Model selection in medical research: a simulation study comparing Bayesian model averaging and stepwise regression. BMC Med Res Methodol 2010;10:108.
[22] Volinsky CT, Madigan D, Raftery AE, Kronmal RA. Bayesian model averaging in proportional hazard models: assessing the risk of a stroke. Appl Stat 1997;48(4):433–48.
[23] Colon-Emeric CS, Pieper CF, Artz MB. Can historical and functional risk factors be used to predict fractures in community-dwelling older adults? Development and validation of a clinical tool. Osteoporos Int Dec 2002;13(12):955–61.
[24] Raftery A, Hoeting J, Volinsky C, Painter I, Yeung KY. BMA: Bayesian model averaging. R package version 3.17.1; 2014.
[25] Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, et al. Multiple genetic loci for bone mineral density and fractures. N Engl J Med May 29 2008;358(22):2355–65.
[26] Richards JB, Rivadeneira F, Inouye M, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet May 3 2008;371(9623):1505–12.
[27] Kim YA, Choi HJ, Lee JY, Han BG, Shin CS, Cho NH. Replication of Caucasian loci associated with bone mineral density in Koreans. Osteoporos Int Oct 2013;24(10):2603–10.
[28] Ham S, Roh TY. A follow-up association study of genetic variants for bone mineral density in a Korean population. Genome Inform Sep 2014;12(3):114–20.
[29] Park SE, Oh KW, Lee WY, et al. Association of osteoporosis susceptibility genes with bone mineral density and bone metabolism related markers in Koreans: the Chungju Metabolic Disease Cohort (CMC) study. Endocr J Nov 28 2014;61(11):1069–78.
[30] Baek WY, Lee MA, Jung JW, et al. Positive regulation of adult bone formation by osteoblast-specific transcription factor osterix. J Bone Miner Res Off J Am Soc Bone Miner Res Jun 2009;24(6):1055–65.
[31] Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, et al. New sequence variants associated with bone mineral density. Nat Genet Jan 2009;41(1):15–7.
[32] Timpson NJ, Tobias JH, Richards JB, et al. Common variants in the region around Osterix are associated with bone mineral density and growth in childhood. Hum Mol Genet Apr 15 2009;18(8):1510–7.
[33] Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO. Mannose-binding lectin and its genetic variants. Genes Immun Mar 2006;7(2):85–94.
[34] Ginaldi L, Di Benedetto MC, De Martinis M. Osteoporosis, inflammation and ageing. Immun Ageing Nov 4 2005;2:14.
[35] Kiseljakovic E, Hasic S, Valjevac A, et al. Association of mannose-binding lectin 2 (mbl2) gene heterogeneity and its serum concentration with osteoporosis in postmenopausal women. Bosn J Basic Med Sci Feb 2014;14(1):25–9.
[36] Morvan F, Boulukos K, Clement-Lacroix P, et al. Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. J Bone Miner Res Jun 2006;21(6):934–45.
[37] Caetano-Lopes J, Rodrigues A, Lopes A, et al. Rheumatoid arthritis bone fragility is associated with upregulation of IL17 and DKK1 gene expression. Clin Rev Allergy Immunol Aug 2014;47(1):38–45.
[38] Xiao SM, Kung AW, Sham PC, Tan KC. Genetic analysis of recently identified osteoporosis susceptibility genes in southern Chinese. J Clin Endocrinol Metab Nov 2013;98(11):E1827–34.
[39] Duncan EL, Danoy P, Kemp JP, et al. Genome-wide association study using extreme truncate selection identifies novel genes affecting bone mineral density and fracture risk. PLoS Genet Apr 1983;7(4):e1001372.
[40] Freedman DA. A note on screening regression equations. Am Stat 1983;37(8):152–5.