Osteoporos Int (2010) 21:579–587
DOI 10.1007/s00198-009-0998-7

ORIGINAL ARTICLE

Genome-wide copy number variation association study suggested *VPS13B* gene for osteoporosis in Caucasians

F.-Y. Deng · L.-J. Zhao · Y.-F. Pei · B.-Y. Sha · X.-G. Liu · H. Yan · L. Wang · T.-L. Yang · R. R. Recker · C. J. Papasian · H.-W. Deng

Received: 22 March 2009 / Accepted: 29 May 2009 / Published online: 13 August 2009

Abstract
Summary Osteoporotic fracture (OF) is a serious outcome of osteoporosis. Important risk factors for OF include reduced bone mineral density and unstable bone structure. This genome-wide copy number variation association study suggested *VPS13B* gene for osteoporosis in Caucasians.
Introduction Bone mineral density (BMD) and femoral neck cross-sectional geometric parameters (FNCSGPs) are under strong genetic control. DNA copy number variation (CNV) is an important source of genetic diversity for human diseases. This study aims to identify CNVs associated with BMD and FNCSGPs.
Methods Genome-wide CNV association analyses were conducted in 1,000 unrelated Caucasian subjects for BMD at the spine, hip, femoral neck, and for three FNCSGPs —cortical thickness (CT), cross-section area (CSA), and buckling ratio (BR). BMD was measured by dual energy X-ray absorptiometry (DEXA). CT, CSA, and BR were estimated using DEXA measurements. Affymetrix 500K arrays and copy number analysis tool was used to identify CNVs.
Results A CNV in *VPS13B* gene was significantly associated with spine, hip and FN BMDs, and CT, CSA, and BR ($p<0.05$). Compared to subjects with two copies of the CNV, carriers of one copy had an average of 14.6%, 12.4%, and 13.6% higher spine, hip, and FN BMD, 20.0% thicker CT, 10.6% larger CSA, and 12.4% lower BR. Thus, a decrease of the CNV consistently produced stronger bone, thereby reducing osteoporotic fracture risk.
Conclusions *VPS13B* gene, via affecting BMD and FNCSGPs, is a novel osteoporosis risk gene.

Keywords Bone geometry · Bone mineral density · Copy number variation · Osteoporosis

Fei-Yan Deng and Lan-Juan Zhao equally contributed to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00198-009-0998-7) contains supplementary material, which is available to authorized users.

F.-Y. Deng · Y.-F. Pei · T.-L. Yang · C. J. Papasian · H.-W. Deng (✉)
School of Medicine, University of Missouri—Kansas City,
Kansas City 64108 MO, USA
e-mail: dengfe@umkc.edu

L.-J. Zhao · R. R. Recker
Osteoporosis Research Center, Creighton University,
Omaha 68131 NE, USA

Y.-F. Pei · X.-G. Liu · H. Yan · L. Wang · H.-W. Deng
The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics,
School of Life Science and Technology,
Xi'an Jiaotong University,
Xi'an, Shannxi 710049, People's Republic of China

B.-Y. Sha · H.-W. Deng
Laboratory of Molecular and Statistical Genetics,
College of Life Sciences, Hunan Normal University,
Changsha,
Hunan 410081, People's Republic of China

Introduction

Osteoporosis is a common skeletal disease in the elderly. The most serious outcome of osteoporosis is osteoporotic fracture (OF) [1]. Bone mineral density (BMD) is a major determinant of both osteoporosis and OF [2] and is commonly used by geneticists to search for genes underlying osteoporosis due to its high heritability (up to 70%). To date, no more than 10% of the total genetic variance in BMD has been explained by identified candidate genes. Consequently, the major genetic factors contributing to BMD variation remain to be identified [3].

Hip fracture is one of the most common OF and is generally considered to be the most severe OF due to its high associated morbidity, mortality [4], and therapeutic cost [5]. An important risk factor for hip fracture is reduced bone strength at the proximal femur [6], which is largely determined by poor femoral neck bone geometry and low BMD [7]. Femoral neck cross-sectional geometric parameters (FNCSGPs) are highly correlated with BMD and play an important role in determining bone strength or OF risk, independent of BMD [8, 9]. FNCSGPs are under strong genetic control, with heritability ranging from 30% to 70% [10, 11]. Although several linkage and association studies have been conducted [10, 12–14], genetic factors responsible for variations in FNCSGPs warrant further investigation.

Copy number variation (CNV) represents a form of structural genomic variation in which the number of copies of a DNA segment, ranging from 1 kb to several Mb, varies in different individuals when compared to a reference genome [15]. According to a study of 270 individuals from four populations with ancestry in Europe, Africa, or Asia, CNVs are widespread, covering approximately 12% (360 Mb) of the human genome [16]. CNVs have been shown to account for nearly 18% of the variation in gene expression, and consequently, it is reasonable to explore the possibility that CNVs play an important role in determining complex traits [17]. Support for this perspective comes in the form of evidence showing that CNVs are associated with certain complex human diseases such as progression of HIV infection, selected autoimmune diseases, and psychiatric disorder autism [18–20]. Recently, a genome-wide CNV association study on hip OF in Chinese suggested the importance of a deletion variant of UGT2B17 gene for pathogenesis of osteoporosis [21].

In the current investigation, we performed a genome-wide association study in Caucasians to identify CNVs that are significantly associated with variations in either BMD or FNCSGPs.

