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Association Analysis of BMD-associated SNPs with Knee Osteoarthritis†

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Abstract

Osteoarthritis (OA) risk is widely recognized to be heritable but few loci have been identified. Observational studies have identified higher systemic bone mineral density (BMD) to be associated with an increased risk of radiographic knee osteoarthritis. With this in mind, we sought

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to evaluate whether well-established genetic loci for variance in BMD are associated with risk for radiographic OA in the Osteoarthritis Initiative (OAI) and the Johnston County Osteoarthritis (JoCo) Project. Cases had at least one knee with definite radiographic OA defined as the presence of definite osteophytes with or without joint space narrowing (KL grade ≥ 2) and controls were absent for definite radiographic OA in both knees (KL grade ≤ 1bilaterally). There were 2014 and 658 Caucasian cases, respectively, in the OAI and JoCo Studies, and 953 and 823 controls. Single nucleotide polymorphisms (SNPs) were identified for association analysis from the literature. Genotyping was carried out on the Illumina 2.5M and 1M arrays in GeCKO and JoCo, respectively and imputation was done. Association analyses were carried out separately in each cohort with adjustments for age, BMI, and sex and then parameter estimates were combined across the two cohorts by meta-analysis. We identified 4 SNPs significantly associated with prevalent radiographic knee OA. The strongest signal (p=0.0009, OR=1.22, 95% CI[1.08–1.37]) maps to 12q3 which contains a gene coding for *SP7*. Additional loci map to 7p14.1 (*TXNDC3*), 11q13.2 (*LRP5*) and 11p14.1 (*LIN7C*). For all four loci the allele associated with higher BMD was associated with higher odds of OA. A BMD risk allele score was not significantly associated with OA risk. This meta-analysis demonstrates that several GWAS-identified BMD SNPs are nominally associated with prevalent radiographic knee OA and further supports the hypothesis that BMD, or its determinants, may be a risk factor contributing to OA development.

Keywords

Genetic Research/Human Association studies; Osteoarthritis

Introduction

Osteoarthritis (OA) is a disease characterized by structural changes in the diarthrodial joints including focal degradation of articular cartilage with related changes in subchondral bone(1). These structural changes are associated with an illness characterized by joint pain that leads to activity limitation, physical disability and decreased health-related quality of life. Knee OA is a highly prevalent condition representing a huge economic burden on health care systems(2,3).

Risk factors associated with the development of knee OA include older age, female gender, higher body mass index (BMI), joint injury and genetic predisposition, among others(4). While local changes in subchondral bone during the development of osteoarthritis are complex(5), multiple observational studies have also found that higher systemic bone mineral density (BMD) is associated with an increased risk of radiographic knee osteoarthritis(6–10). This association between high systemic BMD with knee OA risk is intriguing and raises the question as to whether genetic influences on systemic BMD might also predispose to OA. Recently, Estrada and colleagues reported on new loci associated with BMD measured at the hip and/or lumbar spine in a series of meta-analyses including over 80,000 subjects from 51 studies(11,12). In this report, we use data from two large North American cohorts that have been well characterized for knee OA phenotypes and have undergone genotyping as part of genome-wide association studies (GWAS) to test the

hypothesis that genes associated with systemic BMD would also be associated with the presence of structural knee OA.

Methods

The Osteoarthritis Initiative (OAI)

The Osteoarthritis Initiative (OAI) is a publicly and privately funded prospective longitudinal cohort with a primary objective of identifying risk factors for incidence and progression of tibiofemoral knee OA. Data used in the preparation of this article were obtained from the Osteoarthritis Initiative (OAI) database, which is available for public access at http://www.oai.ucsf.edu/. Specific datasets used are: Baseline version (v.) 0.5, 12-month v.1.5, 24-month v.3.4, 36-month Release v.5.4, 48-month v.6.2. The cohort is a racially and ethnically diverse mix of persons between the ages of 45 and 79 years at baseline. The current analysis includes 2,967 Caucasian participants who have consensus radiographic information from the OAI and genotype data generated as part of the Genetic Components of Knee OA (GeCKO) project. Genome wide genotyping was completed at the Translational Genomics Research Institute (Phoenix, AZ) using the Illumina 2.5M platform. Imputation was completed using Minimac (http://genome.sph.umich.edu/wiki/Minimac) on the 1000 genomes CEU reference panel (June 2011 release).

