Published in final edited form as:
Osteoporos Int. 2012 February ; 23(2): 781–787. doi:10.1007/s00198-011-1626-x.

Genetic variants in the *SOX6* gene are associated with bone mineral density in both Caucasian and Chinese populations

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Abstract

Summary—Given the biological function of *SOX6* and recent genome-wide association finding, we performed a fine-mapping association analyses to investigate the relationship between *SOX6*

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Electronic supplementary material The online version of this article (doi:10.1007/s00198-011-1626-x) contains supplementary material, which is available to authorized users.

Conflicts of interest None.

and BMD both in Caucasian and Chinese populations. We identified many single-nucleotide polymorphisms (SNPs) within or near the *SOX6* gene to be significantly associated with hip bone mineral density (BMD).

Introduction—*SOX6* gene is an essential transcription factor in chondrogenesis and cartilage formation. Recent genome-wide association studies (GWAS) detected a SNP (rs7117858) located at the downstream of *SOX6* significantly associated with hip BMD.

Methods—Given the biological function of *SOX6* and the GWAS finding, we considered *SOX6* as a new candidate for BMD and osteoporosis. Therefore, in this study, we performed a fine-mapping association analyses to investigate the relationship between SNPs within and near the *SOX6* gene and BMD at both hip and spine. A total of 301 SNPs were tested in two independent US Caucasian populations (2,286 and 1,000 unrelated subjects, respectively) and a Chinese population (1,627 unrelated Han subjects).

Results—We confirmed that the previously reported rs7117858-A was associated with reduced hip BMD, with combined *P* value of 2.45×10^{-4}. Besides this SNP, we identified another 19 SNPs within or near the *SOX6* gene to be significantly associated with hip BMD after false discovery rate adjustment. The most significant SNP was rs1347677 located at the intron 3 ($P=3.15\times10^{-7}$). Seven additional SNPs in high linkage disequilibrium with rs1347677 were also significantly associated with hip BMD. SNPs in *SOX6* showed significant skeletal site specificity since no SNP was detected to be associated with spine BMD.

Conclusion—Our study identified many SNPs in the *SOX6* gene associated with hip BMD even across different ethnicities, which further highlighted the importance of the *SOX6* gene influencing BMD variation and provided more information to the understanding of the genetic architecture of osteoporosis.

Keywords

Association; BMD; Osteoporosis; *SOX6*

Introduction

Osteoporosis is a serious public health problem. It is a metabolic skeletal disease mainly characterized by low bone mass and microarchitectural deterioration of bone tissue, with the consequent increase in the risk of fragility fractures [1]. Clinically, bone mineral density (BMD) is the single best predictor of osteoporotic fractures, and has been widely used as a reference standard for the description of osteoporosis [2, 3]. BMD is a highly heritable quantitative trait. Previous studies have demonstrated that approximately 50% to 80% of the variation in BMD can be explained by genetic factors [4–6].

SOX6 gene (SRY-box 6) encodes a member of the D subfamily of sex determining region y-related transcription factors, which is characterized by a conserved DNA-binding domain termed the high-mobility group box. SOX6 is an essential transcription factor in chondrogenesis and cartilage formation [7–10]. This gene was found to cooperate with *Col2a1* in chondrogenesis [11] and mediate BMP2 in the activation and maintenance of chondrogenesis during murine fracture healing [12]. *SOX6* was also found to have

significant differential expression during osteoblast development [13]. Interestingly, in human, recent genome-wide association studies (GWAS) [13, 14] and a replication study [15] have reported that an SNP (rs7117858) located at the downstream of the *SOX6* gene was significantly associated with hip BMD. Given the biological function of *SOX6* and this GWAS finding, we thought that the *SOX6* gene could be a new candidate for elucidating the genetic impact on BMD. We further raise a question that whether other SNPs within the *SOX6* gene are also associated with BMD. Investigating the SNPs within the *SOX6* gene may provide further insights to understand the relationship between this gene and BMD. In addition, replication of genetic associations in independent populations is essential to evaluate a positive finding and to further explore the role of these variants in the complex traits. Therefore, the aim of this study was to conduct a fine-mapping association analyses to investigate the relationship between SNPs located within and near the *SOX6* gene and BMD. Our study was performed in three sample sets from two ethnicities, including two US Midwestern Caucasian populations and a Midwestern Chinese Han population, in order to see whether the variants identified are common or ethnicity-specific.

