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

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.2337/db11-1515

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11855790

Terms of Use
This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Impact of Common Variation in Bone-Related Genes on Type 2 Diabetes and Related Traits

Liana K. Billings,1,2,3 Yi-Hsiang Hsu,4,5,6 Rachel J. Ackerman,1 Josée Dupuis,6,7 Benjamin F. Voight,1,2,8 Laura J. Rasmussen-Torvik,9 Serge Hercberg,10 Mark Lathrop,11 Daniel Barnes,12 Claudia Langenberg,12 Jennie Hui13,14,15 Mao Fu16 Nabila Boutatia-Naji,17 Cecile Lecoeur,17 Ping An,18 Patrik K. Magnusson19 Ida Surakka20,21 Samuli Ripatti,20,21 Lene Christiansen22 Christine Dalgård,23 Lasse Folkersen24 Elin Grundberg,25,26 the MAGIC Investigators,* the DIAGRAM+ Consortium,* the MuTHER Consortium,* the ASCOT Investigators,* the GEFOS Consortium,27,* Per Eriksson,24 Jaakko Kaprio,20,28,29 Kirsten Ohm Kyvik,30,31 Nancy L. Pedersen,19 Ingrid B. Borecki,18 Michael A. Province,19 Beverley Balkau,32 Philippe Froguel,17,33 Alan R. Shuldiner,16,34 Lyle J. Palmer,35 Nick Wareham,12 Pierre Meneton,36 Toby Johnson,37 James S. Pankow,38 Michael L. Willard,39 Karen E. Anderson,40,41 Domenico Laurenti,42,43 Daniel Barnes,12 Liana K. Billings,1,2,3 Yi-Hsiang Hsu,4,5,6 Rachel J. Ackerman,1 Josée Dupuis,6,7 Benjamin F. Voight,1,2,8 Laura J. Rasmussen-Torvik,9 Serge Hercberg,10 Mark Lathrop,11 Daniel Barnes,12 Claudia Langenberg,12 Jennie Hui13,14,15 Mao Fu16 Nabila Boutatia-Naji,17 Cecile Lecoeur,17 Ping An,18 Patrik K. Magnusson19 Ida Surakka20,21 Samuli Ripatti,20,21 Lene Christiansen22 Christine Dalgård,23 Lasse Folkersen24 Elin Grundberg,25,26 the MAGIC Investigators,* the DIAGRAM+ Consortium,* the MuTHER Consortium,* the ASCOT Investigators,* the GEFOS Consortium,27,* Per Eriksson,24 Jaakko Kaprio,20,28,29 Kirsten Ohm Kyvik,30,31 Nancy L. Pedersen,19 Ingrid B. Borecki,18 Michael A. Province,19 Beverley Balkau,32 Philippe Froguel,17,33 Alan R. Shuldiner,16,34 Lyle J. Palmer,35 Nick Wareham,12 Pierre Meneton,36 Toby Johnson,37 James S. Pankow,38 David Karasik,4,6 James B. Meigs,2,6 Douglas P. Kiel,2,4,6 and Jose C. Florez1,2,3,8

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 glycaemic 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.


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 diabetes.

