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Impact of Common Variation in Bone-Related Genes on Type 2 Diabetes and Related Traits

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Exploring genetic pleiotropy can provide clues to a mechanism underlying the observed epidemiological association between type 2 diabetes and heightened fracture risk. We examined genetic variants associated with bone mineral density (BMD) for association with type 2 diabetes and glycemic traits in large well-phenotyped and -genotyped consortia. We undertook follow-up analysis in ~19,000 individuals and assessed gene expression. We queried single nucleotide polymorphisms (SNPs) associated with BMD at levels of genome-wide significance, variants in linkage disequilibrium ($r^2 > 0.5$), and BMD candidate genes. SNP rs6867040, at the *ITGA1* locus, was associated with a 0.0166 mmol/L (0.004) increase in fasting glucose per C allele in the combined analysis. Genetic variants in the *ITGA1* locus were associated with its expression in the liver but not in adipose tissue. *ITGA1* variants appeared among the top loci associated with type 2 diabetes, fasting insulin, β -cell function by homeostasis model assessment, and 2-h post-oral glucose tolerance test glucose and insulin levels. *ITGA1* has demonstrated genetic pleiotropy in prior studies, and

its suggested role in liver fibrosis, insulin secretion, and bone healing lends credence to its contribution to both osteoporosis and type 2 diabetes. These findings further underscore the link between skeletal and glucose metabolism and highlight a locus to direct future investigations. *Diabetes* 61:2176–2186, 2012

Studies show that adults with type 2 diabetes have a higher fracture rate than those without diabetes (1–5). A meta-analysis of 16 studies revealed a 1.7 (95% CI 1.3–2.2) relative risk of hip fracture for people with diabetes compared with those without diabetes (6). The higher fracture rate persisted even after considering factors including, but not limited to, falls, impaired vision, and weight (4). Quantitative computed tomography studies show increased bone porosity in individuals with type 2

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*A complete list of the MAGIC Investigators, the DIAGRAM+ Consortium, the MuTHER Consortium, and the GEFOS Consortium can be found in the Supplementary Data online. A complete list of the ASCOT Investigators can be found in ref. 51.

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diabetes, suggesting that bone integrity is compromised and thereby causing increased bone fragility (7–9), but it remains unclear what may be causing the decreased bone integrity. Despite the generally increased bone mineral density (BMD) of individuals with type 2 diabetes (1), for the same BMD measurement, people with type 2 diabetes have a higher risk of fracture (10). Basic science studies reveal further evidence of a link between bone-derived hormones and glucose regulation. Mice lacking osteocalcin, an osteoblast-specific secreted molecule, have glucose intolerance (11,12).

The relationship between osteoporosis and type 2 diabetes raised by these epidemiological studies, and intriguing new molecular data, hint to a common mechanism implicated in the pathogenesis of both disorders. Discovering genetic determinants that exhibit genetic pleiotropy (defined as one gene influencing multiple phenotypic traits) may point to a common underlying mechanism. Approximately 16.9% of the genes in the National Human Genome Research Institute's catalog of published genome-wide association studies (GWASs) are estimated to be pleiotropic (13). GWASs reveal genetic variants that are associated with BMD (a quantitative endophenotype for osteoporosis and a surrogate for fracture risk) (10,14–18). Some of these loci are also associated with traits seemingly unrelated to BMD (Table 1). However, common genetic variants

influencing BMD have not been studied systematically for association with type 2 diabetes and other glycemic traits.

We therefore performed a comprehensive evaluation of the influence of BMD-related genetic loci on diabetes-related phenotypes. After examining an extensive list of BMD-related single nucleotide polymorphisms (SNPs) for association with type 2 diabetes and quantitative glycemic traits in large GWAS meta-analysis datasets, our top SNPs were selected for in silico replication in additional cohorts, *cis*-gene expression analyses, and BMI association. In this study, we aimed to underscore the genetic determinants that are shared between osteoporosis and type 2 diabetes and provide clues into a common mechanism that may contribute to both diseases. Furthermore, through this systematic exploration, we have generated testable hypotheses for replication by independent cohorts and experimental follow-up.

RESEARCH DESIGN AND METHODS

SNP selection. In total, 1,778 SNPs were collated for association with type 2 diabetes and glycemic traits (Fig. 1). The SNP selection is described below.

A total of 35 SNPs initially were selected based on BMD GWASs in populations of European ancestry (14–17). If multiple SNPs were listed for one gene per trait, SNPs were kept for analysis if the correlation was low (pairwise linkage disequilibrium [LD] $r^2 < 0.5$); if $r^2 \geq 0.5$, only the SNP with the lowest *P* value was kept unless the study indicated that multiple correlated SNPs had a high degree of explanatory power of the variance for the trait. We removed

TABLE 1
BMD loci associated with non-BMD related traits and disease in GWASs

Locus	SNP	Trait/disease	Reference*
<i>MEF2C</i>	rs17421627	Retinal vascular caliber	Ikram MK, <i>PLoS Genetics</i> , 2010
	rs10037512	Height	Lango Allen H, <i>Nature</i> , 2010
	rs770189	Tonometry	Levy D, <i>BMC Medical Genetics</i> , 2007
<i>SOX6</i>	rs297325	BMI	Liu YZ, <i>PLoS One</i> , 2009
<i>MEPE</i>	rs7698623	Ischemic stroke among migraineurs with aura	Schürks M, <i>PLoS One</i> , 2011
<i>MHC</i>	rs2516399	Eosinophil count	Okada Y, <i>PLoS Genetics</i> , 2011
	rs2269426	Eosinophil count	Gudbjartsson DF, <i>Nature Genetics</i> , 2009
	rs3095254	Monocyte count	Okada Y, <i>PLoS Genetics</i> , 2011
	rs9271366	Inflammatory bowel disease	Okada Y, <i>Gastroenterology</i> , 2011
	rs774434	Primary biliary cirrhosis	Mells GF, <i>Nature Genetics</i> , 2011
	rs34704616	Cognitive test performance	Cirulli ET, <i>Eur J Human Genetics</i> , 2010
	rs7743761	Ankylosing spondylitis	Reveille JD, <i>Nature Genetics</i> , 2010
	rs9268866	Ulcerative colitis	Barrett JC, <i>Nature Genetics</i> , 2009
	rs13194053	Schizophrenia	Purcell SM, <i>Nature</i> , 2009
	rs6932590	Schizophrenia	Stefansson H, <i>Nature</i> , 2009
	rs3131296	Schizophrenia	Stefansson H, <i>Nature</i> , 2009
	rs9272346	Type 1 diabetes	WTCCC, <i>Nature</i> , 2007
	rs9268645	Type 1 diabetes	Barrett JC, <i>Nature Genetics</i> , 2009
	rs1265181	Psoriasis	Zhang XJ, <i>Nature Genetics</i> , 2009
	rs6457617	Rheumatoid arthritis	WTCCC, <i>Nature</i> , 2007
<i>ESR1</i>	rs2982694	Sudden cardiac arrest	Aouizerat BE, <i>BMC Cardiovasc Disord</i> , 2011
	rs4869742	Chronic myeloid leukemia	Kim DH, <i>Blood</i> , 2011
	rs3734805	Breast cancer	Fletcher O, <i>J Nail Cancer Inst</i> , 2011
	rs3757318	Breast cancer	Turnbull C, <i>Nature Genetics</i> , 2010
	rs2046210	Breast cancer	Zheng W, <i>Nature Genetics</i> , 2009
	rs543650	Height	Lango Allen H, <i>Nature</i> , 2010
<i>DCDC5</i>	rs6902771	Alcohol dependence	Treutlein J, <i>Arch Gen Psychiatry</i> , 2009
	rs3925584	Serum magnesium levels	Meyer TE, <i>PLoS Genetics</i> , 2010
<i>TNFRSF11A (RANK)</i>	rs3018362	Paget disease	Albagha OM, <i>Nature Genetics</i> , 2011
	rs2957128	Paget disease	Albagha OM, <i>Nature Genetics</i> , 2011
<i>TNFRSF11 (RANKL)</i>	rs2062305	Crohn disease	Franke A, <i>Nature Genetics</i> , 2010