Materials and methods

Subjects

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

Caucasian samples 1 and 2—Caucasian samples 1 and 2 contained 2,286 and 1,000 unrelated adults, respectively. These unrelated subjects from both samples were identified from our established and expanding database containing more than 10,000 subjects. All of the chosen subjects were US Caucasians of Northern European origin living in Midwestern area. Subjects with chronic diseases and conditions that might potentially affect bone mass, structure, or metabolism were excluded. These diseases/conditions included chronic disorders involving vital organs (heart, lung, liver, kidney, brain), serious metabolic diseases (diabetes, hypo- and hyper-parathyroidism, hyperthyroidism, etc.), other skeletal diseases (Paget's disease, osteogenesis imperfecta, rheumatoid arthritis, etc.), chronic use of drugs affecting bone metabolism (hormone replacement therapy, corticosteroid therapy, anti-convulsant drugs), and 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 these exclusions was to minimize the influence of known environmental and therapeutic factors on bone variation. BMD values at hip and spine were measured using Hologic 4500W machines (Hologic Inc., Bedford, MA, USA) that were calibrated daily. The coefficient of variation (CV) values of the dual-energy X-ray absorptiometry (DXA) measurements for spine and hip BMDs were approximately 1.98% and 1.87%, respectively.

Chinese sample—The Chinese sample consisted of 1,627 unrelated subjects. The subjects were recruited from Midwestern Chinese Han adults living in Xi'an and Changsha cities. The exclusion criteria were the same as with Caucasian samples. BMD at hip and

spine was measured using the same model Hologic 4500W machines (Hologic Inc., Bedford, MA, USA) under the same strict protocols used in the Caucasian sample. The CV values of the DXA measurements for spine and hip BMDs were approximately 1.01% and 1.33%, respectively.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. For Caucasian sample 1 and Chinese sample, SNP genotyping was performed using Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA), according to the Affymetrix protocol. Briefly, approximately 250 ng of genomic DNA was digested with restriction enzyme NspI or StyI. Digested DNA was adaptor-ligated and polymerase chain reaction (PCR)-amplified for each sample. Fragment PCR products were then labeled with biotin, denatured, and hybridized to the arrays. Arrays were then washed and stained using Phycoerythrin on Affymetrix Fluidics Station, and scanned using the GeneChip Scanner 3000 7G to quantitate fluorescence intensities. Data management and analyses were conducted using the Genotyping Command Console. Only samples with a minimum call rate of 95% were included. Due to efforts of repeat experiments, all samples met this criteria and the final mean call rate reached a high level of 98.93% for Caucasian sample 1 and 98.96% for Chinese sample. For the Caucasian sample 2, SNP genotyping was performed using the Affymetrix Human Mapping 500K array set, which has been finished in our previous experiments [16]. SNPs that deviated from Hardy–Weinberg equilibrium (HWE, $P<0.0001$) and had a minor allele frequency <0.01 were discarded in each sample set. Thus, 301 SNPs located within and near the *SOX6* gene were included for subsequent association analyses. The basic characteristics of these SNPs are summarized in Supplementary Table 1.

Statistical analyses

Before association analyses, principal component analysis implemented in EIGENSTRAT [17] 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 residues were used for association analyses. For the Caucasian sample 1 and the Chinese sample, a linear regression implemented in PLINK [18] was fitted to test for association assuming an additive inheritance model. For the Caucasian sample 2, imputation was used to evaluate associations for the same SNPs across study populations using different Affymetrix arrays. Thus, IMPUTE program [19] was utilized to impute the genotypes of SNPs detected on Array 6.0 but not on 500K array set based on HapMap data (release 22). To ensure the reliability of the imputation, all of those imputed SNPs have reached a calling threshold of 0.90, i.e., a 90% probability that an imputed genotype is true. SNPTEST [19] was used to test for associations in this sample.

Meta-analysis calculations were done using the METAL software package (http://genome.sph.umich.edu/wiki/METAL_Documentation) taking into account sample size and direction of effect. SNAP was used to characterize linkage disequilibrium (LD) and depict the regional association plot [20]. A raw *P* value of <0.05 in our study was considered

nominally significant, which were further subjected to a false discovery rate (FDR) of Benjamini and Hochberg procedure [21] to account for multiple comparisons.

