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Genome-wide Survey of Runs of Homozygosity Identifies Recessive Loci for Bone Mineral Density in Caucasian and Chinese Populations

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Abstract

Runs of homozygosity (ROHs), in which both parental alleles are identical, have been proposed to have recessive effects on multiple human complex diseases. Osteoporosis is a common complex disease characterized by low bone mineral density (BMD), which is highly heritable. And recessive loci that contribute to BMD variations have been identified. In this study, we performed a genome-wide ROHs association study using our SNP array data from three GWAS samples including 4,900 subjects from Caucasian and Chinese populations. Significant results were further subjected to replication in 3,747 additional subjects. ROHs associated with BMD were also tested for associations with osteoporotic fractures in a GWAS fracture sample. Combining results from all the samples, we identified 697 autosomal regions with ROHs. Among these, we detected genome-wide significant associations between BMD and 6 ROHs, including ROH1q31.3, 1p31.1, 3q26.1, 11q12.1, 21q22.1 and 15q22.3 (combined $P=6.29 \times 10^{-5} - 3.17 \times 10^{-8}$). Especially, ROH1p31.1 was found to be associated with increased risk of osteoporotic hip fractures (odds ratio [OR] 3.71, $P=0.032$). To investigate the functional relevance of the identified ROHs, we performed *cis*-expression quantitative trait locus (eQTL) analysis in lymphoblast cell lines. Three ROHs, including ROH1p31.1, 11q12.1, and 15q22.3, were found to be significantly associated with mRNA expression levels of their nearby genes ($P_{\text{eQTL}} < 0.05$). In summary, our findings reveal that ROHs could play as recessive-acting determinants contributing to the pathogenesis of osteoporosis. Further molecular and functional studies are needed to explore and clarify the potential mechanism.

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Disclosures

All authors state that they have no conflicts of interest.

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Keywords

ROHS; BMD; FRACTURES; ASSOCIATION

Introduction

Osteoporosis is a major public health problem leading to an increased risk of low-trauma osteoporotic fractures (OF).⁽¹⁾ Hip fractures are the most common and severe form of OF associated with high mortality and huge health care costs.⁽²⁾ Clinically, bone mineral density (BMD) serves as a diagnostic index in the assessment of osteoporosis and fracture risk, and is the single best predictor of OF.⁽³⁾ Both BMD and OF are highly heritable, with heritability estimates of 0.6–0.8⁽⁴⁾ for BMD and ~0.5⁽⁵⁾ for OF. As with most complex diseases, although a number of genome-wide association studies (GWASs) on osteoporosis have been successfully performed and over 60 genome-wide significant loci have been reported, these loci collectively account for less than 10% of the overall heritability of BMD.^(6–13) Therefore, additional studies, using innovative methodologies, are required to identify other genetic factors that influence osteoporosis.

A run of homozygosity (ROH) is defined as a contiguous stretch of a genomic sequence without heterozygosity in the diploid state. ROHs could be inherited from a common ancestor many generations back.⁽¹⁴⁾ There is emerging evidence supporting that ROHs may represent a novel type of independent genomic variability.⁽¹⁵⁾ With the achievement of the SNPs array, it has become more feasible and powerful to conduct ROHs mapping, which could provide opportunities to identify recessive loci for complex diseases or traits. Indeed, a series of studies have reported that ROHs are widely but not randomly distributed in the outbred human genomes, and have potential recessive effects on many complex diseases^(16–21), such as schizophrenia,⁽¹⁸⁾ Alzheimer's disease,⁽¹⁷⁾ autism,⁽¹⁶⁾ speech delay in autism,⁽¹⁹⁾ and lung cancer.⁽²⁰⁾ However, to the best of our knowledge, studies have not been performed for genome-wide ROHs mapping for osteoporosis. Osteoporosis could be affected by recessive loci.^(22–24) For instance, variation in *WNT3A* was strongly associated with hip BMD under the recessive model.⁽²⁴⁾ SNP rs312009 in the 5'-flanking region of *LRP5* was associated with BMD under the recessive model.⁽²²⁾ A haplotype in the *SRPYI* gene showed association with an increased risk for osteoporosis in the recessive genetic model.⁽²³⁾ Given that ROHs could act as recessive-acting determinants in the underlying genetic mechanism of osteoporosis, in this study, we adopted ROHs, to perform a genome-wide association study using our current high-density SNP genome-scan data from four GWAS samples of 5,600 subjects. The most promising results were further tested for replication in another sample containing 3,747 subjects, aiming to identify novel variants for osteoporosis.

