Published in final edited form as:
*Bone*. 2013 March ; 53(1): 129–133. doi:10.1016/j.bone.2012.11.028.

# Genome-Wide Association Study Identified *UQCC* Locus for Spine Bone Size in Humans

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## Abstract

Bone size (BS) contributes significantly to the risk of osteoporotic fracture. Osteoporotic spine fracture is one of the most disabling outcomes of osteoporosis. This study aims to identify genomic loci underlying spine BS variation in humans.

We performed a genome-wide association scan in 2,286 unrelated Caucasians using Affymetrix 6.0 SNP arrays. Areal BS ($cm^2$) at lumbar spine was measured using dual energy X-ray absorptiometry scanners. SNPs of interest were subjected to replication analyses and meta-analyses with additional two independent Caucasian populations (N = 1,000 and 2,503) and one Chinese population (N = 1,627).

In the initial GWAS, 91 SNPs were associated with spine BS ($P$<1.0E-4). Eight contiguous SNPs were found clustering in a haplotype block within *UQCC* gene (**u**bi**q**uinol-**c**ytochrome **c**reductase complex chaperone). Association of the above eight SNPs with spine BS were replicated in one Caucasian and one Chinese populations. Meta-analyses (N = 7,416) generated much stronger association signals for these SNPs (e.g., $P$ = 1.86E-07 for SNP rs6060373), supporting association of *UQCC* with spine BS across ethnicities.

This study identified a novel locus, i.e., the *UQCC* gene, for spine BS variation in humans. Future functional studies will contribute to elucidating the mechanisms by which *UQCC* regulates bone growth and development.



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**Keywords**

Spine bone size; GWAS; UQCC

Bone size (BS) is one of the major indexes of bone strength, and BS contributes significantly to the risk of osteoporotic fracture [1, 2]. Osteoporotic fracture at spine is one of the most disabling outcomes of osteoporosis in the elderly.

BS variation is under strong genetic determination, with a heritability of ~0.5–0.6 [3]. Specific genes underlying variation of BS are largely unknown. A limited number of candidate genes, such as *VDR*[4], *COL1A2*[5], and *CYP17*[6], were tested for associations with BS variation. Compared to these candidate gene association studies, which rely on existing biological/functional evidence for selected candidate genes, genome-wide association scan (GWAS) study is hypothesis-free and much more powerful in rapid and systematic identification of genes for complex traits or diseases of interest.

To identify genomic loci underlying variation of spine BS in humans, we performed a GWAS study, by covering 909,622 SNPs across human genome, in a population of 2,286 unrelated Caucasians. We identified **u**bi**q**uinol-**c**ytochrome **c**reductase complex chaperone (*UQCC*) as an important locus associated with spine BS variation in Caucasians. Meta-analyses suggested a general effect of the *UQCC* locus on spine BS across ethnic boundary.

## MATERIALS AND METHODS

### Samples

The study was approved by Institutional Review Board or Research Administration of the involved institutions. Signed informed-consent documents were obtained from all study participants before entering the study. For the study samples CAU-1, CAU-2, and CHN, areal BS ($cm^2$)at lumbar spine ($L_{1–4}$) was measured using Hologicdual energy X-ray absorptiometry (DEXA) scanners. The DEXA scanners were calibrated daily using a standard phantom. The coefficient of variation (CV) of repeated DEXA measurement of BS was about 1.94%.

**Initial GWAS sample (CAU-1)—**A total of 2,286 random unrelated subjects were recruited in Midwestern US in Kansas City, Missouri and Omaha, Nebraska. All identified subjects were Caucasians of European origin. This sample is for discovery of interesting loci for BS variation.

**Replication study sample (CAU-2)—**This sample was composed of 1,000 unrelated Caucasians, which is independent of the initial GWAS sample. This 2$^{nd}$ Caucasian sample was selected from our established and expanding genetic database currently including more than 7,000 subjects and largely recruited in Midwestern US in Omaha, Nebraska. All the selected subjects were Caucasians of European origin.