Results

The basic characteristics of the study subjects are presented in Table 1. We summarized the major association results of *SOX6* with hip BMD in Table 2. After association tests and multiple testing corrections, 20 SNPs showed significant results with hip BMD, with *P* values ranging from 9.12×10^{-4} to 3.15×10^{-7} (Table 2). The directions of effect for each significant SNP were perfectly consistent in Caucasian samples 1, Caucasian sample 2, and Chinese sample. Among these 20 SNPs, 15 SNPs located within the *SOX6* gene, the other 5 SNPs located at the downstream of the *SOX6* gene.

The most significant SNP was rs1347677 located at the intron 3 of *SOX6*, with the *P* values of 2.33×10^{-4}, 2.37×10^{-3}, and 2.69×10^{-2} in Caucasian sample 1, Caucasian sample 2, and Chinese sample, respectively. After meta-analysis by METAL software package, the combined *P* value achieved a highly significant level of 3.15×10^{-7}. The rs1347677-T was associated with reduced hip BMD values with the effect size (beta) of −0.0173, −0.0205, and −0.0094 in Caucasian sample 1, Caucasian sample 2, and Chinese sample, respectively. According to the FASTSNP program (http://fastsnp.ibms.sinica.edu.tw), rs1347677 is located at intronic enhancer region. A change of "G→T" at rs1347677 may lead to creation of binding sites for transcription factors *GATA-1*, *GATA-2*, *S8*, and *CdxA*. There were seven additional SNPs (rs297366, rs10219384, rs11023907, rs12274377, rs10832576, rs12798980, rs1837096, and rs2028162) showed significant association signals with hip BMD around the top significant SNP rs1347677. The *P* values of these SNPs were ranged from 7.32×10^{-4}–1.49×10^{-6} (Table 2). We characterized the LD for these SNPs using the regional association plot. As shown in Fig. 1b, these SNPs were in high LD with the top significant SNP rs1347677.

For the previously reported SNP rs7117858 identified by GWAS [13, 14], significant association was successfully replicated in our study samples, with combined *P* value of 2.45×10^{-4}. The rs7117858-A was associated with reduced hip BMD value with the beta values of −0.01, −0.0063, and −0.015 in Caucasian sample 1, Caucasian sample 2, and Chinese sample, respectively. The frequency of rs7117858-A detected in our study was consistent with that previously reported. Interestingly, beside the SNP rs7117858, we detected another four SNPs (rs1531903, rs7108738, rs16931831, and rs11827785) around rs7117858 significantly associated with hip BMD. The *P* values of these four SNPs were 6.55×10^{-4} to 4.92×10^{-5} (Table 2). These SNPs had high LD with the SNP rs7117858 (Fig. 1a).

Previous reports [14, 15] found that the variation in *SOX6* was skeletal site specific, since the significant signal was only detected from the SNP associated with hip BMD but not with spine BMD. Our study obtained consistent results. No SNP was detected to be significantly associated with spine BMD.

Discussion

SOX6 is a newly identified candidate gene for osteoporosis. A SNP (rs7117858) located at the downstream of the *SOX6* gene was reported to be associated with hip BMD by recent GWAS [13, 14] and a replication study [15]. In addition, our group has detected an SNP rs11023787 in the *SOX6* to be associated with wrist bone mass by GWAS in the Caucasian sample 2 [22]. Our group has also found two SNPs (rs297325 and rs4756846) associated with BMI and hip BMD by bivariate GWAS in the Caucasian sample 2 [23]. However, most GWAS focused only on those SNPs of top-ranking statistical significance, which may ignore some useful information. In this study, combining all the sample sets in our group, we performed a fine-mapping association study to investigate the relationship between SNPs within and near the *SOX6* gene and BMD at both hip and spine. A group of SNPs of the *SOX6* gene were identified to be significantly associated with hip BMD both in the Caucasian and Chinese populations. Our results further supported the potential contribution of *SOX6* to the variation of BMD and the pathogenesis of osteoporosis.