Materials and Methods

Ethics Statement

Each study was approved by the required Institutional Review Board or Research Administration of the institutions involved. Signed informed-consent documents were obtained from all study participants before entering the study.

Subjects

The study was initially performed with a discovery stage for detection of ROHs associated with BMD in our three GWAS samples from white and Chinese ethnicities, including Kansas-city osteoporosis study (KCOS), Omaha osteoporosis study (OOS), and China osteoporosis study (COS). Significant ROHs identified from the discovery stage were further confirmed through a replication stage in an additional independent sample from Framingham Heart Study (FHS). ROHs associated with BMD were also tested for associations with osteoporotic fractures in a GWAS sample from China fracture study (CFS). The description of each study has been detailed in our previous studies.⁽²⁵⁾ Briefly, the KCOS and OOS samples came from population-based cohort, including 2,286 and 987 unrelated US Caucasians of Northern European origin, separately. The COS sample was derived from a population-based cohort of 1,627 unrelated Chinese Han subjects. The CFS sample was from a case-control cohort of Chinese Han origin, including 350 cases with osteoporotic hip fractures and 350 elderly healthy controls. We focused exclusively on hip fractures in order to minimize potential clinical and genetic heterogeneity of the study phenotype. The FHS sample came from a longitudinal and prospective cohort comprising over 16,000 individuals spanning three generations, of European ancestry. Focusing on the first two generations, we identified 3,747 phenotyped individuals. Basic characteristics of all study samples are summarized in Table 1.

Phenotype measurements

For the KCOS, OOS, and COS samples, BMD (g/cm^2) at the total hip for each subject was measured with dual energy x-ray absorptiometry (DXA) using Hologic 4500W machines (Hologic Inc., Bedford, MA, USA) that were calibrated daily. For the FHS sample, BMD at the hip was measured using DXA machine (Lunar DPX-L, Madison, WI, USA).

Genotyping and Quality Control

For the discovery stage, samples from KCOS and COS were genotyped using Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA), according to the Affymetrix protocol. Samples from OOS and CFS were genotyped using the Affymetrix Human Mapping 500K array set. The details of genotyping for each sample have been described in our previous studies.⁽²⁵⁾

For the replication stage, the FHS sample was genotyped using approximately 550,000 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array). For details of the genotyping method, please refer to FHS SHARe at NCBI dbGaP website (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v3.p2).

Quality control of genotype data were implemented with PLINK,⁽²⁶⁾ with the following criteria applied: individual missingness < 5%, SNP call rate < 95%, and Hardy–Weinberg equilibrium (HWE) P -value < 0.0001.

Identification of ROHs

ROHs were identified using the Runs of Homozygosity program in PLINK software.⁽²⁶⁾ In order to avoid segments that were homozygous by chance, we defined an ROH as segments that contained a minimum of 100 consecutive SNPs. Since strong linkage disequilibrium (LD) within 100 kb is common throughout the human genome, we set the minimum length for an ROH at 500 kb to exclude very common ROHs that occur in all individuals in all populations. In addition, the required minimum density in an ROH was set at 50 kb per SNP, and the maximum gap between two consecutive homozygous SNPs was set at 100 kb. PLINK uses a sliding window of 5 Mb (minimum 50 SNPs) across the genome to scan the defined ROHs, it allows five missing SNPs and one heterozygous site per window. All SNPs located in the X chromosome were excluded from the analyses, because male subjects have only one copy of the X chromosome.