**Replication study sample (CAU-3)—**This sample came from the dbGaP Framingham Heart Study (FHS). The sample used herein was composed of 2,503 Caucasians with both phenotype data and genotype data of interest available. Specifically, information of age, height (to next lower ¼ inch originally), weight (to nearest pound originally), whole body scan lumbar bone mineral content (BMC) and bone mineral density (BMD) was extracted from exam 22 or exam 23 for FHS ‘original cohort’ and extracted from exam 6 or exam 7 for FHS ‘offspring cohort’, respectively. Lumbar BS ($cm^2$) was calculated by using lumbar BMC (g) divided by lumbar BMD ($g/cm^2$).

**Replication study sample (CHN)—**This sample was composed of 1,627 unrelated subjects. All the subjects belong to Chinese Han ethnicity.

## SNP Genotyping

**GWAS sample—**Genomic DNA was extracted from peripheral blood white cells using DNA isolation kit (Gentra systems, Minneapolis, MN, USA). SNP genotyping was performed with Affymetrix Genome-Wide Human SNP Array 6.0 following standard protocol of the manufacturer. Fluorescence intensities were quantified using an Affymetrix array scanner 3000 7G. Data management and analyses were performed using the Affymetrix® GeneChip® Command Console®. Contrast quality control (QC) measures how well experiments resolve SNP signals into three genotype clusters. Contrast QC threshold was set at the default value (≥0.4). Subjects with contrast QC less than 0.4 were subject to re-genotyping. The final average contrast QC across the entire sample was as high as 2.32. Out of the initial full-set of 909,622 SNPs, 21,247 SNPs' allele frequencies deviated from Hardy-Weinberg equilibrium ($P < 0.01$), and additional 141,666 SNPs had minor allele frequencies (MAF) less than 1.0%. Therefore, a final set of 746,709 SNPs was retained for subsequent association analyses, and yielded an average spacing of ~4.0 kb throughout the human genome.

**Replication study samples—**For the CAU-2 sample, genotyping of SNPs of interest was performed with the Affymetrix Mapping 250 k Nsp and 250 k Sty arrays in Vanderbilt Microarray Shared Resources (VMSR) (http://array.mc.vanderbilt.edu/) using standard protocol recommended by Affymetrix. For the CAU-3 sample from FHS, SNPs of interest were genotyped using the Affymetrix Mapping 500 k arrays. For the CHN sample, SNPs genotyping was performed with Affymetrix Genome-Wide Human SNP Array 6.0 using standard protocol.

## Statistical Analyses

**GWAS analyses—**Age and gender were used to adjust raw BS measurements using SAS (SAS Institute Inc., Cary, NC). Then, command "--assoc" in the PLINK [7] was used to perform quantitative trait association tests (Wald test), i.e., under additive model to test the phenotypic difference among carriers of three different genotypes for each SNP. Two methods were used to correct for population stratification in the above GWAS association analyses. Firstly, the Genomic Control (GC) method [8] was used to calculate "inflation factor'. The calculated inflation factor in the initial GWAS population is 1.35, which was used to correct the GWAS p values. Secondly, EIGENSTRAT program was employed to perform principal component analyses (PCA). We used ~ 700,000 SNPs to calculate principal components, and ten default main eigenvectors generated by EIGENSTRAT were used as covariates to adjust population stratification in GWAS analyses by PLINK.

**Haplotype block analyses—**Linkage disequilibrium (LD) [standardized $D'$ ($D/D_{max}$)] patterns for SNPs of interest were analyzed using SNP genotypes in the initial GWAS sample, and plotted using the Haploview program [9] (http://www.broad.mit.edu/mpg/haploview/).

**Replication and meta-analyses—**Focused association analyses for 91 SNPs of interest ($P$<1.0E-4 in discovery sample) were performed using PLINK [7] in each of the three replication samples, respectively. Fisher's combined $p$ method [10] was used to integrate association signals from our four study populations (one discovery and three replication samples) and to ascertain the significance of SNPs of interest on spine BS across populations. The following formula was used to calculate the statistic:

$$X^2_{2k} = -2\sum_{i=1}^{k} \log_e(p_i).$$

where, $2k$ is the degree of freedom of the $X^2$ statistic, and $k$ is the number of tests being combined, herein it is 4.