Case/control status for radiographic knee OA (rKOA) was defined using central readings of bilateral knee radiographs obtained at baseline and annually over 4 yearly study visits. Central image assessment data releases were version 0.5 for Baseline, 1.5 for 12-month, 3.4 for 24-month, 5.4 for 36-month, and 6.2 for 48-month. Specifically, rKOA cases were defined as having a Kellgren-Lawrence (KL) grade of 2 or higher in at least one knee, or total knee replacement at any available visit. Controls were defined as having KL grade of 0 or 1 at all available visits through 48 months in both knees.

The Johnston County Osteoarthritis Project (JoCo)

The Johnston County Osteoarthritis Project (JoCo) is an ongoing, community-based study of the occurrence of knee and hip OA in African American and Caucasian residents, aged 45 years and above, in a rural county in North Carolina. A detailed description of participant recruitment has been reported(13). Briefly, participants were recruited by probability sampling, with oversampling of African Americans. A total of 3,068 individuals were recruited at baseline with additional participants recruited in a subsequent wave. The current analysis includes 1,481 Caucasian participants with genotype data and radiographic information. The JoCo sample has been genotyped using the Illumina 1M platform. Imputation was completed using Minimac (http://genome.sph.umich.edu/wiki/Minimac) on the 1000 genomes CEU reference panel (June 2011 release).

Case/control status for radiographic knee OA (rKOA) was defined from radiographs taken at the baseline (1991–1998) and two follow-up visits (1999–2004 and 2006–2010). As in the OAI, rKOA cases were defined as having a KL grade of 2 or higher in at least one knee, or total knee replacement at any available visit whereas controls were defined as having KL grade of 0 or 1 at all available visits in both knees.

SNP selection

We selected for analysis all SNPs robustly associated with femoral neck or lumbar spine BMD at genome-wide levels of significance (n = 64 SNPs from 56 loci) from a large meta-analysis (N=83,894 for discovery and replication studies) conducted through the Genetic Factors of Osteoporosis (GEFOS) consortium(11).

Statistical Analysis

Association analyses for each BMD variant were conducted under an additive model in the two cohorts separately. Analyses for the OAI and JoCo were conducted using PLINK and ProbABEL, respectively, with adjustment for age, BMI, and sex. A principal components term estimated from the full genetic data was also included to account for observed population sub-structure. Inverse variance fixed-effects meta-analysis was carried out using METAL (14) so as to weight the contribution of both studies by the observed standard error. Heterogeneity between studies was also assessed using Cochran's Q statistic. All analyses were conducted with the imputed genotypes.

We estimated that the pooled OAI/JoCo sample of 2672 cases and 1776 controls provided 80% power to detect ORs ranging from 1.10 – 1.19 for BMD-associated SNPs at an alpha = 0.05, and across a range of allele frequencies.

BMD risk score analysis

To assess the combined additive effects of all BMD-associated SNPs on rKOA, we developed a global BMD risk score based on all SNPs that were genome-wide associated with BMD. For the risk score calculations, we used the 62 replicating SNPs from the non-sex-stratified analysis from Estrada et al. We calculated three risk scores: 1) hip BMD, 2) spine BMD, and 3) composite BMD. Hip BMD risk scores are based on 49 SNPs that were genome-wide significant for femoral neck BMD (regardless of their association with spine BMD). Spine BMD risk scores are based on 48 SNPs that were genome-wide significant for lumbar spine BMD (regardless of their association with femoral neck BMD). Composite BMD risk scores are based on all 62 SNPs that were genome-wide significant for either femoral neck BMD or lumbar spine BMD. To calculate the risk scores, we weighted individual allele dosage for each SNP by the effect size reported from the analysis of BMD in the pooled discovery and replication sets in Estrada et al. and then summed the weighted allele dosage for all SNPs within each risk score category. For the composite BMD risk score, SNPs were weighted by the larger of the effect sizes for association with femoral neck BMD and lumbar spine BMD. The coded allele for each SNP was selected as the allele associated with higher BMD, thus a higher BMD risk score indicates a larger number of high BMD SNP variants.