Methods

Research subjects

The study was approved by the necessary Institutional Review Board or Research Administration of involved institutions. Signed informed-consent documents were obtained from all study participants before they enrolled in the study. In total, 1,000 adult US Caucasian subjects (499 men and 501 women) were randomly selected from our established and expanding genetic repertoire, currently containing >6,000 subjects. All 1,000 subjects were self-identified as being of European origin living in Omaha, Nebraska and its surrounding areas. They averaged 50.21± 18.28 years in age, 1.71±0.10 m in height and 80.13± 17.80 kg in weight.

Subjects with chronic diseases and conditions that might potentially affect bone mass, structure, or metabolism were excluded. These diseases and conditions included chronic disorders involving vital organs (heart, lung, liver, kidney, and brain), serious metabolic diseases (diabetes, hypo- and hyperparathyroidism, hyperthyroidism, etc.), other skeletal diseases (Paget disease, osteogenesis imperfecta, rheumatoid arthritis, etc.), chronic use of drugs affecting bone metabolism (hormone replacement therapy, corticosteroid therapy, anti-convulsant drugs), malnutrition conditions (such as chronic diarrhea, chronic ulcerative colitis, etc.), etc. In addition, subjects taking anti-bone-resorptive or bone anabolic agents/drugs such as bisphosphonates were also excluded from this study. The purpose of the above exclusion criteria was to minimize the effect of any known environmental and/or therapeutic factors that influence bone phenotypic variation, which should correspondingly provide for augmentation of genetic effects, relative to environmental effects, in the study sample, and consequently increase statistical power for detecting the genetic factors.

BMD measurements

Areal BMD (grams per squares centimeter) at the spine and hip and areal bone size at femoral neck (FN) were measured using Hologic dual energy X-ray absorptiometry (DEXA) machines (Hologic Inc., Bedford, MA, USA). Herein, hip BMD represents a combined BMD from the femoral neck, trochanter, and interchochanter. The machines were calibrated daily. The coefficient of variation values of the DEXA measurements were 1.98%, 1.87%, 1.87%, and 1.97% for spine BMD, hip BMD, FN BMD, and FN bone size, respectively.

Using DEXA-derived FN BMD and bone size, we estimated three FNCSGPs: cortical thickness (CT), cross-section area (CSA, an indicator of bone axial compression strength), and bulking ratio (BR, an index of cortical instability, indicating the risk of fracture by buckling; the lower the BR, the higher the resistance to buckling). The algorithm and the underlying assumptions regarding FN geometry and structure were communicated earlier [22, 23] and will not be further described herein.

Genome-wide genotyping

Genomic DNA was extracted from peripheral white blood cells using a commercial isolation kit (Gentra systems, Minneapolis, MN, USA). DNA concentration was assessed by a DU530 UV/VIS spectrophotometer (Beckman Coulter, Inc, Fullerton, CA, USA). Before advancing to the genotyping step, the measurements of DNA quantification

were double-checked by pico-green analysis that can detect fluorescent signal enhancement of PicoGreen® dsDNA Quantitation Reagent, which selectively binds to dsDNA. Genotyping with the Affymetrix Mapping 250 k Nsp and Affymetrix Mapping 250 k Sty arrays was performed at the Vanderbilt Microarray Shared Resource at Vanderbilt University Medical Center, Nashville, TN, USA using the standard protocol recommended by the manufacturer. Briefly, for each array, 250 ng of genomic DNA was digested with either Nsp1 or Sty1 and ligated to adapters that recognize the cohesive four base pair overhangs. A generic primer that recognizes the ligated adapter sequence was used to amplify the ligation products in a polymerase chain reaction. The amplified DNA was assayed by agarose gel electrophoresis to verify an average size distribution of 250 to 1,500 bp. The amplified DNA was purified per the manufacturer's protocol and quantitated by absorbance at 260 and 280 nm. Ninety micrograms of purified DNA was fragmented with DNase I and visualized on a 4% agarose gel. Samples with fragment distributions less than 180 bp were hybridized to the appropriate array (Nsp or Sty). Arrays were stained, washed, and scanned per manufacturer's protocol using immunopure streptavidin (Pierce, Milwaukee, WI, USA), biotinylated antistreptavidin antibody (Vector Labs, Burlingame, CA, USA), and R-phycoerythrin streptavidin (Invitrogen, Carlsbad, CA, USA). Fluorescence intensities were quantitated using an Affymetrix array scanner 30007G. Data management and analyses were performed using Affymetrix GeneChip Operating System.

CNV and CNVR determination in autosomes

Subjects with genome-wide genotyping call rates <93% were excluded from further analysis. The call rate was assessed using the Bayesian Robust Linear Model with Mahalanobis distance algorithm [24, 25]. Accordingly, a total of 15 Caucasian subjects were excluded, and the resultant sample of 985 subjects was used for subsequent analysis.

Affymetrix GeneChip® Chromosome Copy Number Analysis Tool (CNAT) Software V.4.0.1 was used to identify chromosomal copy number changes from intensity data. CNAT includes a built-in probe-level quantile normalization of signal intensity for test samples, which is based on the intensities of Perfect Match probes across reference samples. The genomic smoothing bandwidth was set as 100 kb. CNAT implements a Hidden Markov Model (HMM) based algorithm for smoothing and segmentation of the copy number (CN) changes. In this HMM model, there are five hidden states representing different copy number variation: homozygous deletion (CN=0), heterozygous deletion (CN=1), normal diploid (CN=2), single copy gain (CN=3), and amplification (CN≥4). In our analyses, CN<2 was categorized as "loss", CN=2 was defined as "normal", and CN>2 was categorized as "gain". Default values in the CNAT program for the Affymetrix GeneChip® Mapping 500K Array were used for the HMM parameters (0.2 for the priors, 0.07 for standard deviation when CN equal to 2, 0.09 for other CN states, and 10 Mb for the transition decay value).