**Power analyses—**We employed the Genetic Power Calculator (available at http://pngu.mgh.harvard.edu/~purcell/gpc/qtlassoc.html) to assess power of association tests in the discovery cohort of 2,286 unrelated individuals and in the meta-analyses cohorts involving 7,416 unrelated individuals. Herein, we assume strong linkage disequilibrium ($D' = 0.95$) and equal minor allele frequency for QTLs and SNP markers. QTL effect size was tested at 0.5–1.2% variance. The significance level is set at 1.0E-4 and 1.0E-7 for GWAS discovery and meta-analyses replication, respectively.

## RESULTS

Basic characteristics of the four population samples, for discovery and replication respectively, were summarized in Table 1. Quantile-quantile (Q-Q) plot for 746,709 SNPs tested in the initial GWAS showed that the observed *P* values matched the expected *P* values over the range of $1.0 < -\log_{10}(P) < 4.3$ (Figure 1). Figure 2 showed the distribution of GWAS *P* values for the SNPs across the chromosomes. Figure 3 showed the power of association analyses in the GWAS discovery cohort and meta-analyses cohorts. In the discovery cohort, at significance level of 1.0E-4, we have ≥75% power to detect a QTL with additive effect ≥1.0%. With a sample size of 7,416 in our meta-analyses cohorts, at significance level of 1.0E-7, we have ≥99.8% power to detect a QTL with additive effect ≥1.0%.

As presented in Table 2 and Supplemental Table 1, a total of 91 SNPs across the human genome, including 8 SNPs in UQCC (Table 2), had moderate association signals for spine BS with cutoff significance level at $P < 1.0E\text{-}4$. Of these 91 SNPs, 60 SNPs were randomly distributed across the genome. The remaining 31 SNPs were relatively tightly clustered into three distinct genomic regions. Two of these regions failed to be replicated in the CAU-2 and CHN population samples. One of the regions is covered by 11 contiguous intron SNPs in the *SORCS3* gene (sortilin-related VPS10 domain containing receptor 3), with initial *P* values ranging from 3.19E-05 to 8.52E-05. The other region is covered by 12 contiguous intron SNPs in the *PLEKHG1* gene (pleckstrin homology domain containing, family G member 1), with initial *P* values ranging from 6.74E-06 to 7.33E-05 (Supplemental Table 1).

The most interesting region is covered by eight contiguous SNPs (rs6060369, rs6088791, rs6060373, rs2425062, rs2248393, rs4911178, rs1570004, rs6142358), which are located in the intron of *UQCC* gene, with *P* values ranging from 1.64E-05 to 7.11E-05 in initial GWAS tests (Table 2). Correction for population stratification using either GC or PCA methods did not qualitatively alter association results for the eight SNPs (Table 2). The association of the eight SNPs with spine BS was replicated in the CAU-3 and CHN populations. However, none of these SNPs showed significant association with spine BS in the CAU-2 population at nominal level of 0.05. Furthermore, we found that these SNPs exert consistent direction of effects among all the four study population samples. The discrepant association among CAU-1 and CAU-2 implies different effect size of SNPs in the two populations. In addition, heterogeneity tests using the Cochrane's Q statistic and $I^2$ heterogeneity index suggest significant heterogeneity for these SNPs between the CAU-1 and CAU-2 populations (Table 2), which may partially explain the discrepant association between these two populations. Through meta-analyses, the eight SNPs attained much

stronger association signals (combined *P* values) than that in respective test (Table 2), suggesting a general effect of *UQCC* on spine BS across ethnic boundary. Figure 4 illustrates the LD patterns of the eight SNPs in the initial GWAS population. Those SNPs, with strong linkage disequilibrium with each other, fall within one haplotype block in the *UQCC* gene. $R^2$ estimation showed that each of these SNPs explains about 0.7% variation in spine BS in the GWAS population. According to the β coefficient, minor allele was positively associated with spine BS (i.e., carriers of minor allele tend to have large bone size than those with major allele). For example, as showed in Figure 5, carriers of minor allele C of rs6060373 have larger spine bone size than carriers of major allele T.