From the 1Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts; the 2Department of Medicine, Harvard Medical School, Boston, Massachusetts; the 3Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts; the 4Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, Boston, Massachusetts; the 5Molecular and Integrative Physiological Sciences Program, Harvard School of Public Health, Boston, Massachusetts; the 6Framingham Heart Study, Framingham, Massachusetts; the 7Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the 8Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts; the 9Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois; the 10INSERM, National Institute of Agronomic Research, University of Paris, Bologna, France; the 11National Genotyping Center, Atomic Energy Commission, Institute of Genomics, Evry, France; the 12Medical Research Council Epidemiology Unit, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, U.K.; the 13Molecular Genetics, PathWest Laboratory Medicine of Western Australia, Nedlands, Western Australia; the 14School of Population Health and School of Epidemiology and Laboratory Medicine, University of Western Australia, Nedlands, Western Australia; the 15Bassettel Region Population Medical Research Foundation, Sir Charles Gairdner Hospital, Nedlands, Western Australia; the 16Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; the 17National Center for Scientific Research, UMR 8199, Genomics and Metabolic Diseases, Lille Pasteur Institute, Lille Nord de France University, Lille, France; the 18Division of Statistical Genomics and Department of Genetics, Washington University School of Medicine, St. Louis, Missouri; the 19Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden; the 20Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; the 21Public Health Research Unit, National Institute for Health and Welfare, Helsinki, Finland; the 22Danish Twin Registry, Epidemiology, Institute of Public Health, University of Southern Denmark, Odense, Denmark; the 23Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark; the 24Atherosclerosis Research Unit, Department of Medicine, Karolinska Institute, Stockholm, Sweden; the 25Wellcome Trust Sanger Institute, Hinxton, U.K.; the 26Department of Twin Research and Genetic Epidemiology, King’s College London, London, U.K.; the 27Erasmus Medical College (Coordinating Center), Rotterdam, the Netherlands; the 28Unit for Child and Adolescent Mental Health, National Institute for Health and Welfare, Helsinki, Finland; the 29Department of Public Health, University of Helsinki, Helsinki, Finland; the 30Institute of Regional Health Services Research, University of Southern Denmark, Odense, Denmark; the 31Odense Patient Data Explorative Network, Odense University Hospital, Odense, Denmark; the 32CESP Center for Research in Epidemiology and Health of Populations, U1018, Epidemiology of Diabetes, Obesity and Chronic Kidney Disease Over the Life Course, INSERM, Villejuif, France, and Université Paris-Sud 11, UMRS 1018, Villejuif, France; the 33Genomic Medicine, Hammersmith Hospital, Imperial College London, London, U.K.; the 34Geriatric Research, Education and Clinical Center, Baltimore VA Medical Center, Baltimore, Maryland; the 35Ontario Institute for Cancer Research, Toronto, Ontario, Canada; the 36Cordeliers Center of Research, INSERM, Paris, France; the 37Clinical Pharmacology and the Genome Centre, William Harvey Research Institute, Barts and London School of Medicine and Dentistry, Queen Mary University of London, London, U.K.; and the 38Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota.

Corresponding author: Jose C. Florez, jcflorez@partners.org.
Received 27 October 2011 and accepted 9 March 2012.
DOI: 10.2337/db11-1515

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-1515/-/DC1.
*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. 26.

© 2012 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativedcommons.org/licenses/by-nc-nd/3.0/ for details.
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.