We then tested the association between BMD risk score and rKOA. For each of the three BMD risk scores, we divided the distribution of risk scores into quintiles and compared the odds of OA in each of quintiles 2–5 with the odds of OA in the lowest quintile (i.e. low BMD). We tested the association of BMD risk score with rKOA using logistic regression modeling with adjustment for age, sex, and principle component score. In OAI, we used a generalized estimating equation (GEE) logistic regression model to take into account

clustering of study subjects within clinical site using an exchangeable correlation matrix. Study-specific BMD score analyses in the OAI and JoCo were completed using SAS 9.2and R x64 2.14.1, respectively. We then conducted a meta-analysis to combine estimates across both cohorts by using an inverse variance fixed effects approach implemented in R 2.15.1 to weight the contribution of both studies by the observed standard error.

Results

Our study sample included 2,014 rKOA cases and 953 controls from OAI and 658 rKOA cases and 823 controls from JoCo. Participant characteristics are shown in Table 1. Participant characteristics were similar in both studies though there was a slightly higher proportion of women in the JoCo study. In both studies, rKOA cases were slightly older and had higher BMI.

Four of the 62 BMD-associated SNPs were nominally ($p<0.05$) associated with rKOA in the meta-analysis of OAI and JoCo data (Table 2 and Figure 1). The most strongly associated variant (OR=1.22, $p=9.4\times10^{-4}$) was rs2016266, which is located on chromosome 12 near the Sp7 transcription factor (*SP7)* gene. The other three variants rs10226308, rs3736228, and rs10835187 also demonstrated a similar magnitude of association (OR 1.13–1.19). Of note, the association of the rs3736228 variant near the *LRP5* gene has a statistically significant heterogeneity statistic ($I^2=4.0$, $p=0.046$). Three of the four variants, but not the rs10835187 variant, were also significant in models adjusted only for age, sex and principal components.

To evaluate if these four variants were associated with radiographic progression of disease we conducted association analysis in both the OAI and JoCo studies. Radiographic progressors were defined as those with rKOA at baseline who progressed by at least 1 KL grade compared to those with rKOA who did not progress during 48 months. Patients with the maximum KL score at baseline (KL=4) were excluded from analysis since we were unable to measure progression using this definition. We found no significant associations for these four SNPs (data not shown), but had limited power due to smaller sample sizes (107 cases/357 controls in JoCo; 449 cases/1351 controls in the OAI).

Several biological pathways (WNT signaling, endochondral ossification and RANK/RANKL/OPG signaling) have been implicated in the development of both osteoarthritis and osteoporosis. Figure 1 displays the association results for all 62 BMD-associated SNPs, grouped by the assigned pathway of the SNP. Two of the nominally significant variants (rs3736228, rs10226308) are located near genes in the WNT-signaling pathway (*LRP5* and *TXNDC3)*. Moreover, of the 15SNPs in genes assigned to the WNT-signaling pathway, 12 had odds ratios ≥ 1 for their associations with OA, consistent with the notion that high BMD-associated variants in genes in the WNT-signaling pathway are also associated with OA risk.