All SNPs listed were associated with the traits/disease at $P < 1 \times 10^{-5}$ in GWASs. Table was compiled using www.genome.gov (49). The following loci were not associated with non-BMD related traits/disease: *CTNBN1*, *ARHGAP1*, *LRP5*, *MARK3*, *HDAC5*, *SOST*, *SPTBN1*, *STARD3NL*, *SP7*, *FOXL1*, *CRHR1*, *ZBTB40*, *GPR177*, *FLJ42280*, and *TNFRSF11B (OPG)*. *The full reference list can be found in the Supplementary Data online.

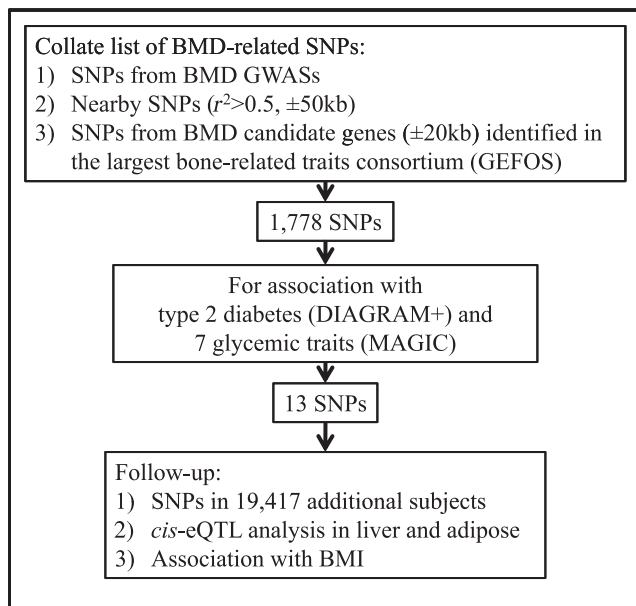


FIG. 1. Study schema. A staged approach was used to examine BMD-related SNPs for association with type 2 diabetes and related traits. BMD-related SNPs were collated from BMD GWASs (14–17), nearby SNPs (± 50 kb) in moderate-to-high LD ($r^2 > 0.5$), and SNPs from candidate genes (± 20 kb) identified in GEFOS (20). A total of 1,778 SNPs were tested for association with type 2 diabetes in DIAGRAM+ (21) and seven glycemic traits in MAGIC (22,24). Thirteen SNPs were taken forward for follow-up in a replication cohort ($N = 19,417$), *cis*-eQTL analysis in liver and adipose tissue, and association with BMI.

rs6696981 (*ZBTB40*), rs4879055 and rs6929137 (*ESR1*), rs699813 and rs6469804 (*TNFRSF11*), rs9594759 (*RANKL*), rs1107748 (*SOST*), rs2566755 (*GPR177*), and rs7781370 (*FLJ42280*) (14,15,17). The final list of 26 BMD genome wide-associated SNPs was examined for association with type 2 diabetes and glycemic traits (Table 2).

Since the index SNP may not be the causal variant and other genetic variants in the region may have a stronger influence on the traits examined, we tested the region around the index variant by selecting SNPs in moderate-to-strong LD ($r^2 > 0.5$). We chose variants in moderate-to-strong LD, rather than all of the variants in this region, to base our exploration on variants with a higher prior probability of true association and reduce the multiple testing burden. All SNPs that were 50 kilobases (kb) upstream and downstream from and in moderate-to-strong LD with the 26 BMD-related SNPs were tested for association with type 2 diabetes and glycemic traits. These SNPs were identified using SNP Annotation and Proxy Search, SNAP (<http://www.broadinstitute.org/mpg/snap/>) (19) (Supplementary Table 1).

In addition to selecting the 26 SNPs associated at genome-wide significance with BMD and the surrounding region, we selected candidate genes that were found to be associated ($P < 2.39 \times 10^{-6}$ after Bonferroni correction) with BMD in the GEFOS (Genetic Factors for Osteoporosis) Consortium (20). This article identifies nine candidate genes, including *ESR1*, *LRP4*, *ITGA1*, *LRP5*, *SOST*, *SPPI1*, *TNFRSF11A* (*RANK*), *TNFRSF11B*, and *TNFRSF11* (*RANKL*). For each gene, we identified all SNPs within and 20 kb upstream and downstream of any transcript of the gene. All SNPs within those boundaries that were genotyped or imputed in the consortia were tested for association with type 2 diabetes and glycemic traits (Supplementary Table 1).

Study populations. We tested SNPs in the DIAGRAM+ (Diabetes Genetics Replication and Meta-analysis) Consortium (21) for association with type 2 diabetes and in MAGIC (Meta-Analyses of Glucose and Insulin-Related Traits Consortium) (22–24) for association with seven glycemic quantitative traits. These traits included fasting glucose, fasting insulin, homeostasis model assessments of β -cell function (HOMA-B) and insulin resistance (HOMA-IR) (25), hemoglobin A1C (HbA_{1c}), and glucose and insulin levels 2 h post-glucose load (2-h glucose and 2-h insulin). The DIAGRAM+ Consortium combined case-control data from eight type 2 diabetes GWASs with up to 42,542 case and 98,912 control subjects of European ancestry (21). MAGIC combined data from multiple GWASs that identified loci that affect quantitative glycemic traits. Its discovery sample included up to 46,186 individuals from 17 population-based cohorts and 4 case-control studies (22–24). It is noteworthy that the Framingham Heart Study (FHS), Diabetes Epidemiology: Collaborative Analysis of Diagnostic

Criteria in Europe (deCODE) Study, Erasmus Rucphen Family Study, and TwinsUK Study provided data to both MAGIC and the BMD datasets from where the genome-wide-associated SNPs were selected. Using FHS as a representative cohort of European descent that contained both BMD and glycemic values, we found phenotypic correlations, r of 0.11–0.16, between bone (femoral neck and lumbar spine BMD) and glycemic traits (glucose and insulin). Since the phenotypic correlation is low, we would not necessarily expect to see a genetic association solely based on the fact that a small portion of the participants were assessed for both traits. In addition, examining the associations using meta-analyses of large consortia, rather than in the subset of overlapping participants, provides a more powerful approach.

The study protocols were approved by the institutional review board of the respective cohorts' institutions, and informed consent was obtained from each subject prior to participation.