CNAT requires reference data sets to be compared with sample sets for estimating the CN state of each SNP locus. For CNV detection on autosomes, samples on the same plate of 94 starting samples were assigned with equal probabilities into test and reference sets. This process yielded 47±2 subjects in a reference set in various sample plates and screened CN changes for all individuals. A total of 500,446 probes, averaging one SNP per 5.8 kb, were used to detect CN change.

At least two consecutive SNPs were used to define a CNV for each subject. The exact boundaries of CNVs cannot be obtained from data produced by the Affymetrix 500K SNP genotyping platform. However, they can be approximated by physical positions (NCBI Build 36.1, March 2006) of the probe pairs having the maximal distance within a CNV, yielding conservatively shorter defined sizes of CNVs than the actual sizes. Individual CNVs were organized into CNV regions (CNVRs), which are stretches of genomic regions covering overlapping CNVs across subjects. A CNVR started with two overlapping CNVs and was bounded by the two SNPs with maximal distance in the two CNVs. The CNVR would be extended when the next overlapping CNV exceeded the previous boundaries. For association analyses, we divided complex CNVRs, which include CNVs with inconsistent boundaries across the entire samples, into several subregions to ensure that (1) each CNV subregion has the same boundary among subjects and (2) each subject will be either gain or loss at each CNV subregion. CNV subregions with frequencies higher than 1% generated by the above procedure were selected for association analyses. Supplement 1 illustrates the relationship among SNPs, CNVs, CNVRs, and how CNV subregions were assigned in this study.

Genome-wide association analysis between CNV and BMD in the samples

First of all, age and sex were tested for their significance with each phenotype. Due to significant effects of age and sex on BMD and FNCSGPs ($p<0.05$), both age and sex were used as covariates to adjust the raw phenotypes. Considering significant genetic correlation of weight and height with BMD and FNCSGPS ([26], personal communication), weight and height were not used as

covariates to adjust each phenotype. The adjusted phenotypes, if not following normal distribution, were further subject to a Box–Cox transformation to conform to a normal distribution.

Analyses of variance (ANOVA) were performed to test for association between CNV subregions and adjusted phenotypes using the software package Helixtree (Golden Helix, Bozeman, MT, USA). The independent variable was the CNV subregion, which was divided into three levels according to the CN (gain, CN>2; normal, CN=2; and loss, CN<2). A raw p value<0.05 was considered nominally significant, which was further subjected to a Bonferroni correction to account for multiple testing in this study, where a significance level of 0.05/N (N=243, i.e., the total number of the tested CNV subregions) was used as a significance threshold for a test (p=0.0002). This significance threshold (p = 0.0002) was considered to be reasonable since the six study phenotypes that we studied are correlated to each other, and the Bonferroni correction is very conservative.

For significant CNVs identified, we performed further gender-stratified ANOVA analyses to test whether the significant CNV has a gender-specific effect on the testing phenotypes. The effect of age was adjusted in the analyses.

Assessment of population stratification

The STRUCTURE program version 2.2 [27] was used to detect possible population stratification of our study sample. Two thousand randomly picked unlinked SNPs with an average distance of 1.2 Mb, which were determined to be in Hardy–Weinberg equilibrium, were used to cluster all subjects using a Markov chain Monte Carlo algorithm. The admixture model and correlated allele frequency model were selected. Potential substructure was estimated based on the cluster pattern of all subjects. Existence of substructure is suggested if the subjects were clustered into two or more groups. We further tested our sample for population stratification using the genomic control method [28]. Based on genome-wide SNP information, we estimated the inflation factor (λ), a measure for population stratification. Ideally, for a homogeneous population with no stratification, the value of λ should be equal or near to 1.0.

Results

Basic characteristics of the sample

The basic characteristics of the 985 Caucasian subjects used for association analyses are summarized in Table 1. Use of the STRUCTURE program revealed that all subjects in this Caucasian sample were clustered together and could not be assigned into any subgroups, indicating that there was no significant population stratification within the sample. Further, the genomic control method estimated the λ value to be 1.007, confirming the results achieved through the Structure 2.2 software, indicating that there was essentially no population stratification in this sample. The relative homogeneity of this study sample eliminates potential spurious associations due to population stratification.

Genome-wide CNVs and CNVRs information in the sample

Table 2 summarizes the information for genome-wide CNVs and CNVRs detected in the Caucasian sample. In these 985 Caucasian subjects, we identified 9,196 CNV calls belonging to a total of 1,352 CNVs. These 1,352 CNVs were merged into 593 CNVRs, covering approximately 7.5% (215 Mb) of the 22 autosomes. For these 593 CNVRs, a total of 243 CNV subregions were assigned in the sample and utilized for subsequent genome-wide association analyses. Information for the 243 CNV subregions is presented in Supplement 2.

Table 1 Basic characteristic of the studied subjects

Traits	Total (n=985)	Female (n=493)	Male (n=492)
Age (years)	50.39 (18.26)	50.30 (17.65)	50.49 (18.87)
Height (cm)	170.79 (9.75)	163.80 (6.53)	177.78 (7.06)
Weight (kg)	80.06 (17.72)	71.25 (15.88)	88.86 (14.88)
SPN BMD (g/cm^2)	1.03 (0.16)	0.99 (0.15)	1.07 (0.17)
Hip BMD (g/cm^2)	0.97 (0.16)	0.91 (0.14)	1.04 (0.15)
FN BMD (g/cm^2)	0.81 (0.14)	0.82 (0.14)	0.81 (0.15)
CSA (cm^2)	2.84 (0.64)	2.85 (0.64)	2.83 (0.64)
CT (cm)	0.16 (0.03)	0.16 (0.03)	0.15 (0.03)
BR	12.02 (2.74)	11.90 (2.74)	12.18 (2.74)