Results of the BMD risk score analysis are shown in Table 3. In the OAI, although the top quintile for BMD risk score was significantly associated with OA (OR = 1.33, 95% C.I.: 1.04–1.70), there was no clear, linear trend of increasing risk of OA with increasing BMD

risk score quintile. Despite this, a nominal association between continuous BMD risk score and OA was observed in the OAI (β=0.326, p=0.033). No evidence of increased OA risk by quintile or with BMD risk score was present in JoCo (p>0.05). On meta-analysis, the association of BMD risk score with OA was negligible (β=0.05, *p*=0.162).

Discussion

Although no individual SNP met stringent levels of statistical significance following multiple comparisons, several of our findings provide support for an association of at least some high BMD-associated SNPs with radiographic knee OA risk. First, of the four nominally associated SNPs, the association of each with OA was in the hypothesized direction (i.e., the higher BMD allele was associated with increased OA risk). Second, if the nominal SNP associations with OA were real, one would expect the associated SNPs to be the ones with the largest effect sizes (i.e., have the largest betas) on BMD. This is in fact what we observed – the 4 nominally associated SNPs all rank among the most strongly associated SNPs for lumbar spine BMD (Supplemental tables 4A and 4B in (11)). Specifically, the effect sizes of these SNPs on spine BMD ranged from 0.05 – 0.08 and only 30 of the 62 BMD-associated SNPs from Estrada et al. had betas as high as 0.05 (i.e., fewer than half of the BMD-associated SNPs). In other words, the four SNPs nominally associated with OA all ranked in the top 50% in terms of their effect sizes on spine BMD, as one would anticipate for true associations.

Although the epidemiologic literature has revealed a consistent association between high systemic BMD and the incidence of radiographic hip and knee OA, the mechanisms for this association are not known. The most intriguing hypothesis is that increased OA risk is a direct consequence of high systemic BMD, so that any factors associated with high systemic BMD will lead to a corresponding increase in OA risk. One possibility is that OA risk is enhanced as a direct consequence of high systemic BMD due to mechanical pressure applied to cartilage from the underlying subchondral cortical plate while the adjacent subarticular surface may become osteoporotic(5). It has also been proposed that (some) of the BMD-associated SNPs do not, in fact, influence BMD per se, but rather influence bone size or bone shape and it is this phenotype that more directly influences OA risk. This speculation arises from the concern that DXA-obtained determinations of high BMD may be artifactual as the primary effect of some BMD-associated genes may be on bone size or bone shape, rather than BMD *per se*, but is only attributed to BMD because of the greater apparent areal rather than volumetric BMD(15). Indeed, data from several studies support the hypothesis that variation in shape of the femoral head influences the risk of radiographic hip OA(16,17) and data are also available that support such an association between variation in tibia and femur surface geometry and knee OA (18–20). Hence, whether the association is mediated by BMD or bone shape or both, the hypothetical model is that the gene influences a bone phenotype, and it is that phenotype that influences OA risk. It is also worth noting that despite this observation of higher BMD, individuals with OA may actually be at higher risk of osteoporotic fracture though this appears to be mediated through an increased risk of falls(21).

An unresolved question is whether OA is initiated by changes occurring in subchondral bone or in articular cartilage. On the one hand, the Wnt/β-catenin signaling pathway influences development and regulation of both bone and cartilage, making disturbances in this pathway an obvious candidate linking BMD and OA risk. Variants in genes within the Wnt-signaling pathway could both impair Wnt signaling or enhance it, an example of the latter being the recently reported variants in *FRZB*, a Wnt antagonist, which have been associated with OA susceptibility in multiple populations (22–26). On the other hand, an association between BMD-associated SNPs and OA may imply that at least some of the effects of OA risk are mediated by high systemic BMD if one assumes that the allelic effects on WNT-signaling would be in the same direction in both bone and cartilage. For example, if an allele that impairs Wnt-signaling in bone (resulting in lower BMD) also impairs Wnt-signaling in cartilage, then one might expect cartilage integrity and/or repair to be compromised and OA risk to be higher (not lower) in people with impaired Wnt-signaling. On the other hand, higher OA risk would track high systemic BMD if the increased OA risk in people with the enhanced Wnt-signaling allele were due to mechanical forces exerted by bone on cartilage.