Testing for association. After the collation of the index, LD-based, and gene-based BMD-related SNPs, we tested 1,778 unique SNPs for association with type 2 diabetes and glycemic traits. We obtained effect estimates and P values from GWAS meta-analyses provided by DIAGRAM+ and MAGIC. We determined which SNPs to examine in follow-up studies by calculating a significance threshold for each group of SNPs selected (index, LD-based, and gene-based). We used a Bonferroni correction for the estimated number of independent tests after taking LD into account determined using a method proposed by Nyholt (26) and Li and Ji (27). For our primary analyses, we used a stricter threshold by accounting for the number of traits tested. We evaluated 26 BMD SNPs for association with type 2 diabetes and seven glycemic traits (26 tests multiplied by 8 traits = 208), yielding thresholds to declare statistical significance at $P = 2.4 \times 10^{-4}$ (0.05/208 tests). For the LD- and gene-based secondary analyses, we corrected for the number of independent SNPs tested but not for the number of traits examined. The P value threshold for the 513 LD-based SNPs (188 independent tests) was 2.6×10^{-4} and for the 1,318 candidate gene-based SNPs (651 independent tests), 7.7×10^{-5} . A study-wide P value of 6.0×10^{-5} for 1,778 total SNPs (830 independent tests) determined significance for the combined meta-analysis (described below).

Follow-up strategy. To follow up the BMD-related SNPs associated with type 2 diabetes and glycemic traits, we combined in silico GWAS data from 12 additional cohorts of 19,417 nondiabetic participants (Amish Family Diabetes Study, Atherosclerosis Risk in Communities Study [ARIC], Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT], Busseton Health Study [BHS], Data From the Epidemiological Study on the Insulin Resistance Syndrome [DESIR] Study, French Obese Study, Family Heart Study [FamHS], Fenland Study, Finnish Twins Study, Swedish Twins Study, GEMINAKAR Study, and the Supplémentation en Vitamines et Minéraux Antioxydants [SU.VI.MAX] Study (detailed in Supplementary Table 2). We then combined the discovery and replication meta-analysis results for overall association using METAL (28).

Follow-up SNPs were examined by *cis*-expression quantitative trait loci (eQTL) analysis in metabolically relevant tissues, liver, and adipose. Liver tissue samples came from the Advanced Study of Aortic Pathology (ASAP) cohort of 211 healthy adults undergoing aortic valve surgery. Each biopsy was taken in RNAlater (Ambion, Austin, TX). RNA quality was analyzed with an Agilent 2100 bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA), and quantity was measured by NanoDrop (Thermo Scientific, Waltham, MA). RNA was purified using the RNeasy Mini kit (QIAGEN, Hilden, Germany), including treatment with RNasefree DNase set (QIAGEN) according to the manufacturer's instructions. Expression profiling was done on the Affymetrix GeneChip Human Exon 1.0 ST array (Affymetrix, Inc., Santa Clara, CA). Expression data were preprocessed using the robust multiarray analysis algorithm with quantile normalization, log₂ transformation, and the "extended" set of meta probe sets. Genotyping of the DNA samples was done using Illumina 610wQuad arrays (Illumina, Inc., San Diego, CA). SNPs were imputed using MACH 1.0 software with a readability strength quality score ≥ 0.6 . Each SNP was encoded as 0, 1, or 2 depending on genotype, and a linear regression model was fitted (29).

Adipose tissue samples came from the Multiple Tissue Human Expression Resource (MuTHER) (30) of 776 healthy female adult twins. RNA was extracted from homogenized subcutaneous adipose tissue samples using TRIzol Reagent (Invitrogen, Grand Island, NY) according to protocol provided by the manufacturer. RNA quality was assessed with the Agilent 2100 BioAnalyzer, and the concentrations were determined using NanoDrop ND-1000 (Thermo Scientific). Whole-genome expression profiling of the samples was performed using the Illumina Human HT-12 V3 BeadChips according to the protocol supplied by the manufacturer. Log₂-transformed expression signals were normalized separately per tissue as follows: quantile normalization was performed across technical replicates of each individual followed by quantile normalization across all individuals. Subject DNA was genotyped using a combination of Illumina arrays (HumanHap300, HumanHap610Q, 1M-Duo, and 1.2MDuo 1M). Untyped HapMap2 (<http://hapmap.ncbi.nlm.nih.gov>) SNPs were imputed using the IMPUTE software package (version 2) (31). Association between all SNPs (minor allele frequency [MAF] $> 5\%$, IMPUTE INFO > 0.8) within a gene or within 1 MB of

TABLE 2
Twenty-six BMD-associated loci for association with diabetes and quantitative glycemic traits

Chr	SNP	Gene	BMD-raising allele/other	Type 2 diabetes		Fasting glucose		Fasting insulin	
				Odds ratio (95% CI)	<i>P</i>	β (mmol/L)	<i>P</i>	β (pmol/L)	<i>P</i>
SNPs associated at genome-wide levels of significance with hip BMD									
3	rs87939	<i>CTNNA1</i>	g/a	1.01 (0.97–1.05)	0.80	−0.0071 (0.004)	0.05	−0.0084 (0.004)	0.03
5	rs1366594	<i>MEF2C</i>	a/c	1.01 (0.97–1.05)	0.59	0.0039 (0.004)	0.30	0.0086 (0.004)	0.03
11	rs7117858	<i>SOX6</i>	g/a	1.04 (0.99–1.09)	0.15	0.0052 (0.004)	0.22	−0.0064 (0.004)	0.14
11	rs7932354	<i>ARHGAP1</i>	t/c	1.05 (1.0–1.10)	0.03	0.0106 (0.004)	0.01	−0.0013 (0.004)	0.76
11	rs3736228*	<i>LRP5</i>	c/t	0.99 (0.94–1.06)	0.97	0.0006 (0.006)	0.92	0.0012 (0.006)	0.85
14	rs2010281*	<i>MARK3</i>	g/a	1.02 (0.98–1.06)	0.35	−0.0027 (0.004)	0.49	−0.003 (0.004)	0.46
17	rs228769	<i>HDAC5</i>	g/c	1.01 (0.96–1.06)	0.80	0.0014 (0.005)	0.75	0.0036 (0.005)	0.45
17	rs7220711	<i>SOST</i>	g/a	1.01 (0.96–1.05)	0.83	0.006 (0.004)	0.12	0.0022 (0.004)	0.58
17	rs1513670*	<i>SOST</i>	c/t	1.00 (0.96–1.05)	0.92	0.0058 (0.004)	0.13	0.006 (0.004)	0.14
SNPs associated at genome-wide levels of significance with spine BMD									
2	rs11898505*	<i>SPTBN1</i>	a/g	1.01 (0.97–1.06)	0.63	0.0024 (0.004)	0.56	0.0075 (0.004)	0.08
4	rs1471403	<i>MEPE</i>	t/c	0.99 (0.95–1.04)	0.78	−0.0005 (0.004)	0.90	−0.0072 (0.004)	0.07
6	rs3130340	<i>MHC</i>	c/t	1.02 (0.97–1.07)	0.39	0.0079 (0.004)	0.07	−0.0083 (0.005)	0.07
6	rs1999805	<i>ESR1</i>	a/g	0.99 (0.95–1.03)	0.57	−0.0054 (0.004)	0.14	−0.0088 (0.004)	0.02
7	rs1524058	<i>STAR3NL</i>	c/t	0.99 (0.95–1.03)	0.61	0.0002 (0.004)	0.95	0.0001 (0.004)	0.98
11	rs16921914	<i>DCDC5</i>	a/g	0.97 (0.93–1.02)	0.20	−0.0058 (0.004)	0.16	0.0064 (0.004)	0.14
12	rs10876432	<i>SP7</i>	g/a	1.02 (0.98–1.07)	0.38	0.0009 (0.004)	0.83	−0.0009 (0.004)	0.84
16	rs10048146	<i>FOXL1</i>	a/g	1.02 (0.97–1.08)	0.51	−0.0074 (0.005)	0.16	−0.0038 (0.006)	0.50
17	rs9303521	<i>CRHR1</i>	g/t	1.00 (0.96–1.05)	0.88	−0.0031 (0.004)	0.43	0.003 (0.004)	0.46
18	rs3018362*	<i>TNFRSF11A</i> (<i>RANK</i>)	g/a	1.02 (0.97–1.06)	0.42	−0.004 (0.004)	0.30	0.0041 (0.004)	0.31
SNPs associated at genome-wide levels of significance with hip and spine BMD									
1	rs7524102	<i>ZBTB40</i>	g/a	1.02 (0.97–1.08)	0.43	0.0068 (0.005)	0.18	0.0079 (0.005)	0.14
1	rs1430742	<i>GPR177</i>	c/t	1.01 (0.96–1.07)	0.59	−0.0005 (0.005)	0.91	0.0026 (0.005)	0.59
6	rs4870044	<i>ESR1</i>	c/t	1.05 (1.00–1.09)	0.05	−0.0015 (0.004)	0.70	−0.0017 (0.004)	0.69
6	rs1038304	<i>ESR1</i>	a/g	1.02 (0.97–1.05)	0.77	−0.0042 (0.004)	0.25	−0.0013 (0.004)	0.72
7	rs4729260	<i>FLJ42280</i>	c/g	0.99 (0.96–1.04)	0.93	0.003 (0.004)	0.46	0.0059 (0.004)	0.16
8	rs4355801	<i>TNFRSF11B</i> (<i>OPG</i>)	g/a	1.04 (0.99–1.08)	0.08	0.0068 (0.004)	0.07	0.003 (0.004)	0.43
13	rs9594738	<i>TNFSF11</i> (<i>RANKL</i>)	c/t	1.01 (0.97–1.05)	0.77	−0.0024 (0.004)	0.52	0.0039 (0.004)	0.31