Data presented are unadjusted means (SD)

SPN spine, *BMD* bone mineral density, *FN* femoral neck, *CSA* cross-section area, *CT* cortical thickness, *BR* buckling ratio

Table 2 Information for the CNVs and CNVRs in the Sample

Statistics	Value
CNV	
Total number of CNV calls	9,196
Total number of CNV calls with gain	4,748
Total number of CNV calls with loss	4,448
Total number of CNVs	1,352
Mean size of CNVs	295 kb
CNVR	
Total number of CNVRs	593
Mean size of CNVRs	361 kb
Mean number of probes per CNVR	58
Genome coverage	
By CNVs, averaged on subjects	2.1 Mb
By CNVRs, combined across subjects	215 Mb

CNV copy number variation, *CNVR* copy number variation region

Genome-wide association analysis of autosomal CNVs in the sample

Figure 1 illustrates the genome-wide association signals for BMD (at spine, hip and FN) and FNCSGPs (CT, CSA, and BR) on all 22 autosomes. The most significant association was detected at CNV_084 (8q22.2), which simultaneously achieved statistical significance for spine BMD, hip BMD, FN BMD, CT, CSA, and BR (p=0.0001, 0.0002, 0.0001, 0.0001, 0.0028, 0.0174, respectively; Table 3). Even after applying the stringent Bonferroni correction, associations remained significant for spine BMD, hip BMD, FN BMD, and CT (Table 3).

In addition to the significant association at CNV_084, prominent association signals (p<0.01) for BMD and/or FNCSGPs were observed for chromosome regions 1p36.21 (CNV_003), 1q12 (CNV_014), 8q22.2 (CNV_083), 9p11.1 (CNV_099), 9q12 (CNV_103), 14q32.33 (CNV_135), 15q11.2 (CNV_144-147,163), 21p11.2 (CNV_228-229), and 21p11.1 (CNV_230). For a more complete listing, we present all nominal significant associations between CNVs and BMD and/or FNCSGPs ($p< 0.05$) detected across the whole genome (Supplement 3); this represents a total of 53 CNV subregions on chromosomes [1, 2, 7–10, 14–17, 21, 22].

Effect of the significant CNV_084

For CNV_084, only CNV losses were observed in the study sample; no CNV gains were detected. Of the 985 analyzed subjects, 16 were carriers of a loss (CN=1), representing a minor copy number frequency of 1.62%.

For subjects with one CN vs. two CNs at CNV_084, we compared raw BMD and FNCSGPs (Fig. 2). For the total sample, compared to two copies of CNV_084, one copy is

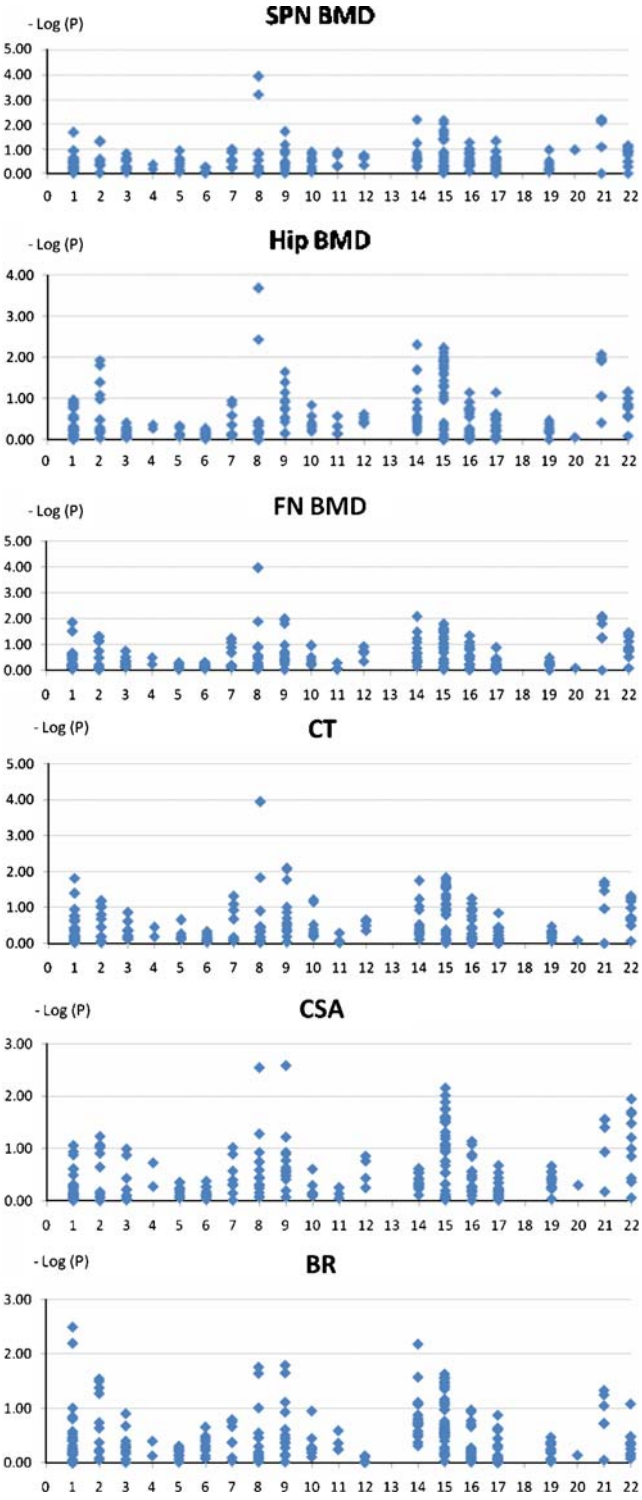

Fig. 1 Genome-wide CNV association results for BMD and FNCSGPs

associated with a higher BMD, CT, CSA, and a lower BR. Specifically, compared with those with two CN, individuals with one CN have an average of 14.6%, 12.4%, and 13.6% higher spine BMD, hip BMD, and FN BMD, respectively, 20.0% thicker CT, 10.6% larger CSA, and 12.4% lower