## DISCUSSION

The present GWAS study represents our continuous effort to identify genes underlying BS variation in humans. Our previous GWAS study on BS, interrogating ~500,000 SNPs covering human genome, identified four SNPs in the *PLCL1* gene for hip BS in female Caucasians [11]. The present GWAS study aimed to identify genetic loci underlying BS at a different skeletal site, i.e., lumbar spine, and with denser SNPs covering human genome. This study was attempted to add new knowledge to genetic basis of BS variation in humans. Through meta-analyses in four independent population samples involving 7,416 subjects, the current study suggested *UQCC* as an important locus determining spine BS variation in humans.

*UQCC* gene encodes a trans-membrane protein ubiquinol-cytochrome c reductase complex chaperone. Biological functions of *UQCC* in bone have yet to be demonstrated. Interestingly, an important candidate gene i.e., growth/differentiation factor 5 (GDF5), which is nearby the *UQCC* locus, is known to be closely relevant to bone biology.

GDF5 is a member of the TGF-beta superfamily of growth factors/signaling molecules that regulate cell growth and differentiation in both embryonic and adult tissues. Several mutations in this gene were associated with skeletal malformations including acromesomelic dysplasia, Hunter-Thompson type, brachydactyly, type C and chondrodysplasia Grebe type [12–14]. A SNP (rs143383) in *GDF5* gene was associated with the susceptibility of osteoarthritis (OA), which is characterized by degeneration of articular cartilage [15].

A GWAS study reported that common variants in the GDF5-UQCC locus contribute to variation of height, a measurable index of skeletal growth and development [16]. A longitudinal study in the northern Finland birth cohort 1966 assessed the associations between height growth and common variants identified by GWAS studies for height, and found that *UQCC* was associated with peak height velocity in infancy [17]. A GWAS meta-analysis evaluated the contribution of height loci to the upper- (trunk) and lower- body (hip axis and femur) skeletal components of height, and found *UQCC* associated with hip axis length [18]. In the initial GWAS population, significant association was also detected between UQCC SNPs and height (P<1.0E-4). The directions of effect for the height trait are similar to that of spine BS trait. Then, we performed association analysis for SNPs and spine BS using height as a covariate. As shown in Table 2, after correction for height, the association signals for spine BS dramatically decreased but remain significant. The data suggest that the UQCC gene play pleiotropic effects on both height and bone size. Height and bone size are indexes of skeletal length and skeletal area, respectively. Our data, taken together, suggested the importance of *UQCC* locus for the growth and development of the human skeleton. To the best of our knowledge, the present GWAS study firstly identified *UQCC* locus for spine BS variation in humans.

In summary, this GWAS study identified a novel locus *UQCC* for spine BS in humans. The *UQCC* locus has ethnic-general effects on spine BS variation. Focused molecular functional studies will contribute to elucidating the mechanisms by which the *UQCC* locus regulates bone growth and development.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The study was partially supported by grants from National Institutes of Health (R01AG026564, R01AR050496, R01AR057049, and R03TW008221), a SCOR (Specialized Center of Research) grant (P50 AR055081) supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and the Office of Research on Women's Health (ORWH), Natural Science Foundation of China (NSFC) (30600364, 30771222, and 30900810). The Framingham Heart Study is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with Boston University (Contract No. N01-HC-25195). This manuscript was not prepared in collaboration with investigators of the Framingham Heart Study and does not reflect the opinions or views of the Framingham Heart Study, Boston University, or NHLBI. Funding for genotyping of the FHS sample was provided by NHLBI Contract N02-HL-64278. Funding support for the Framingham Whole Body DXA dataset was provided by NIH grants R01 AR/AG 41398, R01 AR050066 and R03 AG20321.