**TABLE 1**

| Locus | SNP | Trait/disease | Reference*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MEF2C</td>
<td>rs17421627</td>
<td>Retinal vascular caliber</td>
<td>Ikram MK, PLoS Genetics, 2010</td>
</tr>
<tr>
<td></td>
<td>rs10037512</td>
<td>Height</td>
<td>Lango Allen H, Nature, 2010</td>
</tr>
<tr>
<td></td>
<td>rs770189</td>
<td>Tonometry</td>
<td>Levy D, BMC Medical Genetics, 2007</td>
</tr>
<tr>
<td>SOX6</td>
<td>rs297325</td>
<td>BMI</td>
<td>Liu YZ, PLoS One, 2009</td>
</tr>
<tr>
<td>MEPE</td>
<td>rs7699623</td>
<td>Ischemic stroke among migraineurs with aura</td>
<td>Schürks M, PLoS One, 2011</td>
</tr>
<tr>
<td>MHC</td>
<td>rs2516399</td>
<td>Eosinophil count</td>
<td>Okada Y, PLoS Genetics, 2011</td>
</tr>
<tr>
<td></td>
<td>rs2269426</td>
<td>Eosinophil count</td>
<td>Gudbjartsson DF, Nature Genetics, 2009</td>
</tr>
<tr>
<td></td>
<td>rs3069254</td>
<td>Monocyte count</td>
<td>Okada Y, PLoS Genetics, 2011</td>
</tr>
<tr>
<td></td>
<td>rs8271366</td>
<td>Inflammatory bowel disease</td>
<td>Okada Y, Gastroenterology, 2011</td>
</tr>
<tr>
<td></td>
<td>rs7774434</td>
<td>Primary biliary cirrhosis</td>
<td>Melles GF, Nature Genetics, 2011</td>
</tr>
<tr>
<td></td>
<td>rs34704616</td>
<td>Cognitive test performance</td>
<td>Cirulli ET, Eur J Human Genetics, 2010</td>
</tr>
<tr>
<td></td>
<td>rs7743761</td>
<td>Ankylosing spondylitis</td>
<td>Reveille JD, Nature Genetics, 2010</td>
</tr>
<tr>
<td></td>
<td>rs9268866</td>
<td>Ulcerative colitis</td>
<td>Barrett JC, Nature Genetics, 2009</td>
</tr>
<tr>
<td></td>
<td>rs13194053</td>
<td>Schizophrenia</td>
<td>Purcell SM, Nature, 2009</td>
</tr>
<tr>
<td></td>
<td>rs6932590</td>
<td>Schizophrenia</td>
<td>Stefansson H, Nature, 2009</td>
</tr>
<tr>
<td></td>
<td>rs3131296</td>
<td>Schizophrenia</td>
<td>Stefansson H, Nature, 2009</td>
</tr>
<tr>
<td></td>
<td>rs9272346</td>
<td>Type 1 diabetes</td>
<td>WTCCC, Nature, 2007</td>
</tr>
<tr>
<td></td>
<td>rs9268645</td>
<td>Type 1 diabetes</td>
<td>Barrett JC, Nature Genetics, 2009</td>
</tr>
<tr>
<td></td>
<td>rs1265181</td>
<td>Psoriasis</td>
<td>Zhang XJ, Nature Genetics, 2009</td>
</tr>
<tr>
<td></td>
<td>rs6457617</td>
<td>Rheumatoid arthritis</td>
<td>WTCCC, Nature, 2007</td>
</tr>
<tr>
<td>ESR1</td>
<td>rs2982694</td>
<td>Sudden cardiac arrest</td>
<td>Aouizerat BE, BMC Cardiovasc Disord, 2011</td>
</tr>
<tr>
<td></td>
<td>rs4865742</td>
<td>Chronic myeloid leukemia</td>
<td>Kim DH, Blood, 2011</td>
</tr>
<tr>
<td></td>
<td>rs3734058</td>
<td>Breast cancer</td>
<td>Fletcher O, J Natl Cancer Inst, 2011</td>
</tr>
<tr>
<td></td>
<td>rs3757318</td>
<td>Breast cancer</td>
<td>Turnbull C, Nature Genetics, 2010</td>
</tr>
<tr>
<td></td>
<td>rs2046210</td>
<td>Breast cancer</td>
<td>Zheng W, Nature Genetics, 2009</td>
</tr>
<tr>
<td></td>
<td>rs543650</td>
<td>Height</td>
<td>Lango Allen H, Nature, 2009</td>
</tr>
<tr>
<td></td>
<td>rs6902771</td>
<td>Alcohol dependence</td>
<td>Treutlein J, Arch Gen Psychiatry, 2009</td>
</tr>
<tr>
<td>DCDC5</td>
<td>rs3825584</td>
<td>Serum magnesium levels</td>
<td>Meyer TE, PLoS Genetics, 2010</td>
</tr>
<tr>
<td>TNFRSF11A (RANK)</td>
<td>rs3018362</td>
<td>Paget disease</td>
<td>Albagha OM, Nature Genetics, 2011</td>
</tr>
<tr>
<td></td>
<td>rs2957128</td>
<td>Paget disease</td>
<td>Albagha OM, Nature Genetics, 2011</td>
</tr>
<tr>
<td>TNFSF11 (RANKL)</td>
<td>rs2063205</td>
<td>Crohn disease</td>
<td>Franke A, Nature Genetics, 2010</td>
</tr>
</tbody>
</table>