Table 3 Association between CNV_084 and bone phenotypes in the sample

Phenotype	Phenotypic value		*p* value		
	CNV state = 1	CNV state = 2	Total	Female	Male
SPN BMD	1.18 (0.22) [20]	1.03 (0.16) [965]	**0.0001**	0.0559	0.0003
Hip BMD	1.09 (0.20) [22]	0.97 (0.15) [963]	**0.0002**	0.0096	0.0071
FN BMD	0.92 (0.20) [33]	0.81 (0.14) [952]	**0.0001**	0.0032	0.0103
CT	0.18 (0.04) [27]	0.15 (0.03) [958]	**0.0001**	0.0029	0.0042
CSA	3.13 (0.77) [27]	2.83 (0.64) [958]	0.0030	0.0150	0.0510
BR	10.71 (2.92) [27]	12.04 (2.73) [958]	0.0170	0.1140	0.0710

Presented are mean (SD) [observation number]. In the total sample, age and gender were adjusted. In the gender-stratified analyses, age was adjusted as a covariant. Marked in bold are data that remained significant after Bonferroni correction

BR. These consistent findings suggest that a reduced copy number at CNV_084 is associated with stronger bone and a lower risk of OF. As shown in Table 3, with the exception of BR in the female sample, gender-stratified analyses revealed that CNV_084 is associated with all of the study phenotypes in both female and male subgroups ($p<0.05$).

Table 4 shows that CNV_084 covers seven analyzed intron SNPs (rs6468665, rs10098153, rs7818037, rs6991283, rs7459833, rs7842393, rs10808361) of the VPS13B (vacuolar protein sorting 13 homolog B) gene. This CNV extends 79,871 bp, ranging from intron 4 to 16 within the VPS13B gene.

Discussion

CNV is a genetic polymorphism recently recognized to be associated with human complex diseases, presumably via a dosage effect on gene expression. Using the Affymetrix Genechip® 500K array, we performed a genome-wide CNV study in a large Caucasian sample for two important bone strength phenotypes—BMD and FNCSGPs. We studied BMD at the spine, hip, and femoral neck. FNCSGPs were studied using three indexes, CT, CSA, and BR, which are all newly studied geometric variables associated with hip fracture [22, 23, 29]. CT and CSA are proportional to FN

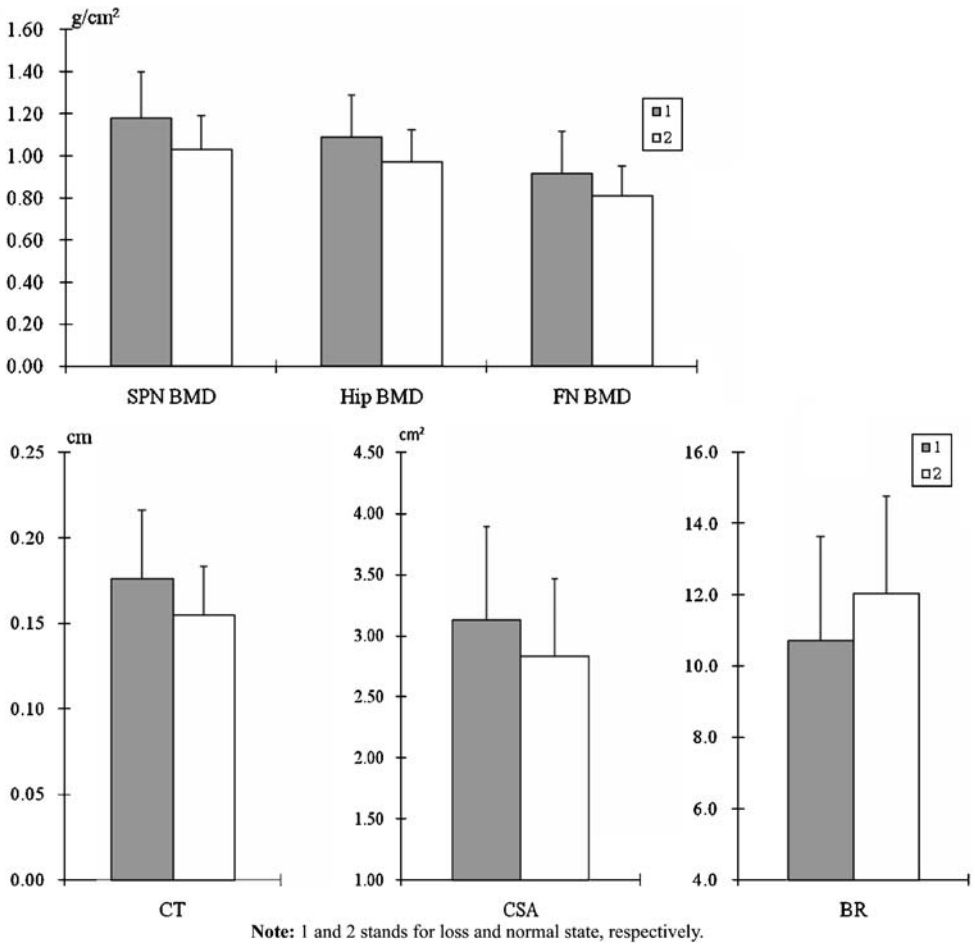

Fig. 2 The average raw BMD and FNCSGPs in subgroups stratified by copy number at CNV_084. *1* and *2* stands for loss and normal state, respectively