Continued on p. 2180

the gene transcription start or end site and normalized expression values were performed using the polygenic linear model incorporating a kinship matrix in GenABEL followed by the ProbABEL mmscore score test with imputed genotypes. Age and experimental batch were included as cofactors.

We also tested SNPs that were associated with fasting glucose for association with BMI using in silico GWAS data from the GIANT (the Genetic Investigation of Anthropometric Traits) Consortium (32) and for association with femoral neck and lumbar spine BMD in GEFOS (16).

RESULTS

A total of 26 SNPs associated with BMD at genome-wide levels of significance were tested for association with type 2 diabetes and seven continuous glycemic parameters. None of the SNPs reached the a priori *P* value threshold of 2.4×10^{-4} using conservative Bonferroni correction. Three SNPs were nominally associated (*P* < 0.05) with two diabetes-related traits: the hip BMD-raising allele (G) of SNP rs87939 (*CTNNA1*) was nominally associated with lower fasting insulin and lower HOMA-IR, the hip BMD-raising allele (A) of SNP rs1366594 (*MEF2C*) was associated with higher fasting insulin and higher HOMA-IR, and the spine BMD-raising allele (A) of SNP rs1999805 (*ESR1*) was associated with lower fasting insulin and lower HOMA-IR (Table 2).

We examined 513 SNPs in moderate-to-strong LD ($r^2 \geq 0.5$) with the BMD index SNPs for association with type 2

diabetes and glycemic traits. None of the SNPs reached our prespecified *P* value threshold ($P = 2.6 \times 10^{-4}$). The G allele at SNP rs2070852 (*ARHGAP1*), a near-perfect proxy for the index SNP rs7932354 (T) ($r^2 = 0.96$), was associated with higher fasting glucose ($\beta = 0.0104$ mmol/L [SE 0.004], $P = 9.0 \times 10^{-3}$) (as would be predicted by the nominal association of the index SNP with the same trait). The minor alleles of three SNPs, rs4081640, rs2371445, and rs2371446, in strong LD ($r^2 > 0.8$) with the index SNP rs487939 (*CTNNA1*), were associated with lower fasting insulin (−0.016 [0.005], $P < 0.002$) and HOMA-IR (−0.016 [0.005], $P < 0.005$) at slightly higher levels of significance compared with the index SNP. Likewise, the major alleles of three SNPs at *ESR1* (rs3020348, rs3020349, and rs2982554) were associated with lower fasting insulin (−0.01 [0.004], $P < 0.01$) at a slightly higher level of significance than the index SNP rs1999805 ($r^2 > 0.9$). No other SNPs correlated with the BMD-related index SNPs achieved significance levels <0.01 (Supplementary Table 1).

We examined 1,318 SNPs from nine BMD candidate genes for association with type 2 diabetes and glycemic traits (Supplementary Table 1). Thirteen SNPs at the locus *ITGA1* were associated with fasting glucose at significance levels below our prespecified (Bonferroni-corrected) threshold of