Finally, it is also possible that the high BMD-associated SNPs do indeed ‘cause’ high BMD, but they also stimulate the development of osteophytes, which are then detected by radiography and misclassified as evidence of OA. However, this explanation is at odds with recent findings from the Multicenter Osteoarthritis Study (MOST) that high BMD is also associated with the development of joint space narrowing in knees without OA(6).

A strength of our study is the detailed radiologic assessment of osteoarthritis that we obtained in over 2,600 subjects and the harmonized case and control definitions we employed across the OAI and JoCo cohorts(27). While we detected nominal levels of association between 4 SNPs and OA risk, the effects of all 4 were in the hypothesized direction and these 4 loci were also among those having the strongest effect sizes on BMD, and thus would be hypothesized to have the strongest effects on OA risk.

In conclusion, our study provides evidence for an association between high BMD-associated alleles and enhanced risk of OA. Although several interpretations of our findings are possible, one attractive interpretation is that OA risk is influenced at least in part by genes associated with BMD.

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LMYA, YL and MSY were responsible for statistical analyses. JR was responsible for reading radiographs for JoCo. DD and SK were responsible for generating genetic data. CKK, MCN, CBE, RDJ, MCH, JMJ and BDM

were responsible for OAI, JoCo and GeCKO study design and oversight. All authors were involved in hypothesis generation, analysis design and preparation of the manuscript.

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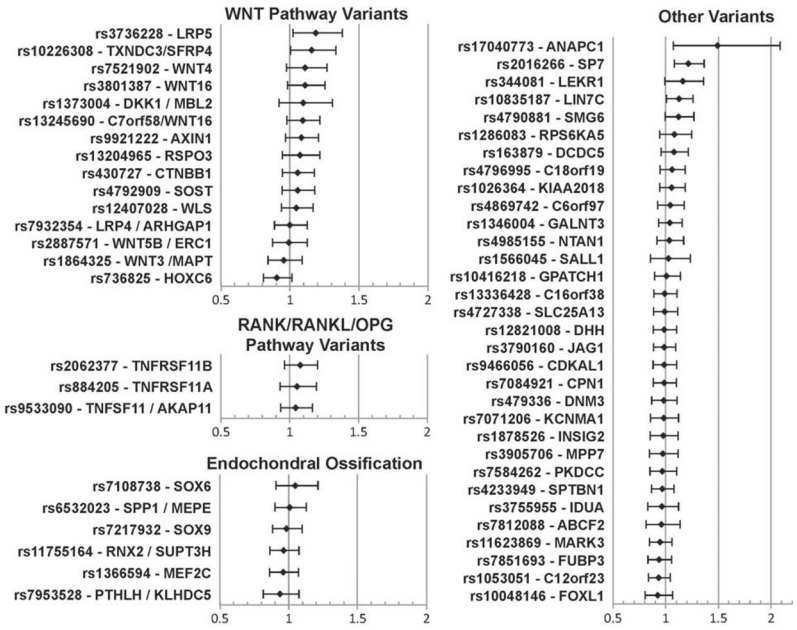