Table 4 Basic information for the SNPs covering CNV_084

Probe set	dbSNP RS ID	Chr. region	Physical position	Allele[a]	MAF[b] (Caucasian)	MAF[c] (present)	Gene name	Gene region
SNP_A-2312363	rs6468665	8q22.2	100181088	G/A	0.15	0.16	VPS13B	Intron 4
SNP_A-2307906	rs10098153	8q22.2	100187297	T/C	0.15	0.16	VPS13B	Intron 5
SNP_A-2296088	rs7818037	8q22.2	100188363	G/C	0.20	0.21	VPS13B	Intron 5
SNP_A-2086716	rs6991283	8q22.2	100189139	C/T	0.14	0.16	VPS13B	Intron 5
SNP_A-4282840	rs7459833	8q22.2	100205307	C/T	0.19	0.21	VPS13B	Intron 8
SNP_A-1879552	rs7842393	8q22.2	100207232	G/A	0.15	0.16	VPS13B	Intron 8
SNP_A-2024722	rs10808361	8q22.2	100260959	G/C	0.21	0.21	VPS13B	Intron 16

[a] The second allele is the minor allele in the sample

[b] The frequency in SNP annotation database

[c] The frequency in the studied sample

SNPs within CNV's usually are not in Hardy–Weinberg equilibrium; thus, association between these SNPs and the study phenotypes were not evaluated. CNV_084 was located within variation_3743 (chr8:100,098,024…100,265,857) archived in Database of Genomic Variants (http://projects.tcag.ca/variation/)

BMD and are negatively correlated with hip fracture risk [22, 29], whereas BR is an index for bone structural instability [22, 30, 31] that is positively associated with hip fracture. In this study, we found that loss of CNV_084 is associated with higher bone strength. Compared with carriers with a normal copy number for CNV_084, subjects with CNV_084 losses have denser bone mass at the spine, hip, and FN and a more stable bone structure indicated by higher CTs and CSAs and a lower BR. Our findings suggest that loss of copy number CNV_084 is associated with stronger bone, thus reducing the risk of osteoporotic fracture. This association was detected in both women and men, suggesting the gender generality of its effect. The only gene overlapping with CNV_084 is *VPS13B*, thus implicating this gene as a new susceptibility gene for osteoporosis in Caucasians.

The *VPS13B* gene contains 62 exons and spans~864 kb on 8q22.2. It is a member of VPS13 gene family, which encodes a potential trans-membrane protein and has *VPS13p* as its closest yeast homologue [32]. A number of functional motifs have been predicted for the *VPS13B* gene [33], and it has been proposed that VPS13B might be involved in vesicle-mediated sorting and intracellular protein transport.

The *VPS13B* gene is more commonly referred to as *COH1* since multiple studies have suggested that mutations of this gene cause Cohen syndrome (OMIM 216550) [33–35], an uncommon autosomal recessive disorder characterized by growth delay, slender limbs with narrow hands and feet, short stature, mental retardation, microcephaly, severe myopia, progressive chorioretinal dystrophy, and truncal obesity [34, 35]. Mutations of the *COH1* gene are thought to prevent cells from producing a functional COH1 protein. Alaluusua et al. evaluated bone loss from the panoramic radiographs in Cohen syndrome patients and age- and sex-matched controls and found that Cohen syndrome patients were more likely to have alveolar bone loss and more extensive bone loss than controls [36]. Furthermore, Kivitie-Kallio et al. reported that marked kyphosis was relatively common in COH patients [34, 35]. The skeletal abnormalities consistently identified in Cohen syndrome patients suggest a potential role for the *VPS13B* gene in bone metabolism.

The *VPS13B* gene has a complicated pattern of alternative splicing, which uses four different termination codons (exons 8, 17, 28, and 62) and three alternative in-frame splicings (exons 28, 28b, and 31) [32]. The *VPS13B* gene is widely expressed in human tissues [32], with some tissue-specific differences in expression of the various transcript variants. In skeletal muscle and brain, variant 1 (containing exon 28 instead of 28b) is the main transcript, different from that expressed in other tissues where variant 2 (containing exon 28b instead of 28) is the predominant transcript [32]. Due to its complex multi-domain structure, various isoforms of the VPS13B gene product are thought to have different functions [33].

In the current study, we found a strong association between CNV_084 and both BMD and FNCSGPs. The region represented by CNV_084 flanks from intron 4 to intron 16 of the *VPS13B* gene and CNV_084 fully or partially overlaps with sequences encoding various *VPS13B* transcripts. To date, at least seven mutations have been identified in this CNVR [34, 37–41]. Interestingly, in an isolated Greek island population, an intragenic *COH1* deletion spanning exon 6 to exon 16 was found to be causative for Cohen syndrome [34]. Furthermore, characteristics such as short stature, kyphosis and/or scoliosis, slender limbs with narrow hands and feet, etc. were fairly consistently

observed in patients with this deletion. Consequently, it appears reasonable to speculate that CNV_084 may play an important role in regulation of bone development and metabolism. However, the molecular mechanism, by which BMD and bone geometry structure is influenced by CNV_084, must be delineated by further focused functional studies. It should be noted that only heterozygous loss of CNV_084 was detected in our sample, and this loss did not lead to Cohen syndrome presumably because Cohen syndrome is inherited in an autosomal recessive manner.