TABLE 2
Continued

HOMA-B		HOMA-IR		HbA _{1c}		2-h glucose		2-h insulin		Ref
β	<i>P</i>	β	<i>P</i>	β (%)	<i>P</i>	β (mmol/L)	<i>P</i>	β (mmol/L)	<i>P</i>	
-0.0026 (0.003)	0.42	-0.0103 (0.004)	0.01	-0.0042 (0.006)	0.49	0.003 (0.019)	0.87	-0.0073 (0.012)	0.54	(16)
0.0032 (0.003)	0.34	0.01 (0.004)	0.02	-0.0001 (0.006)	0.99	0.0167 (0.02)	0.40	-0.004 (0.012)	0.75	(16)
-0.0076 (0.004)	0.04	-0.0052 (0.005)	0.26	-0.0071 (0.007)	0.33	0.0075 (0.022)	0.73	-0.0205 (0.014)	0.14	(16)
-0.0044 (0.004)	0.22	0.0013 (0.004)	0.76	-0.0002 (0.007)	0.98	-0.0028 (0.02)	0.89	0.0089 (0.013)	0.49	(16)
0.0 (0.006)	0.99	0.0024 (0.007)	0.71	0.0018 (0.009)	0.85	-0.0172 (0.03)	0.57	0.0136 (0.019)	0.47	(17)
0.0003 (0.004)	0.94	-0.0015 (0.004)	0.73	-0.0014 (0.007)	0.84	-0.0085 (0.02)	0.67	-0.0036 (0.012)	0.77	(15)
0.0029 (0.004)	0.47	0.0028 (0.005)	0.56	0.0063 (0.008)	0.41	0.0071 (0.023)	0.76	-0.0066 (0.015)	0.65	(16)
-0.0004 (0.003)	0.92	0.0032 (0.004)	0.44	0.0052 (0.006)	0.42	-0.0079 (0.019)	0.68	-0.0153 (0.012)	0.21	(15)
0.0045 (0.004)	0.21	0.0078 (0.004)	0.07	0.0019 (0.006)	0.76	0.0017 (0.02)	0.93	-0.0002 (0.013)	0.99	(15)
-0.0006 (0.004)	0.87	0.0073 (0.004)	0.10	0.0167 (0.007)	0.01	-0.0522 (0.021)	0.01	-0.0243 (0.013)	0.05	(16)
-0.0029 (0.004)	0.40	-0.0061 (0.004)	0.15	0.0035 (0.006)	0.58	0.0014 (0.02)	0.94	0.0 (0.012)	1.00	(16)
-0.0032 (0.004)	0.40	-0.0057 (0.005)	0.23	0.0096 (0.008)	0.23	0.0245 (0.023)	0.28	-0.0125 (0.014)	0.37	(14)
-0.0051 (0.003)	0.13	-0.0086 (0.004)	0.03	0.0115 (0.006)	0.06	0.024 (0.02)	0.23	0.001 (0.012)	0.93	(14)
0.0013 (0.003)	0.71	0.0013 (0.004)	0.75	-0.0052 (0.006)	0.39	-0.0106 (0.02)	0.59	0.0073 (0.013)	0.57	(16)
0.0068 (0.004)	0.06	0.0046 (0.005)	0.30	0.0058 (0.007)	0.42	-0.0167 (0.021)	0.42	-0.0046 (0.014)	0.74	(16)
-0.0022 (0.004)	0.56	-0.002 (0.005)	0.67	0.0019 (0.007)	0.78	-0.0166 (0.021)	0.43	-0.0064 (0.013)	0.62	(15)
-0.0013 (0.005)	0.78	-0.0036 (0.006)	0.53	0.0116 (0.01)	0.24	-0.0467 (0.026)	0.08	-0.0159 (0.018)	0.38	(16)
0.0015 (0.004)	0.66	0.0026 (0.004)	0.54	-0.0052 (0.006)	0.41	0.0491 (0.02)	0.01	0.0221 (0.013)	0.09	(16)
0.0068 (0.003)	0.05	0.0034 (0.004)	0.42	0.0082 (0.007)	0.22	0.0117 (0.02)	0.55	-0.0039 (0.012)	0.75	(15)
0.0013 (0.005)	0.78	0.0067 (0.006)	0.23	0.0022 (0.008)	0.79	-0.0235 (0.026)	0.37	-0.0134 (0.016)	0.40	(14)
0.0015 (0.004)	0.72	0.0033 (0.005)	0.51	-0.0119 (0.008)	0.12	-0.03 (0.024)	0.22	-0.0225 (0.015)	0.14	(16)
-0.0024 (0.004)	0.50	-0.0025 (0.004)	0.56	0.0024 (0.007)	0.73	-0.022 (0.021)	0.30	0.0112 (0.013)	0.39	(14)
0.0015 (0.003)	0.65	-0.002 (0.004)	0.61	0.006 (0.006)	0.31	0.0096 (0.020)	0.62	0.0128 (0.012)	0.28	(14)
0.0043 (0.004)	0.25	0.0054 (0.004)	0.23	0.0004 (0.007)	0.95	0.0243 (0.021)	0.25	0.0002 (0.013)	0.99	(16)
-0.0029 (0.003)	0.38	0.0033 (0.004)	0.41	-0.0032 (0.006)	0.60	0.0267 (0.019)	0.16	-0.0132 (0.012)	0.27	(17)
0.0058 (0.003)	0.09	0.006 (0.004)	0.14	0.0113 (0.006)	0.06	-0.016 (0.019)	0.40	0.0101 (0.012)	0.41	(14)

SEs are shown below the effect estimate; conversion factor (mmol/L \times 18 = mg/L). Ref, article where the genome-wide association for the respective SNP was described. *SNP also is associated with low trauma fracture. Chr, chromosome.

7.7×10^{-5} , of which 8 were below the study-wide significance threshold (Table 3 and Fig. 2). By assembling an in silico replication sample of 19,417 individuals, we achieved >75% power ($\alpha = 0.05$) to detect 1 SD difference in fasting glucose. Therefore, the top 13 *ITGA1* SNPs were examined for association with fasting glucose in the 12 additional cohorts. The major C allele of SNP rs6867040 was nominally associated with higher fasting glucose ($P = 0.03$) in a directionally consistent manner. None of the 13 SNPs reached genome-wide significance ($P < 5 \times 10^{-8}$) in the combined meta-analysis (Table 3). It is notable that variants in this locus, *ITGA1*, were noted to be among the top 10 most significant associations for five additional traits: type 2 diabetes, fasting insulin, HOMA-B, and 2-h glucose and insulin levels (Table 4).

To investigate the mechanism by which *ITGA1* might influence type 2 diabetes and related traits, we examined the effect of these 13 SNPs on *cis*-gene expression of *ITGA1* in liver and adipose tissue using eQTL analysis. *ITGA1* expression was measured in adipose tissue using a 50-base pair probe (chromosome 5:52,284,986–52,285,035) available on the Illumina array and in liver tissue with a set of probes covering the length of the *ITGA1* region (including the gene *PELO*) on the Affymetrix array. The major allele of six SNPs was associated with increased expression (β ranged from

0.089 to 0.107 [SE 0.043–0.044]) of *ITGA1/PELO* in liver tissue at $P < 0.05$, but no SNPs were associated with *ITGA1* expression in adipose tissue (Table 5). Of note, in adipose tissue, the major alleles of the 13 SNPs were highly associated with lower *PELO* expression (effect estimates ~ 0.05 [SE ~ 0.01], lowest $P < 2.0 \times 10^{-4}$). To determine whether *PELO* or *ITGA1* gene expression was driving the association seen in liver tissue of the *ITGA1* expression, we examined probes for each exon individually. We noted that for all of the genetic variants, the SNPs appeared to have a stronger association with the *ITGA1*-specific probes than *PELO*-specific probes (an example figure of one of the SNPs, rs10512997, is provided in the Supplementary Data). *ITGA1* and *PELO* are both expressed in liver, adipose, and pancreatic islets, although *ITGA1* appears to have higher expression in these tissues (Supplementary Data).

We examined 13 SNPs in *ITGA1* for association with BMI in the GIANT Consortium and BMD in the GEFOS Consortium. The major allele of seven SNPs was associated with higher BMI at $P < 0.05$ (Table 5). None of these SNPs were associated with femoral neck and lumbar spine BMD, although they trended toward lowering BMD.

TABLE 3
SNPs in *ITGA1* associated with fasting glucose Stage 1 and taken forward for replication

SNP	Function	Effect/other allele	Stage 1 (up to 46,262 participants)		Replication (Stage 2) (up to 19,417 participants)		Combined (up to 64,188 participants)	
			β (SE)	<i>P</i> value	β (SE)	<i>P</i> value	β (SE)	<i>P</i> value
rs6881900	Intronic enhancer	a/g	0.0167 (0.004)	3.1×10^{-5}	0.0092 (0.006)	0.14	0.0151 (0.003)	9.1×10^{-6}
rs17209725	Intronic	c/t	0.0164 (0.004)	3.9×10^{-5}	0.0109 (0.006)	0.08	0.0154 (0.003)	6.2×10^{-6}
rs17209760	Intronic enhancer	c/g	0.0164 (0.004)	3.9×10^{-5}	0.0108 (0.006)	0.08	0.0154 (0.003)	6.3×10^{-6}
rs10512997	Intronic	c/t	0.0164 (0.004)	3.9×10^{-5}	0.0088 (0.006)	0.15	0.0148 (0.003)	1.4×10^{-5}
rs7716758	Upstream	a/t	0.0165 (0.004)	4.1×10^{-5}	0.0113 (0.006)	0.07	0.0156 (0.003)	5.1×10^{-6}
rs12188019	Intronic enhancer	t/c	0.0163 (0.004)	4.3×10^{-5}	0.0109 (0.006)	0.08	0.0154 (0.003)	6.7×10^{-6}
rs10940273	Intronic	c/a	0.0176 (0.004)	4.5×10^{-5}	0.0103 (0.007)	0.15	0.0165 (0.004)	9.6×10^{-6}
rs6878212	Intronic	t/a	0.0163 (0.004)	4.7×10^{-5}	0.0109 (0.006)	0.08	0.0153 (0.003)	6.8×10^{-6}
rs6867040	Intronic enhancer	c/t	0.0165 (0.004)	6.7×10^{-5}	0.0142 (0.007)	0.03	0.0166 (0.004)	2.3×10^{-6}
rs6450088	Intronic	a/g	0.0158 (0.004)	6.7×10^{-5}	0.0104 (0.006)	0.10	0.0148 (0.003)	1.4×10^{-5}
rs12153381	Intronic enhancer	c/t	0.0157 (0.004)	6.9×10^{-5}	0.0092 (0.006)	0.14	0.0144 (0.003)	1.6×10^{-5}
rs10512998	Intronic enhancer	a/t	0.0156 (0.004)	7.2×10^{-5}	0.0092 (0.006)	0.14	0.0143 (0.003)	1.7×10^{-5}
rs11886	Intronic	t/g	0.0156 (0.004)	7.4×10^{-5}	0.0094 (0.006)	0.13	0.0144 (0.003)	1.7×10^{-5}
rs13179969*	Intronic	g/a	-0.0013 (0.004)	0.76				