The *SOX6* gene is expressed in various tissues, most abundantly in skeletal muscle. *SOX6* is a key transcription factor in chondrogenesis and cartilage formation [7–10]. During embryonic development, trunk, limbs and the majority of the craniofacial skeleton are developed through endochondral bone formation. The process of endochondral bone formation is a complex one that contains multiple stages, including mesenchymal cells differentiating into cartilage cells, pre-hypertrophic chondrocytes, and hypertrophic chondrocytes, as well as mesenchymal cells surrounding hypertrophic chondrocytes differentiating into osteoblasts [24]. A series of molecular signals play important roles in regulating these multiple stages, including genes in the SOX family. For example, SOX6 has been shown to induce chondrocyte hypertrophy and permit formation of prehypertrophic and hypertrophic zones [9]. $Sox6^{-/-}$ mice are born with cartilage defects [24]. Recently, Hsu et al. have found significant differential expression for *SOX6* during osteoblast development [13]. Taking into account our association findings and the above lines of evidence, *SOX6* might affect BMD or osteoporosis through regulating endochondral bone formation. However, the above mechanism is still speculative and needs extensive functional studies for final validation.

It is necessary to evaluate the association findings in different populations from different ethnicities, since the genomic variation is greater when compared across ethnicities. Our study successfully replicated the association between the previously identified SNP rs7117858 and hip BMD [13–15], which demonstrated the validity of the initial finding. Besides this SNP, we also identified another 19 SNPs of *SOX6* significantly associated with hip BMD both in the Caucasian and Chinese populations and the effect directions were perfectly consistent, suggesting that the *SOX6* gene might be a common variant for BMD even across different ethnicities.

Our results, together with previous findings [13–15] showed that the association between variations of *SOX6* and BMD was skeletal site specific. Significant association signals were only detected for hip BMD but not for spine BMD. Hip fracture and spine fracture occurred most frequently in human; however, the genetic correlation of these two sites was less 0.8 in

both females and males [25]. The *SOX6* gene might belong to the 20% of genetic determinations of hip BMD that was not shared with spine.

In summary, we performed a fine-mapping association analysis for the *SOX6* gene and BMD. We confirmed that the SNP rs7117858 located at the downstream of *SOX6* was significantly associated with hip BMD both in Chinese Han and Caucasian populations. Importantly, we detected 15 SNPs located in the introns of *SOX6* and 4 more SNPs located at the downstream of *SOX6* to be significantly associated with hip BMD. Our findings further highlighted the importance of the *SOX6* gene influencing BMD variation and provided more information to the understanding of the genetic architecture of osteoporosis.

Supplementary Table 1

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from NIH (R01 AR050496, R21 AG027110, R01 AG026564, R01 AR057049-01A1 and R21 AA015973) and a SCOR (Specialized Center of Research) grant (P50 AR055081). The study was also funded by grants from National Natural Science Foundation of China (81000363, 31000554), the PhD. Programs Foundation of Ministry of Education of China (20100201120058), the Fundamental Research Funds for the Central Universities, Shanghai Leading Academic Discipline Project (S30501), a grant from Ministry of Education to ShangHai University of Science and Technology, and startup funds from University of Shanghai for Science and Technology and Xi'an Jiaotong University, and the Ministry of Education of China.