In summary, this is the first genome-wide CNV association study for BMD and FNCSGPs in Caucasians. Our data, supported by previous associations between *VPS13B* mutations and skeletal abnormalities, strongly suggest that *VPS13B* is a novel candidate gene for osteoporosis.

Acknowledgment We thank Dr. Tian-Bo Jin for help in CNV and CNVR determination. Investigators of this work were partially supported by grants from NIH (R01 AR050496-01, R21 AG027110, R01 AG026564, R21 AA015973, and P50 AR055081), the Cancer and Smoking Disease Research Bone Biology Program, and Nebraska tobacco settlement biomedical research development award, both supported by the State of Nebraska. The study also benefited from grants from National Science Foundation of China, Huo Ying Dong Education Foundation, Xi'an Jiaotong University, and the Ministry of Education of China.

Conflicts of interest None.

References

1. Ray NF, Chan JK, Thamer M, Melton LJ 3rd (1997) Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res 12:24–35
2. Cummings SR, Black D (1995) Bone mass measurements and risk of fracture in Caucasian women: a review of findings from prospective studies. Am J Med 98:24S–28S
3. Liu YJ, Shen H, Xiao P, Xiong DH, Li LH, Recker RR, Deng HW (2006) Molecular genetic studies of gene identification for osteoporosis: a 2004 update. J Bone Miner Res 21:1511–1535
4. Cauley JA, Thompson DE, Ensrud KC, Scott JC, Black D (2000) Risk of mortality following clinical fractures. Osteoporos Int 11:556–561
5. Fleurence RL, Iglesias CP, Torgerson DJ (2006) Economic evaluations of interventions for the prevention and treatment of osteoporosis: a structured review of the literature. Osteoporos Int 17:29–40
6. Wehren LE, Magaziner J (2003) Hip fracture: risk factors and outcomes. Curr Osteoporos Rep 1:78–85
7. Cheng XG, Lowet G, Boonen S, Nicholson PH, Brys P, Nijs J, Dequeker J (1997) Assessment of the strength of proximal femur in vitro: relationship to femoral bone mineral density and femoral geometry. Bone 20:213–218
8. Ammann P, Rizzoli R (2003) Bone strength and its determinants. Osteoporos Int 14(Suppl 3):S13–S18
9. Pulkkinen P, Partanen J, Jalovaara P, Jamsa T (2004) Combination of bone mineral density and upper femur geometry improves the prediction of hip fracture. Osteoporos Int 15:274–280
10. Shen H, Long JR, Xiong DH, Liu YJ, Liu YZ, Xiao P, Zhao LJ, Dvornyk V, Zhang YY, Rocha-Sanchez S, Liu PY, Li JL, Deng HW (2005) Mapping quantitative trait loci for cross-sectional geometry at the femoral neck. J Bone Miner Res 20:1973–1982
11. Slemenda CW, Turner CH, Peacock M, Christian JC, Sorbel J, Hui SL, Johnston CC (1996) The genetics of proximal femur geometry, distribution of bone mass and bone mineral density. Osteoporos Int 6:178–182
12. Demissie S, Dupuis J, Cupples LA, Beck TJ, Kiel DP, Karasik D (2007) Proximal hip geometry is linked to several chromosomal regions: genome-wide linkage results from the Framingham Osteoporosis Study. Bone 40:743–750
13. Moffett SP, Zmuda JM, Oakley JI, Beck TJ, Cauley JA, Stone KL, Lui LY, Ensrud KE, Hillier TA, Hochberg MC, Morin P, Peltz G, Greene D, Cummings SR (2005) Tumor necrosis factor-alpha polymorphism, bone strength phenotypes, and the risk of fracture in older women. J Clin Endocrinol Metab 90:3491–3497
14. Rivadeneira F, Houwing-Duistermaat JJ, Beck TJ, Janssen JA, Hofman A, Pols HA, Van Duijn CM, Uitterlinden AG (2004) The influence of an insulin-like growth factor I gene promoter polymorphism on hip bone geometry and the risk of nonvertebral fracture in the elderly: the Rotterdam Study. J Bone Miner Res 19:1280–1290
15. Feuk L, Carson AR, Scherer SW (2006) Structural variation in the human genome. Nat Rev Genet 7:85–97
16. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME (2006) Global variation in copy number in the human genome. Nature 444:444–454
17. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, Redon R, Bird CP, de Grassi A, Lee C, Tyler-Smith C, Carter N, Scherer SW, Tavare S, Deloukas P, Hurles ME, Dermitzakis ET (2007) Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 315:848–853
18. Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J, Mangion J, Roberton-Lowe C, Marshall AJ, Petretto E, Hodges MD, Bhangal G, Patel SG, Sheehan-Rooney K, Duda M, Cook PR, Evans DJ, Domin J, Flint J, Boyle JJ, Pusey CD, Cook HT (2006) Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature 439:851–855
19. Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G, Nibbs RJ, Freedman BI, Quinones MP, Bamshad MJ, Murthy KK, Rovin BH, Bradley W, Clark RA, Anderson SA, O'Connell RJ, Agan BK, Ahuja SS, Bologna R, Sen L, Dolan MJ, Ahuja SK (2005) The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science (New York NY) 307:1434–1440
20. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimaki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M (2007) Strong association of de novo copy number mutations with autism. Science (New York NY) 316:445–449
21. Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, Zhou Q, Pan F, Chen Y, Zhang ZX, Dong SS, Xu XH, Yan H, Liu X, Qiu C, Zhu XZ, Chen T, Li M, Zhang H, Zhang L, Drees BM, Hamilton JJ, Papasian CJ,