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: CTNNB1, ARHGA1P1, LRP5, MARK3, HDAC5, SOST, SPTB1, STARD3NL, SP7, FOXL1, CRHR1, ZBTB40, GPR177, FLJ42280, and TNFRSF11B (OPG). *The full reference list can be found in the Supplementary Data online.
BONE-RELATED GENES AND GLYCEMIC TRAITS

Collate list of BMD-related SNPs:
1) SNPs from BMD GWASs
2) Nearby SNPs (r²>0.5, ±50kb)
3) SNPs from BMD candidate genes (±20kb) identified in the largest bone-related traits consortium (GEFOS)

For association with type 2 diabetes (DIAGRAM+) and 7 glycemc traits (MAGIC)

1,778 SNPs

Follow-up:
1) SNPs in 19,417 additional subjects
2) cis-eQTL analysis in liver and adipose tissue
3) Association with BMI

rs669681 (ZBTB40), rs878055 and rs692137 (ESR1), rs6905813 and rs4646084 (TNFRSF11), rs6994759 (RANKL), rs1107748 (SOST), rs3260755 (GP1B1), and rs7781370 (FLJ42280) (14, 15, 17). The association of these traits examined. The 

rs669681 (ZBTB40), rs878055 and rs692137 (ESR1), rs6905813 and rs4646084 (TNFRSF11), rs6994759 (RANKL), rs1107748 (SOST), rs3260755 (GP1B1), and rs7781370 (FLJ42280) (14, 15, 17). The final list of 26 BMI genome-wide-associated SNPs was examined for association with type 2 diabetes and glycemc traits (Table 2).

Follow-up strategy. To follow up the BMD-related SNPs associated with type 2 diabetes and glycemc 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], Bosselton Health Study [BBS], 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). DNA quality was assessed with a NanoDrop 1000 (Thermo Scientific, Waltham, MA). RNA was prepared using the RNeasy Mini kit (Qiagen, Hilden, Germany), including treatment with RNasefree DNase (Qiagen). 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 on technical replicates of each individual followed by quantile normalization across all individuals. Subject DNA was genotyped using a combination of Illumina arrays (Illumina, Inc., San Diego, CA) and was genotyped in 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).
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 BMI 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 $2.4 \times 10^{-8}$ 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 rs879930 (CTNNB1) 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 ($P = 2.6 \times 10^{-8}$). 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 ($\beta = 0.0104 \text{ mmol/L [SE 0.004]}$, $P = 9.0 \times 10^{-5}$) (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 rs2487939 (CTNNB1), 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 rs2082554) 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 $P = 2.6 \times 10^{-8}$.