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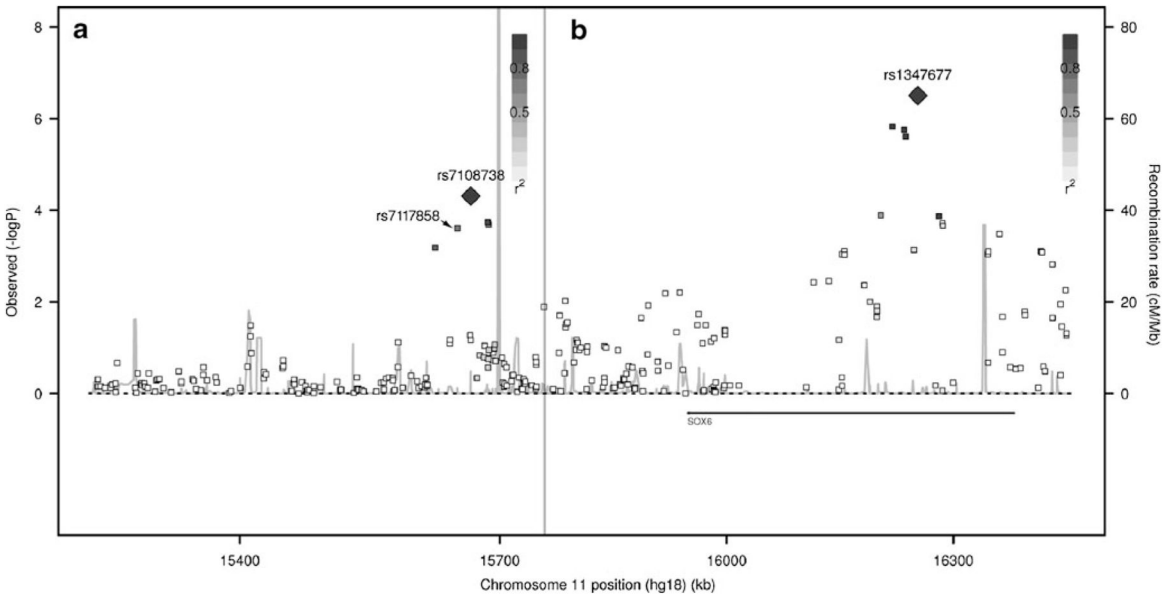

Fig. 1.

Regional association plot for *SOX6* on chromosome 11. In the *left of grey line* (**a**), r^2 of pairwise LD is calculated between rs7108738 and other SNPs in the left of grey line. In the *right of grey line* (**b**), r^2 of pairwise LD is calculated between rs1347677 and other SNPs in the right of grey line. There is no LD between rs7108738 and rs1347677

Table 1

Basic characteristics of the study subjects

Trait	Caucasian sample 1	Caucasian sample 2	Chinese sample
Number	2,286	1,000	1,627
Age (years)	51.37 (13.76)	50.23 (18.24)	34.49 (13.24)
Weight (kg)	75.27 (17.54)	80.16 (17.79)	60.12 (10.48)
Height (cm)	166.35 (8.47)	170.83 (9.74)	164.25 (8.16)
Female/male	1727/558	501/499	825/802
Hip BMD (g/cm^2)	0.968 (0.175)	0.973 (0.156)	0.920 (0.134)
Spine BMD (g/cm^2)	1.025 (0.157)	1.032 (0.164)	0.947 (0.127)

Data are shown as mean (standard deviation, SD).