Recker RR, Song XP, Cheng J, Deng HW (2008) Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. Am J Hum Genet 83:663–674
22. Duan Y, Beck TJ, Wang XF, Seeman E (2003) Structural and biomechanical basis of sexual dimorphism in femoral neck fragility has its origins in growth and aging. J Bone Miner Res 18:1766–1774
23. Beck TJ (2003) Measuring the structural strength of bones with dual-energy X-ray absorptiometry: principles, technical limitations, and future possibilities. Osteoporos Int 14:S81–S88
24. Rabbee N, Speed TP (2006) A genotype calling algorithm for Affymetrix SNP arrays. Bioinformatics 22:7–12
25. Di X, Matsuzaki H, Webster TA, Hubbell E, Liu G, Dong S, Bartell D, Huang J, Chiles R, Yang G, Shen MM, Kulp D, Kennedy GC, Mei R, Jones KW, Cawley S (2005) Dynamic model based algorithms for screening and genotyping over 100 K SNPs on oligonucleotide microarrays. Bioinformatics 21:1958–1963
26. Sun X, Lei SF, Deng FY, Wu S, Papacian C, Hamilton J, Recker RR, Deng HW (2006) Genetic and environmental correlations between bone geometric parameters and body compositions. Calcif Tissue Int 79:43–49
27. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
28. Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55:997–1004
29. Filardi S, Zebaze RM, Duan Y, Edmonds J, Beck T, Seeman E (2004) Femoral neck fragility in women has its structural and biomechanical basis established by periosteal modeling during growth and endocortical remodeling during aging. Osteoporos Int 15:103–107
30. Beck TJ, Stone KL, Oreskovic TL, Hochberg MC, Nevitt MC, Genant HK, Cummings SR (2001) Effects of current and discontinued estrogen replacement therapy on hip structural geometry: the study of osteoporotic fractures. J Bone Miner Res 16:2103–2110
31. Beck TJ, Oreskovic TL, Stone KL, Ruff CB, Ensrud K, Nevitt MC, Genant HK, Cummings SR (2001) Structural adaptation to changing skeletal load in the progression toward hip fragility: the study of osteoporotic fractures. J Bone Miner Res 16:1108–1119
32. Velayos-Baeza A, Vettori A, Copley RR, Dobson-Stone C, Monaco AP (2004) Analysis of the human VPS13 gene family. Genomics 84:536–549
33. Kolehmainen J, Black GC, Saarinen A, Chandler K, Clayton-Smith J, Traskelin AL, Perveen R, Kivitie-Kallio S, Norio R, Warburg M, Fryns JP, de la Chapelle A, Lehesjoki AE (2003) Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. Am J Hum Genet 72:1359–1369
34. Bugiani M, Gyftodimou Y, Tsimpouka P, Lamantea E, Katzaki E, d'Adamo P, Nakou S, Georgoudi N, Grigoriadou M, Tsina E, Kabolis N, Milani D, Pandelia E, Kokotas H, Gasparini P, Giannoulia-Karantana A, Renieri A, Zeviani M, Petersen MB (2008) Cohen syndrome resulting from a novel large intragenic COH1 deletion segregating in an isolated Greek island population. Am J Med Genet 146A:2221–2226
35. Kivitie-Kallio S, Norio R (2001) Cohen syndrome: essential features, natural history, and heterogeneity. Am J Med Genet 102:125–135
36. Alaluusua S, Kivitie-Kallio S, Wolf J, Haavio ML, Asikainen S, Pirinen S (1997) Periodontal findings in Cohen syndrome with chronic neutropenia. J Periodontol 68:473–478
37. Hennies HC, Rauch A, Seifert W, Schumi C, Moser E, Al-Taji E, Tariverdian G, Chrzanowska KH, Krajewska-Walasek M, Rajab A, Giugliani R, Neumann TE, Eckl KM, Karbasiyan M, Reis A, Horn D (2004) Allelic heterogeneity in the COH1 gene explains clinical variability in Cohen syndrome. Am J Hum Genet 75:138–145
38. Katzaki E, Pescucci C, Uliana V, Papa FT, Ariani F, Meloni I, Priolo M, Selicorni A, Milani D, Fischetto R, Celle ME, Grasso R, Dallapiccola B, Brancati F, Bordignon M, Tenconi R, Federico A, Mari F, Renieri A, Longo I (2007) Clinical and molecular characterization of Italian patients affected by Cohen syndrome. J Hum Genet 52:1011–1017
39. Kolehmainen J, Wilkinson R, Lehesjoki AE, Chandler K, Kivitie-Kallio S, Clayton-Smith J, Traskelin AL, Waris L, Saarinen A, Khan J, Gross-Tsur V, Traboulsi EI, Warburg M, Fryns JP, Norio R, Black GC, Manson FD (2004) Delineation of Cohen syndrome following a large-scale genotype-phenotype screen. Am J Hum Genet 75:122–127
40. Mochida GH, Rajab A, Eyaid W, Lu A, Al-Nouri D, Kosaki K, Noruzinia M, Sarda P, Ishihara J, Bodell A, Apse K, Walsh CA (2004) Broader geographical spectrum of Cohen syndrome due to COH1 mutations. J Med Genet 41:e87
41. Seifert W, Holder-Espinasse M, Spranger S, Hoeltzenbein M, Rossier E, Dollfus H, Lacombe D, Verloes A, Chrzanowska KH, Maegawa GH, Chitayat D, Kotzot D, Huhle D, Meinecke P, Albrecht B, Mathijssen I, Leheup B, Raile K, Hennies HC, Horn D (2006) Mutational spectrum of COH1 and clinical heterogeneity in Cohen syndrome. J Med Genet 43:e22