Eight SNPs in *ITGA1* were associated with fasting glucose below our study-wide *P* value threshold ($P = 6.0 \times 10^{-5}$, in boldface type) in the 21 discovery cohorts of MAGIC. The top 13 SNPs were promoted for follow-up with fasting glucose in 12 additional cohorts with in silico genotype data. A combined analysis was then performed. SNP function was determined using FastSNP search (50). β s are expressed in mmol/L (conversion: mmol/L \times 18 = mg/L). Boldfaced alleles are the major allele per HapMap CEU. *rs13179969 major allele (G) was associated with lower lumbar spine BMD ($\beta = -0.07$ g/cm²) in a candidate gene study at study-wide significance ($P = 9.6 \times 10^{-7}$) (20).

DISCUSSION

By exploring genetic pleiotropy, we revealed a locus that may provide clues to a mechanism underlying the observed epidemiological association between type 2 diabetes and heightened fracture risk. We compiled a comprehensive list of BMD-related SNPs composed of genetic variants associated with BMD at levels of genome-wide significance, variants in moderate-to-strong LD with the index SNPs, and

SNPs in BMD candidate genes. By examining these BMD-related SNPs for association with type 2 diabetes and glycemic traits, we discovered that SNPs in the *ITGA1* locus, a BMD candidate gene, are suggestively associated with fasting glucose at study-wide levels of significance. The major alleles of these 13 highly correlated SNPs (CEU HapMap [Utah residents with ancestry from northern and western Europe] $r^2 > 0.7$) consistently raised fasting

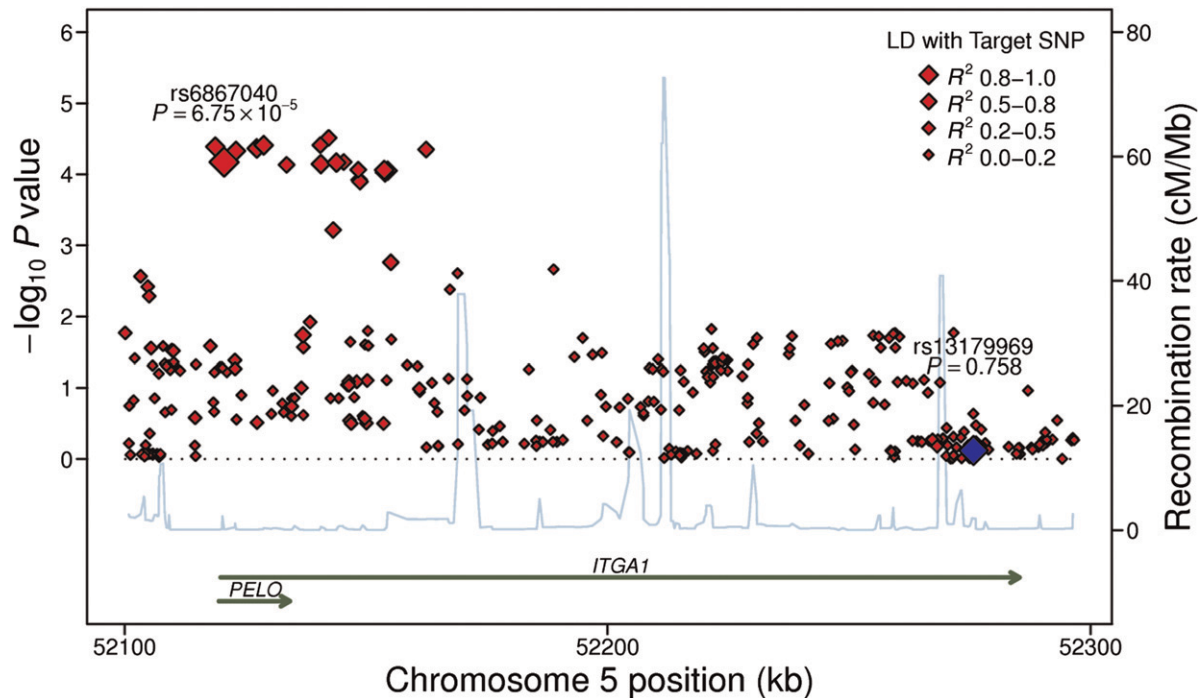


FIG. 2. SNPs at BMD-associated *ITGA1* associated with fasting glucose. Thirteen SNPs (red diamonds) in *ITGA1* were associated with fasting glucose levels ($P < 7.7 \times 10^{-5}$) in the MAGIC discovery cohorts, with 1 SNP (rs6867040) replicating at nominal significance ($P < 0.05$) in 12 replication cohorts. SNP rs13179969 (blue diamond) (*ITGA1*) was associated with lumbar spine BMD in GEFOS at 9.6×10^{-7} (20). This SNP is not associated with fasting glucose in MAGIC. LD is indicated by size of the diamond.

TABLE 4

Top 10 BMD-related SNPs, direction of effect, and level of significance for association with type 2 diabetes and glyce- mic traits