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## Highlights

- Bone size (BS) contributes significantly to the risk of osteoporotic fracture.
- A genome-wide association scan was performed in 2,286 unrelated Caucasians.
- Contiguous SNPs within *UQCC* gene were associated with BS.
- Subsequent meta-analyses (N = 7,416) generated stronger association signals for these SNPs.
- We identified a novel locus (*UQCC* gene) for spine BS variation in humans.

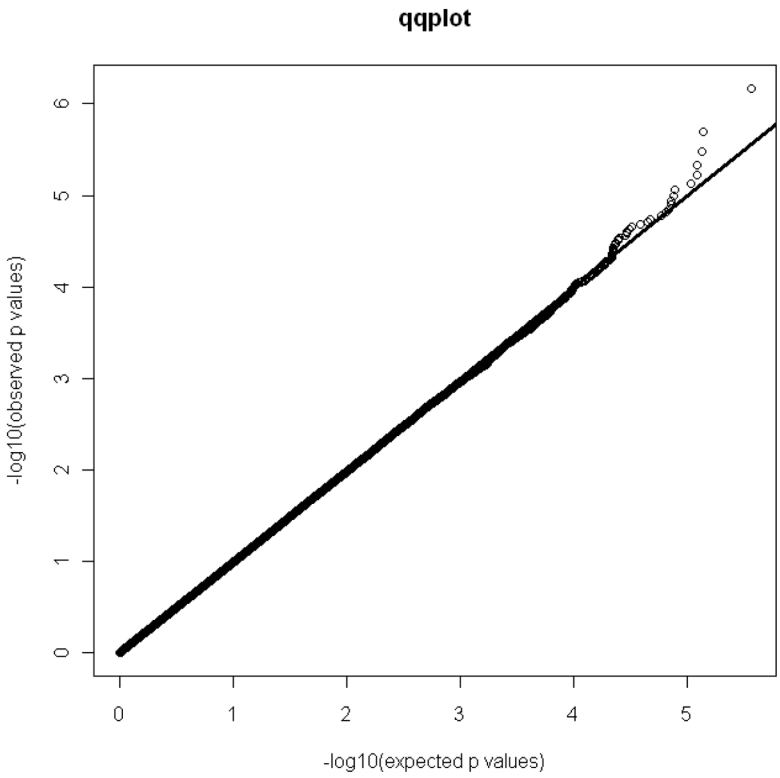


**Figure 1. Quantile-Quantile Plot of the GWAS P Values**

The observed P values match the expected P values under null distributions over the range of [1< −log10 (P) <4.3].

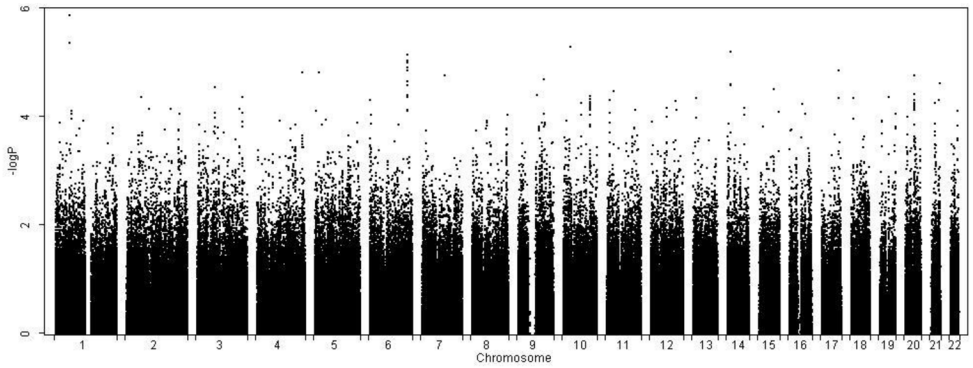


**Figure 2. Distributions of GWAS P Values across the Genome in the Initial GWAS Study**
The −log10P values for 746,709 SNPs are plotted against physical positions on successive chromosomes.