Association analyses between ROHs and phenotypes

We used the following procedure to examine associations between ROHs and phenotypes. First, individual ROHs were divided into different ROH groups by using the homozyg-group command in the Runs of Homozygosity program. An ROH group contains individual ROHs with overlapping regions, i.e. all the individual ROHs in a group include a consensus core region. SNPs in the consensus core region must be allelically matched. Allelic matching was confirmed when at least 95% of jointly non-missing and homozygous SNPs were identical. Next, each ROH group containing more than 1% subjects in each sample were kept for subsequent association analyses. In addition, because copy number changes can influence the accuracy of SNP genotyping calls, we excluded ROH groups from the analyses if they overlapped with regions containing copy number variations, which were analyzed by previous studies.^(25,27) Before association analyses, principal component analysis implemented in EIGENSTRAT⁽²⁸⁾ was used to correct for potential population stratification that may lead to spurious association results. The first ten principal components emerging from the EIGENSTRAT analyses, along with sex, height, weight and age, were used as covariates to adjust the raw BMD values in each sample. The residuals were used for association analyses.

For the discovery stage, linear regression implemented in PLINK⁽²⁶⁾ was used to test for associations between ROHs and BMD. The Benjamini and Hochberg (BH) procedure⁽²⁹⁾ was used for multiple-testing adjustment. ROHs with adjusted $P < 0.05$ in the discovery stage were selected for replication in the FHS sample. FBAT (Family-Based Association Tests)⁽³⁰⁾ was used to examine associations in family-based sample. In order to assess the effect direction of interested ROHs, we randomly select one subject from each family to constitute a sub-sample to calculate effect direction. A nominally significant association threshold (two-sided $P < 0.05$) was set in the replication stage to ensure that the overall significant association is robust across populations. Data from the discovery and replication samples were combined using meta-analysis implemented in the METAL software package

(<http://www.sph.umich.edu/csg/abecasis/Metal/>), taking into account sample size and direction of effect. For the validation analyses in the CFS sample, logistic regression in PLINK⁽²⁶⁾ was used to examine associations between ROHs and hip fractures, taking into account potential covariates such as age, sex, height and weight.

Expression quantitative trait locus (eQTL) analysis

We examined associations between ROH regions and mRNA expression levels of nearby genes, to ascertain whether the ROH regions identified affected expression of their nearest transcript. As variants may have long-range functional connections with genes,⁽³¹⁾ we included genes located in the 500k extension of boundaries of each ROH region. Gene expression information was obtained from human lymphoblastoid cell lines (LCLs) of 210 unrelated individuals from HapMap populations in the NCBI Gene Expression Omnibus.^(32,33) The sample included 60 Caucasian individuals (CEU, of northern and western European ancestry), and 90 East Asian individuals (45 Han Chinese and 45 Japanese individuals). SNP genotyping was conducted using the Affymetrix Human Mapping 500K array and the data were derived from the corresponding HapMap Phase III dataset. ROHs were identified using the same approach as described above. The t-test was used to compare gene expression levels in cells with these ROHs versus cells without them.

Functional annotation

In order to evaluate the potential regulatory function of the ROH regions identified, we used ENCODE data from the UCSC genome database (15 November 2014)⁽³⁴⁾ to inspect the annotated genomic features, including histone modification by ChIP-seq, chromatin state segmentation analysis, and transcription factor obtained experimentally by ChIP-seq. The data from GM12878 (B-lymphoblastoid) cell line were used.

Results

The study was initially performed with a discovery stage in three GWAS BMD samples from in-house studies, in which, two samples consisted of Caucasian subjects, and the other two samples consisted of Chinese Han subjects. We first screened all ROH regions in these three samples. In total, 782 ROHs with consensus core region were identified across all the three samples. Among these ROHs, 85 ROHs overlapped with copy number variations and were excluded from the subsequent association analyses. Finally, 697 ROHs were used to test for associations with hip BMD. Combining results from these three GWAS samples in the discovery stage, we identified 11 ROHs significantly associated with hip BMD after FDR correction for multiple testing. The basic characteristics, including chromosome positions and frequencies, which mean the proportion of subjects with the specific ROH, of these 11 ROHs are presented in Table 2.