Type 2 diabetes				HbA _{1c}				Fasting insulin			
E/O		P		E/O		P		E/O		P	
<i>ITGA1</i> (Chr 5)				<i>DUSP3</i> (Chr 17)				<i>ESR1</i> (Chr 6)			
rs17208683	a/g	↑	0.002	rs1230397	t/c	↓	0.004	rs3020410	a/c	↑	0.001
rs11745801	a/g	↑	0.003	rs4793026	a/g	↓	0.005	rs9341052	a/g	↑	0.004
rs17274300	g/t	↑	0.007	rs17742347	t/c	↑	0.006	rs9371564	a/g	↑	0.006
<i>TNFRSF11B</i> (Chr 8)				rs3785810				<i>CTNNB1</i> (Chr 3)			
rs9642843	a/c	↑	0.007	rs11713	a/g	↑	0.006	rs4081640	t/g	↓	0.001
rs7829123	a/c	↑	0.007	rs1234612	t/c	↓	0.006	rs2371445	a/g	↓	0.002
rs7835846	c/t	↑	0.007	<i>TNFRSF11B</i> (Chr 8)				rs2371446	t/g	↓	0.002
rs12677975	c/t	↑	0.009	rs12675217	a/g	↓	0.006	<i>SOST</i> (Chr 17)			
rs11573849	g/t	↑	0.010	rs9642843	a/c	↓	0.007	rs17610252	a/t	↓	0.004
rs11573828	c/t	↑	0.010	rs7829123	a/c	↓	0.008	<i>ITGA1</i> (Chr 5)			
<i>LRP4</i> (Chr 11)				<i>LRP5</i> (Chr 11)				rs2452868			
rs13448	c/t	↑	0.007	rs7924398	t/c	↑	0.007	rs2938789	t/c	↓	0.007
HOMA-B				HOMA-IR							
E/O		P		E/O		P					
<i>ITGA1</i> (Chr 5)				<i>CTNNB1</i> (Chr 3)							
rs1466445	t/c	↑	0.0006	rs4081640	t/g	↓	0.003				
rs2452864	a/g	↓	0.0008	rs2371445	a/g	↓	0.003				
rs2934215	t/g	↓	0.001	rs2371446	t/g	↓	0.005				
rs2934216	a/g	↑	0.001	rs430727	t/c	↓	0.005				
rs2456216	a/g	↑	0.001	<i>ESR1</i> (Chr 6)							
rs2047067	a/g	↑	0.001	rs9479129	t/c	↓	0.004				
rs2452869	t/c	↑	0.001	rs9371564	a/g	↑	0.006				
rs2447869	t/c	↑	0.001	rs3020410	a/c	↑	0.007				
rs9686276	a/c	↑	0.001	<i>MEF2C</i> (Chr 5)							
rs10038838	a/c	↑	0.001	rs430727	t/c	↑	0.005				
				rs10037512	t/g	↑	0.007				
2-h glucose				2-h insulin							
E/O		P		E/O		P					
<i>ESR1</i> (Chr 6)				<i>ITGA1</i> (Chr 5)							
rs827420	a/g	↓	0.005	rs17274300	t/g	↑	0.0008				
rs712221	a/t	↑	0.06	rs17208683	a/g	↑	0.001				
rs1514348	t/g	↑	0.06	rs11745801	a/g	↑	0.001				
rs827419	a/c	↑	0.06	<i>ESR1</i> (Chr 6)							
rs1709184	t/c	↑	0.05	rs3798758	a/c	↓	0.002				
rs1709182	t/c	↑	0.06	rs926848	t/c	↓	0.003				
<i>TNFRSF11B</i> (Chr 8)				rs1801132							
rs4876868	a/g	↓	0.005	rs9341086	a/c	↓	0.003				
rs11573856	t/c	↑	0.01	rs827419	a/c	↑	0.004				
rs11573869	a/g	↓	0.01	rs1709182	t/c	↑	0.005				
<i>ITGA1</i> (Chr 5)				rs1709184							
rs7730842	t/c	↑	0.01								

Underlined SNPs are in moderate-to-strong LD with SNPs associated with BMD in GWASs. Top fasting glucose SNPs are listed in Table 3. E/O, effect/other allele. Chr, chromosome. Arrows indicate the direction of effect. Gene name is indicated followed by the chromosomal location in parentheses.

glucose in the discovery and replication stages. In addition, genetic variants of *ITGA1* appear among the top 10 genetic variants for association with five additional traits: type 2 diabetes, fasting insulin levels, HOMA-B, 2-h glucose levels, and 2-h insulin levels. The major alleles at these SNPs appear to be associated with higher *ITGA1* expression in the liver and higher BMI. We highlight that genetic variation in *ITGA1* may not only explain increased bone fragility but also contribute to fasting glucose levels.

ITGA1 encodes the α -1 subunit integrin, which heterodimerizes to form the α 1 β 1-integrin cell surface receptor for laminin and collagen. Integrins are transmembrane

glycoproteins involved in cell adhesion to the extracellular matrix. They are also signaling molecules for regulation of apoptosis, gene expression, cell proliferation, invasion and metastasis, and angiogenesis (33). Less is known about the *PELO* gene in humans, which overlaps the *ITGA1* sequence at the 5' end (Fig. 2) and has been more extensively studied in *Drosophila*. Human and *Drosophila* homologs share 70% sequence identity. *PELO* is thought to be involved in mitosis and meiosis (e.g., spermatogenesis) in many tissues (34), but its involvement in bone and glucose disease is unknown.

The *ITGA1* locus was initially chosen for our study because it was found to contain an intronic SNP, rs13179969,

TABLE 5
Association of *ITGA1* genetic variation with *ITGA1* RNA expression and BMI

SNP	Effect/other allele	Association of SNPs with <i>ITGA1</i> eQTL				Association of SNPs with BMI	
		Liver		Adipose		BMI	
		β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>
rs6867040*	c/t	0.073 (0.043)	0.09	-0.018 (0.013)	0.21	0.012 (0.005)	0.03
rs7716758	a/t	0.064 (0.043)	0.15	-0.015 (0.014)	0.16	0.011 (0.005)	0.04
rs12188019*	t/c	0.071 (0.043)	0.10	-0.018 (0.013)	0.17	0.011 (0.005)	0.04
rs17209725*	c/t	0.084 (0.043)	0.05	-0.018 (0.013)	0.18	0.011 (0.005)	0.04
rs6878212*	t/a	0.07 (0.043)	0.11	-0.018 (0.013)	0.17	0.011 (0.005)	0.04
rs17209760*	c/g	0.084 (0.043)	0.05	-0.018 (0.013)	0.18	0.011 (0.005)	0.04
rs6450088	a/g	0.094 (0.043)	0.03	-0.018 (0.014)	0.34	0.009 (0.005)	0.07
rs11886*	t/g	0.089 (0.043)	0.04	-0.015 (0.013)	0.25	0.01 (0.005)	0.06
rs10940273	c/a	0.078 (0.044)	0.08	-0.018 (0.014)	0.20	0.015 (0.006)	0.007
rs10512998	a/t	0.107 (0.043)	0.01	-0.015 (0.013)	0.28	0.01 (0.005)	0.05
rs12153381	c/t	0.1 (0.043)	0.02	-0.015 (0.013)	0.26	0.01 (0.005)	0.05
rs6881900	a/g	0.099 (0.043)	0.02	-0.015 (0.013)	0.28	0.01 (0.005)	0.05
rs10512997	c/t	0.095 (0.043)	0.03	-0.015 (0.013)	0.28	0.01 (0.005)	0.05

*SNP overlies both *ITGA1* and *PELO* gene. The boldfaced *P* values denote nominal significance ($P < 0.05$).

whose G major allele had been associated with lower lumbar spine BMD at levels of study-wide significance ($P = 9.6 \times 10^{-7}$) (20). This SNP was not associated with fasting glucose in our study, nor is it in strong LD with the 13 SNPs followed up in this study ($r^2 < 0.05$, HapMap CEU) (Fig. 2). Despite low LD between these SNPs, they point to a locus, *ITGA1*, in which in vivo and in vitro models have a suggested role in both bone disease and glucose homeostasis. Null *ITGA1* mice have impaired fracture healing and cartilage remodeling (35), although it is not yet clear what role this gene product has on BMD or bone structure in animal models. Furthermore, integrins have been examined in an effort to culture and expand human β -cells for human transplantation ex vivo (36). The $\alpha 1\beta 1$ -integrins appear to play a role in β -cell insulin secretion, migration, and mesenchymal transformation (37).