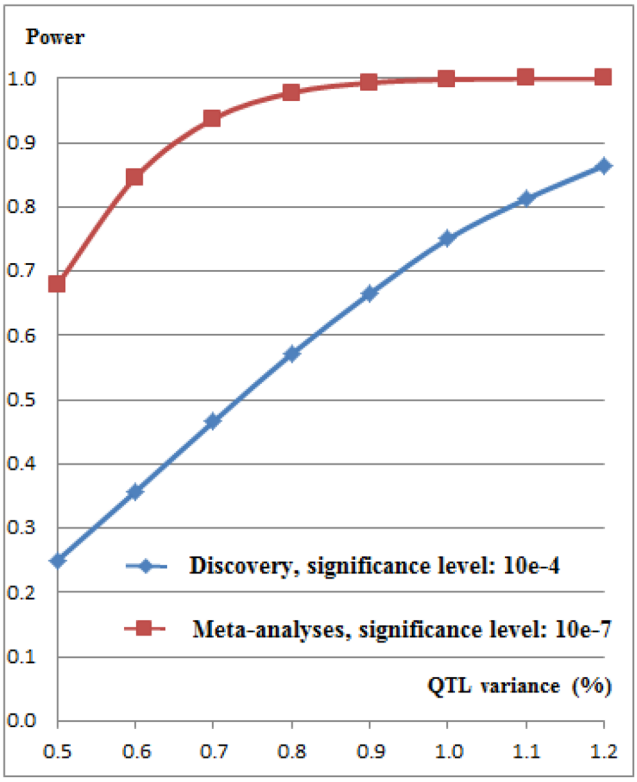


**Figure 3. Power of Association Tests in Discovery Cohort and Meta-analyses Cohorts**

The power analyses were performed for association tests of quantitative trait loci (QTLs) with additive effects and assuming strong linkage disequilibrium ($D'=0.95$) and equal minor allele frequency for QTLs and SNP markers.