Figure 1.
Forest plots by pathway

Table 1

Summary characteristics of the OAI and JoCo populations

	OAI (n = 2,967)		JoCo (n = 1,481)	
	Cases* (N=2,014)	Controls (N=953)	Cases* (N=658)	Controls (N=823)
% women	55.7%	54.0%	61.1%	61.2%
Age (yrs)	66.6 (9.0)	63.4 (9.2)	66.6 (10. 6)	61.5 (9.5)
BMI (kg/m2)	29.1 (4.8)	27.2 (4.5)	31.2 (6.2)	28.7 (5.5)
Height (cm)	169 (9)	169 (9)	164 (10)	164 (9)
Weight (kg)	82.9 (16.1)	77.3 (15.9)	84.9 (20.4)	78.9 (18.9)
Femoral Neck BMD (g/cm^2)	-	-	0.69(0.14)	0.71(0.12)
Spine BMD (g/cm^2)	-	-	1.05(0.20)	1.01(0.18)
Hip BMD risk score**	2.51 (0.22)	2.50 (0.21)	2.37 (0.22)	2.36 (0.22)
Spine BMD risk score**	2.84 (0.24)	2.82 (0.24)	2.75 (0.23)	2.75 (0.24)
Composite BMD risk score**	3.48 (0.27)	3.46 (0.27)	3.32 (0.27)	3.32 (0.27)

Mean and (Std. Deviation) are presented unless otherwise noted.

BMD=Bone Mineral Density; BMD is not available in the OAI study.

* Cases defined as knee KL ≥ 2 or knee replacement, either knee. Controls defined as KL = 0 or 1 in both knees.

** Hip BMD risk score is based on 49 SNPs that were genome wide significant for hip BMD; spine BMD risk score is based on 48 SNPs that were genome wide significant for spine BMD; and composite BMD risk score is based on 62 SNPs that were genome wide significant for either hip or spine BMD (see text).

Table 2

BMD-Associated SNPs also Associated with OA on Meta-analysis in the OAI and JoCo

				Association with lumbar spine BMD***		Effects of BMD-associated SNPs on OA						
						OAI (N=2624)		JoCo (N=976)		Meta-analysis		
SNP (Closest Gene)	Chr (position*)	A1**/A2	A1 Freq.	Beta	P	OR	P	OR	P	OR	P	Heterogeneity I^2 (P)
rs2016266 *(SP7)*	12 (52,014,222)	G/A	0.33	0.05	2.95×10^{-20}	1.13	0.048	1.21	0.026	1.22	9.4×10^{-4}	0.99 (0.32)
rs3736228 *(LRP5)*	11 (67,957,871)	C/T	0.84	0.08	2.08×10^{-26}	1.04	0.640	1.46	6.45×10^{-4}	1.19	0.025	**4.00 (0.046)**†
rs10835187 (*LIN7C*)	11 (27,505,677)	C/T	0.44	0.05	4.14×10^{-7}	1.02	0.736	1.25	7.21×10^{-3}	1.13	0.038	3.20 (0.07)
rs10226308 *(TXNDC3)*	7 (37,904,947)	G/A	0.19	0.06	6.4×10^{-13}	1.23	0.007	1.00	0.965	1.16	0.042	2.30 (0.129)

All analyses for OA are adjusted for age, BMI, sex and principal components of population structure.

BMI=Body Mass Index

* Chromosome Position is hg18

** A1 is the coded allele

*** Skeletal site with the largest effect size from Estrada et. al is reported which is the lumbar spine for selected variants. All loci are also significant for femoral neck ($p<0.05$).

† Significant between-study heterogeneity was detected.

Table 3

Association between OA and composite BMD risk score

	Unadjusted Odds Ratios (95% Confidence Intervals)		
	OAI	**JoCo**	**Combined**
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.08 (0.85–1.37)	1.15 (0.83–1.59)	1.10 (0.91–1.34)
Quintile 3	1.26 (0.99–1.60)	0.99 (0.71–1.37)	1.15 (0.95–1.40)
Quintile 4	1.05 (0.83–1.34)	1.16 (0.84–1.61)	1.09 (0.90–1.32)
Quintile 5	1.33 (1.04–1.70)	1.04 (0.75–1.43)	1.21 (1.00–1.47)
Beta, P-value for effect of risk score on OA, by logistic regression	0.326, P=0.033*	0.036, P=0.367**	0.05, P=0.162

Composite risk score consists of all BMD associated variants.

† Effect of risk score, modeled as a continuous variable, on OA using logistic regression.

* Adjusted for age, sex, BMI, study site, and PCs

** Adjusted for age, sex, BMI and PCs