### TABLE 2

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

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>Gene</th>
<th>BMD-raising allele/other</th>
<th>Type 2 diabetes (Odds ratio (95% CI))</th>
<th>Fasting glucose (β (mmol/L) P)</th>
<th>Fasting insulin (β (pmol/L) P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rs87939</td>
<td>CTNNB1</td>
<td>g/a</td>
<td>1.01 (0.97–1.05) 0.80</td>
<td>-0.0071 (0.004) 0.05</td>
<td>-0.0084 (0.004) 0.03</td>
</tr>
<tr>
<td>5</td>
<td>rs1366594</td>
<td>MEF2C</td>
<td>a/c</td>
<td>1.01 (0.97–1.05) 0.59</td>
<td>0.0039 (0.004) 0.30</td>
<td>0.0086 (0.004) 0.03</td>
</tr>
<tr>
<td>11</td>
<td>rs7117858</td>
<td>SOX6</td>
<td>g/a</td>
<td>1.04 (0.99–1.09) 0.15</td>
<td>0.0052 (0.004) 0.22</td>
<td>-0.0064 (0.004) 0.14</td>
</tr>
<tr>
<td>11</td>
<td>rs7932354</td>
<td>ARHGAP1</td>
<td>t/c</td>
<td>1.05 (1.0–1.10) 0.03</td>
<td>0.0106 (0.004) 0.01</td>
<td>-0.0013 (0.004) 0.76</td>
</tr>
<tr>
<td>11</td>
<td>rs3736228*</td>
<td>LRP5</td>
<td>c/t</td>
<td>0.99 (0.94–1.06) 0.97</td>
<td>0.0006 (0.006) 0.92</td>
<td>0.0012 (0.006) 0.85</td>
</tr>
<tr>
<td>14</td>
<td>rs2010281*</td>
<td>MARK3</td>
<td>g/a</td>
<td>1.02 (0.98–1.06) 0.35</td>
<td>-0.0027 (0.004) 0.49</td>
<td>-0.003 (0.004) 0.46</td>
</tr>
<tr>
<td>17</td>
<td>rs228769</td>
<td>HDAC5</td>
<td>g/c</td>
<td>1.01 (0.96–1.06) 0.80</td>
<td>0.0014 (0.005) 0.75</td>
<td>0.0036 (0.005) 0.45</td>
</tr>
<tr>
<td>17</td>
<td>rs7220711</td>
<td>SOST</td>
<td>g/a</td>
<td>1.01 (0.96–1.05) 0.83</td>
<td>0.006 (0.004) 1.12</td>
<td>0.0022 (0.004) 0.58</td>
</tr>
<tr>
<td>17</td>
<td>rs1513670*</td>
<td>SOST</td>
<td>c/t</td>
<td>1.00 (0.96–1.05) 0.92</td>
<td>0.0058 (0.004) 0.13</td>
<td>0.006 (0.004) 0.14</td>
</tr>
</tbody>
</table>

SNPs associated at genome-wide levels of significance with spine BMD

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>Gene</th>
<th>BMD-raising allele/other</th>
<th>Type 2 diabetes (Odds ratio (95% CI))</th>
<th>Fasting glucose (β (mmol/L) P)</th>
<th>Fasting insulin (β (pmol/L) P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>rs11885805*</td>
<td>SPTBN1</td>
<td>a/g</td>
<td>1.01 (0.97–1.06) 0.63</td>
<td>0.0024 (0.004) 0.56</td>
<td>0.0075 (0.004) 0.08</td>
</tr>
<tr>
<td>4</td>
<td>rs1471403</td>
<td>MEPE</td>
<td>t/c</td>
<td>0.99 (0.95–1.04) 0.78</td>
<td>0.0005 (0.004) 0.90</td>
<td>-0.0072 (0.004) 0.07</td>
</tr>
<tr>
<td>6</td>
<td>rs3103540</td>
<td>MHC</td>
<td>c/t</td>
<td>1.02 (0.97–1.07) 0.39</td>
<td>0.0079 (0.004) 0.03</td>
<td>0.0083 (0.005) 0.07</td>
</tr>
<tr>
<td>6</td>
<td>rs1999805</td>
<td>ESR1</td>
<td>a/g</td>
<td>0.95 (0.95–1.05) 0.76</td>
<td>0.0064 (0.004) 0.14</td>
<td>-0.0008 (0.004) 0.02</td>
</tr>
<tr>
<td>11</td>
<td>rs1224058</td>
<td>STARD3NL</td>
<td>c/t</td>
<td>0.99 (0.95–1.05) 0.61</td>
<td>0.0005 (0.004) 0.95</td>
<td>0.00001 (0.004) 0.98</td>
</tr>
<tr>
<td>11</td>
<td>rs16921914</td>
<td>DCDC5</td>
<td>a/g</td>
<td>0.97 (0.93–1.02) 0.20</td>
<td>-0.0058 (0.004) 0.16</td>
<td>0.0064 (0.004) 0.14</td>
</tr>
<tr>
<td>12</td>
<td>rs10876432</td>
<td>SP7</td>
<td>g/a</td>
<td>1.02 (0.98–1.07) 0.38</td>
<td>0.0009 (0.004) 0.83</td>
<td>0.0009 (0.004) 0.84</td>
</tr>
<tr>
<td>16</td>
<td>rs10048146</td>
<td>FOXL1</td>
<td>a/g</td>
<td>1.02 (0.97–1.08) 0.51</td>
<td>-0.0074 (0.005) 0.16</td>
<td>0.0038 (0.006) 0.50</td>
</tr>
<tr>
<td>17</td>
<td>rs9305321</td>
<td>CHKH</td>
<td>g/t</td>
<td>1.00 (0.96–1.05) 0.88</td>
<td>-0.0031 (0.004) 0.43</td>
<td>0.003 (0.004) 0.46</td>
</tr>
<tr>
<td>18</td>
<td>rs3018362*</td>
<td>TNFRSF11A</td>
<td>g/a</td>
<td>1.02 (0.97–1.06) 0.42</td>
<td>-0.004 (0.004) 0.30</td>
<td>0.0041 (0.004) 0.31</td>
</tr>
</tbody>
</table>