We subsequently validated above 11 ROHs through a replication stage in an independent sample from the FHS. Six ROHs were successfully replicated with $P < 0.05$, and the results are summarized in Table 3. Combining data from the discovery and replication samples together, the P values of these 6 ROHs ranged from 6.29×10^{-5} to 3.17×10^{-8} . Of these, three ROHs were significantly associated with increased risk of osteoporosis, including

ROH1p31.1 (combined $P=3.77\times 10^{-6}$, $\beta = -0.025$ to -0.054), ROH3q26.1 (combined $P=6.74\times 10^{-6}$, $\beta = -0.013$ to -0.048) and ROH11q12.1 (combined $P=1.65\times 10^{-5}$, $\beta = -0.021$ to 0.038). The hip BMD values of subjects carrying these three ROHs were significantly smaller than that of subjects without these ROHs. And the other three ROHs were significantly associated with decreased risk of osteoporosis, including ROH1q31.3 (combined $P = 3.17\times 10^{-8}$, $\beta = 0.015$ to 0.052), ROH21q22.1 (combined $P = 1.80\times 10^{-5}$, $\beta = 0.025$ to 0.046) and ROH15q22.3 (combined $P = 6.29\times 10^{-5}$, $\beta = 0.034$ to 0.044). The hip BMD values of subjects with these three ROHs were significantly greater than that of subjects without these ROHs.

We further examined associations between hip fractures and the above 6 ROHs identified to be associated with hip BMD in the CFS sample (Table 4). The effects of these six ROHs on hip fractures were totally consistent with their associations with hip BMDs values. Especially, ROH1p31.1 was found to be significantly associated with increased risk of hip fractures ($P = 0.032$), and the odds ratio (OR) was estimated to be 3.71. ROH15q22.3 was found to be nearly associated with hip fractures ($P = 0.078$, OR = 0.59).

To investigate the functional relevance of the identified ROHs, we performed eQTL analysis in Caucasian and East Asian samples from HapMap data, respectively. As shown in Table 5, three ROHs were detected to be significantly associated with mRNA expression levels of their nearby genes, including ROH1p31.1 (P_{eQTL} of 0.034 with *LPHN2* in Japanese), ROH11q12.1 (P_{eQTL} of 0.003 and 0.021 with *OR5F1* in Caucasian and Japanese, respectively), and ROH15q22.3 (P_{eQTL} of 0.039 with *CSNK1G1* in Japanese; P_{eQTL} of 0.028 with *SNXI* in Chinese; P_{eQTL} of 0.004 with *TRIP4* in Japanese; and P_{eQTL} of 0.045 with *USP3* in Caucasian).

We further used ENCODE data to evaluate the potential regulatory function of the identified ROHs. Interesting results were obtained for ROH15q22.3 and ROH1p31.1. As shown in Figure 1, analysis of histone modification marks derived from the Gm12878 cell line identified activating H3K4me1, H3K4me2, and H3K27ac marks specifically enriched in the ROH15q22.3 region (Figure 1A.a), and H3K4me1, H3K4me2, H3K4me3, and H3K27ac marks enriched in the ROH1p31.1 region (Figure 1B.a), respectively. Analysis of chromatin state segmentation identified 5 strong enhancers in the ROH15q22.3 region (Figure 1A.b), and 7 strong enhancers in the ROH1p31.1 region (Figure 1B.b), respectively.^(35,36) Moreover, a number of transcription factors were identified to bind these two ROH regions (Figure 1A.c and 1B.c).

Discussion

ROHs could represent a novel type of independent genomic variability and contribute recessive effect to human complex diseases.^(17–19,37) Osteoporosis is a classic complex disease and previous studies have identified recessive loci that were associated with BMD variation.^(22–24) In this study, using our available GWAS samples, we conducted ROHs association mapping and successfully identified six ROHs, which were strongly associated with osteoporosis. Our samples came from two ethnicities, which could identify phylogenetically oldest common ROHs across different populations. Our results revealed

that ROHs could act as recessive-acting determinants in the underlying genetic mechanism of osteoporosis.