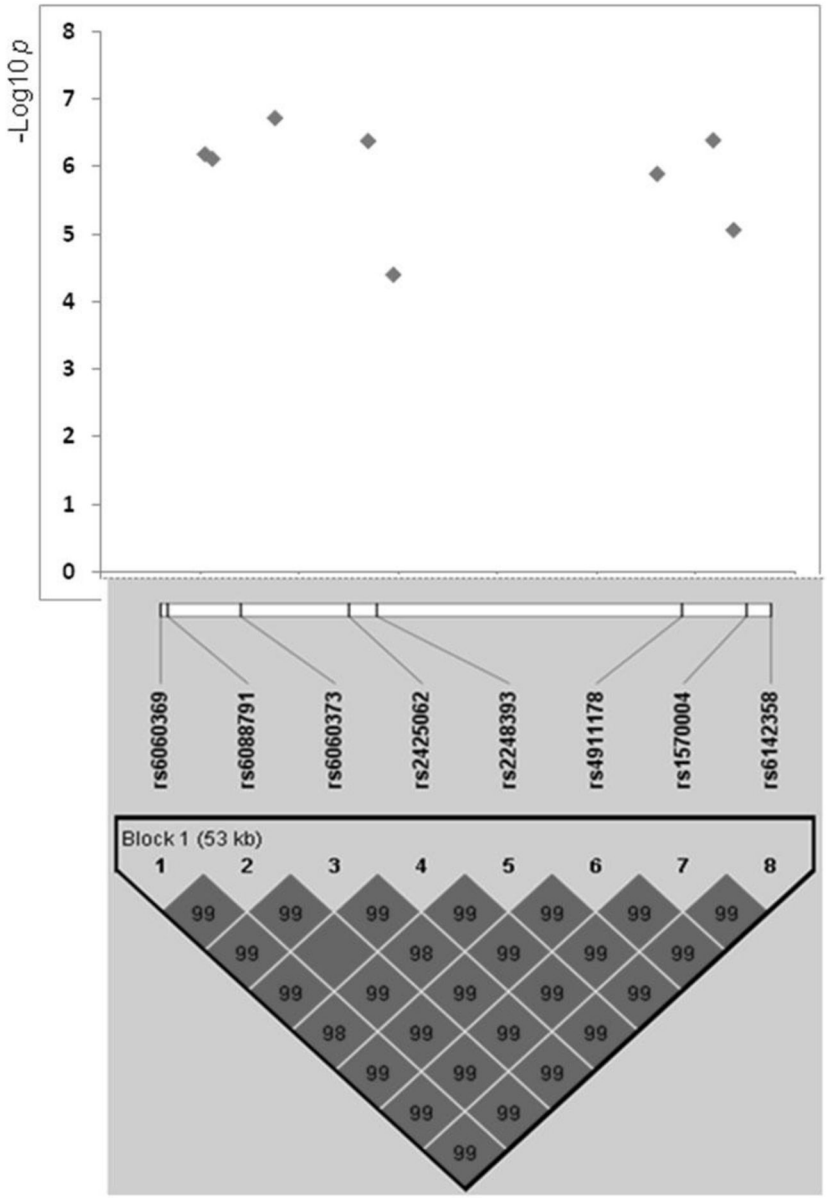


**Figure 4. LD Pattern of the Eight SNPs in UQCC in the Initial GWAS Sample**

P is the P value of meta-analysis. The LD patterns for the 8 SNPs were analyzed and plotted using the Haploview program.

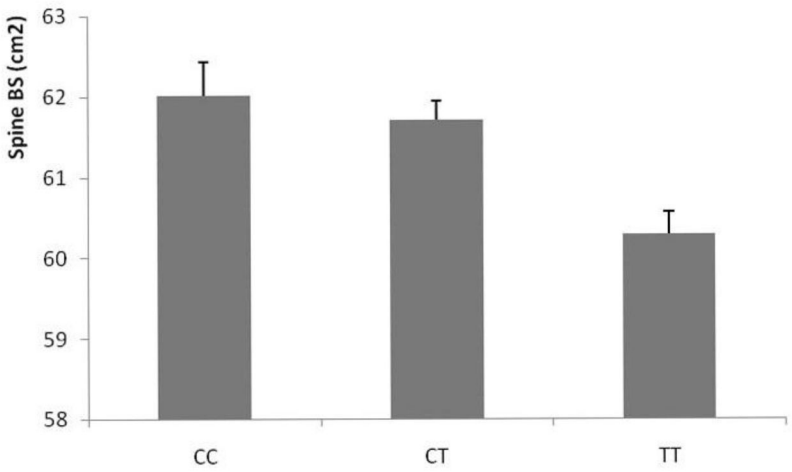


**Figure 5. Genotypic Effects of rs6060373 on Spine Bone Size in Initial GWAS Sample**
Mean and S.E. are presented.

**Table 1**

Basic Characteristics of the Four Study Samples

| Study Sample | | Sample Size | Age (years) | Height (m) | Weight (kg) | Spine BS ($cm^2$) |
|---|---|---|---|---|---|---|
| | Total | 2286 | 51.4(13.8) | 1.66(0.08) | 75.3(17.5) | 61.3(7.7) |
| **CAU-1** | Female | 1728 | 51.6(12.9) | 1.63(0.06) | 71.4(16.0) | 58.6(5.7) |
| | Male | 558 | 50.7(16.1) | 1.76(0.07) | 87.1(16.7) | 69.6(7.3) |
| | Total | 1000 | 50.3(18.3) | 1.71(0.10) | 80.2(17.8) | 63.8(8.7) |
| **CAU-2** | Female | 501 | 50.1(17.7) | 1.64(0.06) | 71.2(15.9) | 60.0(5.7) |
| | Male | 499 | 50.5(18.9) | 1.78(0.07) | 89.0(14.9) | 69.6(7.3) |
| | Total | 2503 | 63.9(11.6) | 1.34(0.65) | 71.6(12.8) | 66.3(13.2) |
| **CAU-3** | Female | 1580 | 64.1(11.6) | 1.29(0.63) | 66.6(11.5) | 64.2(12.5) |
| | Male | 923 | 63.5(11.7) | 1.42(0.66) | 80.1(10.1) | 70.1(13.6) |
| | Total | 1627 | 34.5(13.2) | 1.64(0.08) | 60.1(10.5) | 60.4(7.0) |
| **CHN** | Female | 825 | 37.5(13.8) | 1.58(0.05) | 54.6(8.1) | 56.1(5.0) |
| | Male | 802 | 31.4(11.9) | 1.70(0.06) | 65.7(9.6) | 64.7(6.1) |

Abbreviations: BS, bone size.