SNPs associated at genome-wide levels of significance with hip and spine BMD

Continued on p. 2180
The effect of these 13 SNPs on gene expression was measured using eQTL analysis. In liver and adipose tissue, the major allele was associated with increased expression (β) ranging from -0.0057 to 0.0125 (Table 2). 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 allele of the 13 SNPs were highly associated with lower PELO expression (effect estimates ~0.05 [SE ~0.01], lowest P < 2.0 × 10⁻⁵). 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 13 SNPs were associated with femoral neck and lumbar spine BMD, although they trended toward lowering BMD.

TABLE 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>HOMA-B</th>
<th>HOMA-IR</th>
<th>HbA1c</th>
<th>2-h glucose</th>
<th>2-h insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
<td>β (%)</td>
<td>P</td>
</tr>
<tr>
<td>β (mmol/L)</td>
<td>P</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued

<table>
<thead>
<tr>
<th>β</th>
<th>P</th>
<th>β</th>
<th>P</th>
<th>β (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (mmol/L)</td>
<td>P</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEs are shown below the effect estimate; conversion factor (mmol/L × 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 (α = 0.05) to detect a 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 × 10⁻⁵) 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).
TABLE 3
SNPs in ITGA1 associated with fasting glucose Stage 1 and taken forward for replication