To evaluate the functional role of the identified ROHs, we conducted eQTL analysis in human LCLs using public HapMap data. Three ROHs (ROH1p31.1, ROH1q12.1 and ROH15q22.3) could affect the mRNA expression levels of their nearby genes in Caucasian or East Asian population, which implied some extent of ethnic differentiation. Using ENCODE data, we further assessed the potential regulatory function of the identified ROHs. Two regions overlapped with ROH15q22.3 and ROH1p31.1 were identified to be enriched in a set of regulatory elements, such as a number of strong enhancers and activating histone modification marks (H3K4me1, H3K4me2, and H3K27ac), which suggests that the presence of the specific ROH might inhibit the histone modification and thus down-regulate the expression levels of corresponding genes. Overall, these evidence suggests the functional importance of the ROHs, which is worth for further studies to investigate the detailed mechanisms.

According to the NCBI Gene Expression Omnibus database (GSE35473), genes whose expression levels were affected by the identified ROHs have been detected to be expressed in human monocytes. Monocytes are important for osteoporosis by serving as osteoclast progenitor cells^(38,39) and producing cytokines for osteoclastogenesis and bone resorption.⁽⁴⁰⁾ Among these genes affected by ROHs, some may have potential connection with bone or osteoporosis. For instance, *SNX1* is involved in the negative regulation of ligand-induced EGFR phosphorylation,⁽⁴¹⁾ and enhanced degradation of EGF receptors.⁽⁴²⁾ Mice with reducing osteoblastic EGFR activity, exhibited a remarkable decrease in tibial trabecular bone mass with abnormalities in trabecular number and thickness, and decrease in osteoblast number and mineralization activity and an increase in osteoclast number.⁽⁴³⁾ *OR5F1* encodes olfactory receptor, which is located on olfactory sensory neurons in the olfactory epithelium.⁽⁴⁴⁾ A recent study showed that patients with Kallmann syndrome, which related to defective development of the olfactory system, were associated with specific ethmoid bone abnormalities.⁽⁴⁵⁾ *TRIP4* gene encodes thyroid hormone receptor interactor 4. Previous studies have identified a direct effect of thyroid-stimulating hormone on the skeleton and the pathogenesis of osteoporosis.^(46–48)

Previous studies have identified a number of recessive genes influencing osteoporosis, including *VDR*,⁽⁴⁹⁾ *LRP5*,⁽²²⁾ and *WNT3A*,⁽²⁴⁾ et al. We examined associations of ROHs including these recessive genes with BMD and fractures in our study. We confirmed several recessive genes at the replication level. For example, ROH overlapped with *LRP5* was shown to be associated with fractures ($P = 0.049$). ROH located at 67kb downstream of *VDR* was associated with BMD ($P = 0.038$). We also compared the results for ROHs detected in our study with dozens of genes identified by previous BMD GWASs and meta-analysis studies.^(6–13) Thirteen genes reported by previous GWASs were located at ROH regions. Among which, ROH overlapped with *RSPO3* was found to be associated with BMD ($P = 0.007$). ROHs overlapped with *WNT16/C7orf58* ($P = 0.016$), *CPN1* ($P = 0.032$), *LRP5* ($P = 0.049$) and *MARK3* ($P = 0.007$) were found to be associated with fractures, respectively. These data could serve as a reference for future investigators.

We estimated the statistical power of our study using the Genetic Power Calculator.⁽⁵⁰⁾ Assuming that a marker is in strong linkage disequilibrium ($D' = 0.9$) with a functional variant that accounts for 1.0% variation of a phenotype, our discovery and replication samples can achieve >90% power at an α -level of 10^{-4} and 0.05, respectively, which is large enough to detect a genetic variant. The fracture sample has 64% power at an α -level of 0.05. Inadequate statistical power of the fracture sample might be a reason that only one BMD-associated ROH was detected to be associated with fractures. For the eQTL analysis, since the sample size of HapMap data for each population is relatively small, limited power (>20%) could influence the detection of additional associations. Nevertheless, our results confirm the relevance between ROHs and gene expression, which provides supplementary insights that are indirectly informative for osteoporosis.

There is a potential limitation of our study. SNPs in the region with heterozygosity deletion might be considered as homozygosity. In order to obtain precise results for ROHs, we excluded ROHs that overlapped with copy number polymorphisms from the analyses. However, this approach was not able to exclude other genetic factors influencing the accuracy of ROHs detection, such as uniparental isodisomy. Further exploration of these ROHs with more detailed sequencing will be required.