Note: Presented are mean (S.D.).

**Table 2**

SNPs in the *UQCC* Gene Associated with Spine Bone Size

| **SNP ID** | | **rs6060369** | **rs6088791** | **rs6060373** | **rs2425062** | **rs2248393** | **rs4911178** | **rs1570004** | **rs6142358** |
|---|---|---|---|---|---|---|---|---|---|
| **Physical Position** | | Chr20:33370575 | Chr20:33371323 | Chr20:33377622 | Chr20:33386980 | Chr20:33389517 | Chr20:33416034 | Chr20:33421667 | Chr20:33423722 |
| **Function** | | intron | intron | intron | intron | intron | intron | intron | intron |
| **Allele** [a] | | G/A | G/A | C/T | G/A | G/C | C/T | A/T | G/A |
| **MAF** | | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |
| **Discovery in CAU-1** | *P* value | 4.67E-05 | 3.53E-05 | 1.64E-05 | 7.11E-05 | 5.17E-05 | 6.00E-05 | 5.70E-05 | 6.13E-05 |
| | β | 0.73 | 0.74 | 0.79 | 0.79 | 0.73 | 0.72 | 0.72 | 0.72 |
| | $R^2$ | 0.73% | 0.75% | 0.83% | 0.69% | 0.72% | 0.71% | 0.71% | 0.70% |
| | Adj-*P* [b] | 6.44E-05 | 4.93E-05 | 2.35E-05 | 9.67E-05 | 7.11E-05 | 8.21E-05 | 7.81E-05 | 8.38E-05 |
| | Adj-*P* [c] | 2.44E-04 | 1.93E-04 | 2.09E-04 | 3.79E-04 | 4.61E-04 | 4.22E-04 | 3.81E-04 | 4.03E-04 |
| | Adj-*P* [d] | 3.89E-02 | 3.16E-02 | 3.58E-02 | 5.11E-02 | 5.32E-02 | 5.32E-02 | 4.83E-02 | 4.80E-02 |
| **Heterogeneity between CAU-1 and CAU-2** | Q | 0.0007 | 0.0019 | 0.0004 | 0.0000 | NA | 0.0058 | 0.0001 | NA |
| | $I^2$ | 91.36 | 89.62 | 92.05 | 94.02 | NA | 86.88 | 93.62 | NA |
| **Replication** | CAU-2 | 7.02E-02 | 1.11E-01 | 6.18E-02 | 6.02E-02 | NA | 1.45E-01 | 6.37E-02 | NA |
| | CAU-3 | 7.13E-03 | 6.87E-03 | 6.21E-03 | 5.18E-03 | NA | 1.10E-02 | 5.20E-03 | NA |
| | CHN | 1.37E-02 | 1.48E-02 | 1.23E-02 | 8.59E-03 | 5.61E-02 | 9.61E-03 | 9.79E-03 | 9.15E-03 |
| **Meta-analysis** | ***P* value** | 6.42E-07 | 7.52E-07 | 1.86E-07 | 4.08E-07 | 3.99E-05 | 1.26E-06 | 3.98E-07 | 8.63E-06 |

Abbreviations: MAF, minor allele frequency; NA, not available due to missing SNP genotype data.

[a] The first allele is minor allele.

[b] Adj-*P* is the *P* value of association test after adjusting for population stratification (Genomic Control).

[c] Adj-*P* is the *P* value of association test after adjusting for population stratification (EIGENSTRAT).

[d] Adj-*P* is the *P* value of association test after adjusting for population stratification (EIGENSTRAT) and height.

The direction of genotypic effects is consistent in discovery and replication samples.

The heterogeneity was tested by using the Cochrane's Q statistic and $I^2$ heterogeneity index.