The mechanism by which *ITGA1* may influence fasting glucose is not entirely clear. Fasting glucose is an estimate of hepatic glucose production after an overnight fast and can indicate hepatic and peripheral insulin resistance (38). Our follow-up gene expression studies suggest that *ITGA1* genetic variation may affect fasting glucose via the liver rather than adipose tissue. We found that the major alleles of six of the SNPs tested were correlated with increased hepatic expression of *ITGA1* ($P < 0.05$). In addition, the same top three SNPs were associated with both type 2 diabetes and 2-h insulin level, suggesting that the mechanism may involve insulin resistance. Some studies suggest a role of integrins in insulin resistance (39). Integrins are thought to play a key role in the evolution of liver fibrosis brought on by inflammation as seen in insulin resistance-associated nonalcoholic steatohepatitis (39).

In mice, the influence of *ITGA1* and *ITGA2* (encoding the $\alpha 2$ component of $\alpha 2\beta 1$ -integrin) on the effect of inflammation on insulin resistance in muscle induced by a high-fat diet has been examined recently (40). The high-fat diet induced extracellular matrix changes by increasing collagen accumulation in muscle. The *itga2*^{-/-} mice on a high-fat diet had lower basal glucose than *itga2*^{+/+} mice, suggesting that the extracellular matrix-integrin signaling plays a role in insulin resistance in muscle. The same observation was not seen for *itga1*^{-/-} and *itga1*^{+/+} mice. Given our study's gene expression findings and the role of integrins in the liver

in response to inflammation and insulin resistance, further investigation of the liver in *itga1* null mice in response to inflammation could reveal more information about the role of *ITGA1* in hepatic glucose production. Ultimately, our study remains hypothesis generating and highlights a novel locus that links BMD and fasting glucose that warrants further investigation.

This study suggests that *ITGA1* may exhibit genetic pleiotropy through its association with BMD and fasting glucose. True pleiotropy is difficult to confirm, especially if a causal relationship exists between fasting glucose and BMD, as such a finding suggests the possibility of a mediating effect of one phenotype on the other. The evidence of such a causal relationship between fasting glucose and bone density is not completely consistent. Although in vitro studies show that chronic hyperglycemia may impair osteoblast function (41,42), clinical studies demonstrate that individuals with type 2 diabetes have lower bone turnover (43), which usually indicates a more optimal skeletal state. On the other hand, those with poorly controlled diabetes have been shown to have improvement in BMD measured by bone densitometry after 1 year of tightened control (44). Therefore, it is not possible to clearly establish a direct link between hyperglycemia and BMD (45). Likewise, if there was a common intermediate phenotype driving the relationship between BMD and fasting glucose, then our findings may not indicate true genetic pleiotropy. BMI could be considered a potential intermediate phenotype because it is correlated with both type 2 diabetes pathogenesis and BMD (46,47). We examined the *ITGA1*-related SNPs for association with BMI in the GIANT Consortium (32). Several of the variants reached a nominal level of significance (lowest $P = 0.007$) for association with BMI (Table 4). These data suggest that *ITGA1* may act on BMD or fasting glucose through the intermediate phenotype of BMI. Although the *ITGA1* locus has not been associated with BMI in the past, the intronic SNP rs7723398 ($r^2 < 0.3$ per CEU with the SNPs followed up in this study) has been found to be associated with another anthropometric trait, brachial circumference ($P = 9.7 \times 10^{-6}$), in a Croatian population (48).

The strengths of our study include the comprehensive bone-related SNP selection from recently published GWAS data and the ability to test them in very large, well-phenotyped

type 2 diabetes and glycemic traits consortia. We were able to replicate our findings from the discovery phase in an additional ~19,000 individuals. We also followed up the genetic variants with eQTL analysis and other related traits. Our results may help explain the, as yet not quite well understood, epidemiological link between type 2 diabetes and bone disease. This study has highlighted the necessity to examine genetic variants not reaching the genome-wide significance threshold because this may uncover potential findings buried in the *P* value distribution. Given that the MAGIC discovery dataset has been published since the completion of our analyses, further studies like ours can be pursued (www.magicinvestigators.org). Furthermore, the BMD-related locus that was associated with fasting glucose was selected from a candidate gene study. This illustrates the importance of examining candidate genes in discovering genetic pleiotropy rather than solely examining loci associated at levels of genome-wide significance.

We are limited by having chosen SNPs from GWASs examining only BMD. Even though BMD is predictive of fracture in people with type 2 diabetes (10), studies show that individuals with type 2 diabetes have a higher risk of fracture despite higher BMD in general (4). By examining genetic variants related to BMD only, we may miss the non-BMD related genetic contribution to fracture risk. In addition, our findings do not explain the observed paradox of generally higher BMD and yet higher fracture risk among people with type 2 diabetes (4). A direct genetic test of this paradox using *ITGA1* SNPs is not possible because the SNPs that influence fasting glucose and BMD at this locus are not correlated. In addition, a follow-up study examining fracture-related genetic variants for association with type 2 diabetes and glycemic traits will be warranted when large fracture GWASs become available. In a similar manner, the examination of glycemia-related SNPs for association with BMD and fracture phenotypes may further explain the relationship between bone disease and type 2 diabetes, and these studies are currently under way.

Despite the large sample size, none of the SNPs reached genome-wide significance in the combined analysis. We may need a larger sample size to determine if the *ITGA1* SNPs that were associated with fasting glucose will replicate in other populations and attain genome-wide significance because our replication sample may have been too small to detect the association found in the discovery stage. We estimate that we need an additional 12,000 participants to see an association between the *ITGA1* SNPs and fasting glucose at the same effect sizes seen in the discovery stage. Fortunately, ongoing deployment of the custom-made Metabo-Chip (comprising >200,000 SNPs related to cardiovascular disease, obesity, and type 2 diabetes) across many thousands of samples with relevant phenotypes may provide sufficient power to uncover novel associations at genome-wide significance levels. The *ITGA1* SNPs rs6881900 and rs10940273, found to be associated with fasting glucose in our study, are present in the Metabo-Chip. This provides an exciting opportunity to understand the relationship of *ITGA1* with glycemic traits, as well as other metabolic phenotypes in cardiovascular disease and obesity.

In sum, we have identified a new locus candidate, *ITGA1*, influencing both fasting glucose and BMD, that may begin to explain the genetic contribution to the epidemiological observations linking type 2 diabetes and osteoporosis. The ongoing analysis of Metabo-Chip genotypes across large samples will help determine if *ITGA1* proves to be a new locus associated with fasting glucose at levels of genome-wide significance.

New insights into the genetic pleiotropy of both disease states may further underscore the link between skeletal and glucose metabolism, highlight the complexity of this relationship, provide a focus for future investigations, raise awareness for adverse effects in one system while treating another, and reveal potential targets for disease therapies in both diseases.

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L.K.B. wrote the manuscript and researched data. Y.-H.H. researched data, contributed to discussion, and reviewed and edited the manuscript. R.J.A. formatted the tables and reviewed and edited the manuscript. J.D. performed the meta-analysis and reviewed and edited the manuscript. B.F.V., L.J.R.-T., S.H., M.L., D.B., C.La., J.H., M.F., N.B.-N., C.Le., P.A., P.K.M., I.S., S.R., L.C., C.D., J.K., K.O.K., N.L.P., I.B.B., M.A.P., B.B., P.F., A.R.S., L.J.P., N.W., P.M., T.J., and J.S.P. researched and provided data from their respective cohorts and reviewed and edited the manuscript. L.F., E.G., and P.E. researched and provided eQTL analysis and reviewed and edited the manuscript. D.K., J.B.M., and D.P.K. contributed to discussion and reviewed and edited the manuscript. J.C.F. contributed to discussion and wrote the manuscript. L.K.B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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