In conclusion, using data from 9,347 individuals, we identified 6 ROHs significantly associated with osteoporosis. Our findings provide a strong support that ROHs could play as recessive-acting determinants contributing to the pathogenesis of osteoporosis, which opens a new avenue for discovering missing heritability for osteoporosis. Further molecular and functional studies will be needed for confirmation and clarification of the potential mechanism.

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Figure 1.

Functional annotation for ROH15q22.3 and ROH1p31.1. The data were obtained from the ENCODE project in Gm12878 cells obtained from the UCSC genome browser (15 November 2014). (A) ROH15q22.3: (a) Three histone modification marks (H3K4me1, H3K4me2, and H3K27ac) were identified. (b) Five strong enhancers were predicted. (c) Six transcription factor binding sites (TFBSs) for c-Jun, c-Myc, Max, NFKB, Pol2, and TR4 were enriched. (B) ROH1p31.1: (a) Four histone modification marks (H3K4me1, H3K4me2, H3K4me3, and H3K27ac) were identified. (b) Seven strong enhancers were predicted. (c) Seven TFBSs for c-Fos, c-Jun, c-Myc, JunD, Max, NFKB, and Pol2 were enriched.

Table 1

Basic characteristics of the study subjects

Sample	Sample Size	Female (%)	Age (yrs)	Height (m)	Weight (kg)	Hip BMD (g/cm ²)
KCOS	2,286	75.9	51.4(13.8)	1.66(0.08)	75.27(17.54)	0.97(0.17)
OOS	987	49.6	50.3(18.3)	1.71(0.10)	80.10(17.72)	0.97(0.16)
COS	1,627	50.7	34.5(13.2)	1.64(0.08)	60.12(10.48)	0.92(0.13)
CFS-case	350	64.6	69.4(7.41)	1.63(0.12)	59.15 (12.05)	–
CFS-control	350	50.6	69.5 (6.09)	1.59(0.10)	59.61 (10.84)	–
FHS	3,747	57.3	60.3(10.7)	1.66(0.10)	77.00(16.99)	0.95(0.17)

Notes: Data were presented as mean (SD). Abbreviations: KCOS, Kansas-city osteoporosis study; OOS, Omaha osteoporosis study; COS, China osteoporosis study; CFS, China fracture study; FHS, Framingham heart study.

Table 2

Basic characteristics of 11 significant ROHs

ROH	Chr	Position ^d		KCOS			OOS			COS			<i>P</i> _{combined} ^b	<i>P</i> _{adjusted}
		Start	End	Freq	Beta	<i>P</i>	Freq	beta	<i>P</i>	Freq	beta	<i>P</i>		
ROH1q31.3	1	192279836	192370694	0.144	0.015	0.039	0.024	0.052	0.046	0.044	0.038	0.006	7.17×10^{-5}	0.041
ROH10q11.2	10	69756164	69973521	0.112	0.020	0.013	0.042	0.050	0.011	0.168	0.029	0.084	1.43×10^{-4}	0.041
ROH17q22	17	47697111	47983255	0.128	-0.010	0.195	0.028	-0.073	0.002	0.020	-0.052	0.010	2.15×10^{-4}	0.041
ROH4p15.1	4	33074782	33314955	0.688	-0.009	0.130	0.754	-0.019	0.166	0.544	-0.037	4.48×10^{-4}	2.87×10^{-4}	0.041
ROH15q22.3	15	61969611	61976270	0.022	0.034	0.055	0.058	0.044	0.008	0.020	0.038	0.056	3.59×10^{-4}	0.041
ROH3q26.1	3	164400134	164706138	0.060	-0.027	0.014	0.048	-0.013	0.510	0.026	-0.048	0.008	4.30×10^{-4}	0.043
ROH8q11.2	8	50810643	50924141	0.342	0.004	0.424	0.234	0.022	0.020	0.024	0.059	0.002	5.02×10^{-4}	0.049
ROH21q22.1	21	32806261	32930538	0.056	0.031	0.006	0.024	0.046	0.065	0.020	0.025	0.213	5.74×10^{-4}	0.049
ROH3q25.3	3	159380085	159758744	0.070	-0.025	0.015	0.130	-0.013	0.261	0.026	-0.037	0.036	6.46×10^{-4}	0.049
ROH1p31.1	1	79445126	79859144	0.048	-0.025	0.037	0.048	-0.054	0.059	0.036	-0.028	0.060	7.17×10^{-4}	0.049
ROH11q12.1	11	55946817	56028414	0.078	-0.021	0.032	0.024	-0.035	0.193	0.028	-0.038	0.023	7.89×10^{-4}	0.049