Table 2

Significant association results for 20 SNPs in SOX6 with hip BMD

SNP	Position	Genic position	A1/A2	Caucasian sample 1				Caucasian sample 2				Chinese sample				$P_{combine}$
				Freq	BETA	SE	*P* value	Freq	BETA	SE	*P* value	Freq	BETA	SE	*P* value	
rs1531903	15625402	Downstream	C/G	0.160	0.0089	0.0049	7.09×10^{-2}	0.159	0.0132	0.0074	7.35×10^{-2}	0.181	0.0120	0.0051	1.89×10^{-2}	6.55×10^{-4}
rs7117858	15651038	Downstream	G/A	0.206	0.0100	0.0045	2.72×10^{-2}	0.220	0.0063	0.0067	3.46×10^{-1}	0.181	0.0151	0.0051	3.20×10^{-3}	2.45×10^{-4}
rs7108738	15666660	Downstream	C/A	0.177	0.0092	0.0048	5.45×10^{-2}	0.169	0.0105	0.0076	1.70×10^{-1}	0.153	0.0199	0.0055	3.27×10^{-4}	4.92×10^{-5}
rs16931831	15686130	Downstream	T/C	0.167	0.0094	0.0048	5.14×10^{-2}	0.174	0.0105	0.0071	1.40×10^{-1}	0.179	0.0152	0.0051	3.02×10^{-3}	1.84×10^{-4}
rs11827785	15687087	Downstream	C/A	0.199	0.0108	0.0046	1.83×10^{-2}	0.212	0.0072	0.0068	2.90×10^{-1}	0.180	0.0142	0.0051	5.60×10^{-3}	2.07×10^{-4}
rs7118395	16155641	Intron6	C/T	0.499	0.0085	0.0037	2.15×10^{-2}	0.492	0.0009	0.0053	8.60×10^{-1}	0.294	0.0125	0.0043	3.82×10^{-3}	7.83×10^{-4}
rs2028162	16204051	Intron4	G/A	0.260	0.0136	0.0042	1.19×10^{-3}	0.242	0.0062	0.0070	3.71×10^{-1}	0.302	0.0090	0.0043	3.39×10^{-2}	1.30×10^{-4}
rs1837096	16219450	Intron3	G/A	0.199	0.0161	0.0046	4.73×10^{-4}	0.205	0.0179	0.0067	7.60×10^{-3}	0.303	0.0093	0.0043	2.89×10^{-2}	1.49×10^{-6}
rs12798980	16234929	Intron3	C/G	0.197	0.0166	0.0046	3.44×10^{-4}	0.204	0.0173	0.0067	1.04×10^{-2}	0.305	0.0090	0.0042	3.41×10^{-2}	1.75×10^{-6}
rs10832576	16236880	Intron3	A/T	0.199	0.0170	0.0046	2.33×10^{-4}	0.205	0.0175	0.0067	9.38×10^{-3}	0.305	0.0080	0.0043	6.14×10^{-2}	2.45×10^{-6}
rs12274377	16247709	Intron3	A/T	0.425	−0.0079	0.0037	3.56×10^{-2}	0.422	−0.0133	0.0057	2.00×10^{-2}	0.420	−0.0065	0.0041	1.12×10^{-1}	7.32×10^{-4}
rs1347677	16252988	Intron3	G/T	0.196	0.0173	0.0047	2.33×10^{-4}	0.200	0.0205	0.0067	2.37×10^{-3}	0.304	0.0094	0.0043	2.69×10^{-2}	3.15×10^{-7}
rs11023907	16281025	Intron3	T/G	0.196	0.0120	0.0048	1.24×10^{-2}	0.205	0.0161	0.0068	1.73×10^{-2}	0.300	0.0080	0.0043	6.62×10^{-2}	1.36×10^{-4}
rs10219384	16285545	Intron3	T/C	0.424	−0.0085	0.0037	2.28×10^{-2}	0.410	−0.0142	0.0055	1.06×10^{-2}	0.412	−0.0073	0.0040	7.01×10^{-2}	1.93×10^{-4}
rs297366	16286125	Intron3	C/T	0.425	−0.0082	0.0037	2.70×10^{-2}	0.413	−0.0134	0.0055	1.54×10^{-2}	0.412	−0.0077	0.0040	5.42×10^{-2}	2.19×10^{-4}
rs297326	16346308	Intron1	T/G	0.289	0.0040	0.0040	3.21×10^{-1}	0.269	0.0183	0.0060	2.46×10^{-3}	0.487	0.0089	0.0040	2.42×10^{-2}	7.98×10^{-4}
rs297324	16345860	Intron1	A/G	0.291	0.0047	0.0040	2.38×10^{-1}	0.272	0.0185	0.0059	1.81×10^{-3}	0.505	0.0076	0.0040	5.46×10^{-2}	9.12×10^{-4}
rs297339	16360859	Intron1	A/G	0.289	0.0044	0.0041	2.89×10^{-1}	0.271	0.0191	0.0059	1.37×10^{-3}	0.494	0.0099	0.0040	1.45×10^{-2}	3.29×10^{-4}
rs10832606	16416136	Intron1	C/T	0.292	0.0041	0.0040	3.02×10^{-1}	0.270	0.0193	0.0059	1.19×10^{-3}	0.488	0.0081	0.0039	3.90×10^{-2}	7.86×10^{-4}
rs11023944	16417484	Intron1	A/C	0.293	0.0044	0.0040	2.68×10^{-1}	0.271	0.0195	0.0059	1.02×10^{-3}	0.505	0.0075	0.0039	5.41×10^{-2}	8.25×10^{-4}

Freq, frequency is shown for allele A1. For the Caucasian sample 1 and the Chinese sample, a linear regression implemented in PLINK was fitted to test for association assuming an additive inheritance model. For the Caucasian sample 2, IMPUTE program was utilized to impute the genotypes of SNPs detected on Array 6.0 but not on 500K array set based on HapMap data (release 22). SNPTEST was used to test for associations in this sample. Meta-analysis was done using the METAL software package taking into account sample size and direction of effect ($P_{combine}$).