Notes: Abbreviations: OOS, Omaha osteoporosis study; KCOS, Kansas-city osteoporosis study; COS, China osteoporosis study; CFS, China fracture study; FHS, Framingham heart study. Freq: frequency

^dPosition was relative to the hg18 version of the human genome.

^b*P*_{combined} means that the *P* value was combined by including the three GWAS BMD samples (KCOS, OOS, and COS) in the discovery stage.

All *P* values listed in Table 2 are two-sided.

Table 3

Association results of 6 significant ROHs

ROH	KCOS			OOS			COS			FHS		
	beta	P		beta	P		beta	P		beta	frequency	$P_{combined}^b$
ROH1q31.3	0.015	0.039		0.052	0.046		0.038	0.006		7.60×10^{-5}	0.120	3.17×10^{-8}
ROH1p31.1	-0.025	0.037		-0.054	0.059		-0.028	0.060		1.44×10^{-3}	0.114	3.77×10^{-6}
ROH3q26.1	-0.027	0.014		-0.013	0.510		-0.048	0.008		4.60×10^{-3}	0.064	6.74×10^{-6}
ROH11q12.1	-0.021	0.032		-0.035	0.193		-0.038	0.023		6.88×10^{-3}	0.082	1.65×10^{-5}
ROH21q22.1	0.031	0.006		0.046	0.065		0.025	0.213		9.29×10^{-3}	0.028	1.80×10^{-5}
ROH15q22.3	0.034	0.055		0.044	0.008		0.038	0.056		7.60×10^{-5}	0.024	6.29×10^{-5}

Notes: Abbreviations: OOS, Omaha osteoporosis study; KCOS, Kansas-city osteoporosis study; COS, China osteoporosis study; FHS, Framingham heart study.

^a All P values listed in Table 3 are two-sided.

^b $P_{combined}$ means that the P value was combined by including all the four BMD samples (KCOS, OOS, COS, and FHS) in the discovery and replication stages..

Table 4

Association results of 6 significant ROHs with osteoporotic hip fractures

ROH	Frequency	OR (95% CI)	<i>P</i>
ROH1q31.3	0.020	0.44 (0.14–1.44)	0.164
ROH1p31.1	0.114	3.71 (1.03–13.35)	0.032
ROH3q26.1	0.064	1.24 (1.04–2.07)	0.204
ROH11q12.1	0.138	1.26 (0.92–1.72)	0.247
ROH21q22.1	0.058	0.72 (0.29–1.81)	0.488
ROH15q22.3	0.024	0.59 (0.33–1.07)	0.078

Table 5

Results of expression quantitative trait locus (eQTL) analysis

ROH	genes	Caucasian			Chinese			Japanese		
		frequency	beta	<i>P</i> _{eQTL}	frequency	beta	<i>P</i> _{eQTL}	frequency	beta	<i>P</i> _{eQTL}
ROH1p31.1	LPHN2	0.116	-0.082	0.444	0.022	-0.134	0.078	0.022	-0.146	0.034
ROH1q12.1	OR5F1	0.100	-0.078	0.003	0.312	-0.017	0.297	0.346	0.039	0.021
ROH15q22.3	CSNK1G1	0.066	0.014	0.703	0.466	0.005	0.808	0.022	-0.045	0.039
	SNX1	0.066	0.013	0.920	0.466	-0.150	0.028	0.022	0.057	0.623
	TRIP4	0.066	-0.055	0.663	0.466	-0.118	0.092	0.022	-0.463	0.004
	USP3	0.066	-0.284	0.045	0.466	-0.083	0.179	0.022	-0.113	0.171