<table>
<thead>
<tr>
<th>SNP</th>
<th>Function</th>
<th>Stage 1 (up to 46,262 participants)</th>
<th>Replication (Stage 2) (up to 19,417 participants)</th>
<th>Combined (up to 64,188 participants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6881900</td>
<td>Intronic enhancer</td>
<td>a/g</td>
<td>β (SE) 0.0167 (0.004) 3.1×10⁻⁵ P value 0.0092 (0.006) 0.14</td>
<td>β (SE) 0.0151 (0.003) 9.1×10⁻⁶</td>
</tr>
<tr>
<td>rs17209725</td>
<td>Intronic</td>
<td>c/t</td>
<td>β (SE) 0.0164 (0.004) 3.9×10⁻⁵ P value 0.0109 (0.006) 0.08</td>
<td>β (SE) 0.0154 (0.003) 6.2×10⁻⁶</td>
</tr>
<tr>
<td>rs17209760</td>
<td>Intronic enhancer</td>
<td>e/g</td>
<td>β (SE) 0.0164 (0.004) 3.9×10⁻⁵ P value 0.0108 (0.006) 0.08</td>
<td>β (SE) 0.0154 (0.003) 6.3×10⁻⁶</td>
</tr>
<tr>
<td>rs10512997</td>
<td>Intronic</td>
<td>e/t</td>
<td>β (SE) 0.0164 (0.004) 3.9×10⁻⁵ P value 0.0088 (0.006) 0.15</td>
<td>β (SE) 0.0148 (0.003) 1.4×10⁻⁵</td>
</tr>
<tr>
<td>rs7716758</td>
<td>Upstream</td>
<td>a/t</td>
<td>β (SE) 0.0115 (0.004) 4.1×10⁻⁵ P value 0.0113 (0.006) 0.07</td>
<td>β (SE) 0.0156 (0.003) 5.1×10⁻⁶</td>
</tr>
<tr>
<td>rs12188019</td>
<td>Intronic enhancer</td>
<td>t/c</td>
<td>β (SE) 0.0163 (0.004) 4.3×10⁻⁵ P value 0.0109 (0.006) 0.08</td>
<td>β (SE) 0.0154 (0.003) 6.7×10⁻⁶</td>
</tr>
<tr>
<td>rs10940273</td>
<td>Intronic</td>
<td>g/a</td>
<td>β (SE) 0.0176 (0.004) 4.5×10⁻⁵ P value 0.0103 (0.007) 0.15</td>
<td>β (SE) 0.0165 (0.004) 9.6×10⁻⁶</td>
</tr>
<tr>
<td>rs6878212</td>
<td>Intronic</td>
<td>t/a</td>
<td>β (SE) 0.0163 (0.004) 4.7×10⁻⁵ P value 0.0109 (0.006) 0.08</td>
<td>β (SE) 0.0153 (0.003) 6.8×10⁻⁶</td>
</tr>
<tr>
<td>rs6867040</td>
<td>Intronic enhancer</td>
<td>c/t</td>
<td>β (SE) 0.0165 (0.004) 6.7×10⁻⁵ P value 0.0142 (0.007) 0.03</td>
<td>β (SE) 0.0166 (0.004) 2.3×10⁻⁶</td>
</tr>
<tr>
<td>rs6450088</td>
<td>Intronic</td>
<td>a/g</td>
<td>β (SE) 0.0158 (0.004) 6.7×10⁻⁵ P value 0.0104 (0.006) 0.10</td>
<td>β (SE) 0.0148 (0.003) 1.4×10⁻⁵</td>
</tr>
<tr>
<td>rs12153381</td>
<td>Intronic enhancer</td>
<td>c/t</td>
<td>β (SE) 0.0157 (0.004) 6.9×10⁻⁵ P value 0.0092 (0.006) 0.14</td>
<td>β (SE) 0.0144 (0.003) 1.6×10⁻⁵</td>
</tr>
<tr>
<td>rs10512998</td>
<td>Intronic enhancer</td>
<td>a/t</td>
<td>β (SE) 0.0156 (0.004) 7.2×10⁻⁵ P value 0.0092 (0.006) 0.14</td>
<td>β (SE) 0.0143 (0.003) 1.7×10⁻⁵</td>
</tr>
<tr>
<td>rs11886</td>
<td>Intronic</td>
<td>t/g</td>
<td>β (SE) 0.0156 (0.004) 7.4×10⁻⁵ P value 0.0094 (0.006) 0.13</td>
<td>β (SE) 0.0144 (0.003) 1.7×10⁻⁵</td>
</tr>
<tr>
<td>rs13179969</td>
<td>Intronic</td>
<td>g/a</td>
<td>β (SE) -0.0013 (0.004) 0.37</td>
<td>β (SE) -0.0013 (0.004) 0.37</td>
</tr>
</tbody>
</table>

Eight SNPs in ITGA1 were associated with fasting glucose below our study-wide P value threshold (P = 6.0×10⁻⁵, 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). βs are expressed in mmol/L (conversion: mmol/L × 18 = mg/L). Boldfaced alleles are the major allele per HapMap CEU. *rs13179969 major allele (G) was associated with lower lumbar spine BMD (β = -0.07 g/cm²) in a candidate gene study at study-wide significance (P = 9.6×10⁻⁶) (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] r² > 0.7) consistently raised fasting glucose levels (P < 7.7×10⁻⁵) 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 9.6×10⁻⁶ (20). This SNP is not associated with fasting glucose in MAGIC. LD is indicated by size of the diamond.

FIG. 2. SNPs at BMD-associated ITGA1 associated with fasting glucose. Thirteen SNPs (red diamonds) in ITGA1 were associated with fasting glucose levels (P < 7.7×10⁻⁵) 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 9.6×10⁻⁶ (20). This SNP is not associated with fasting glucose in MAGIC. LD is indicated by size of the diamond.
BONE-RELATED GENES AND GLYCEMIC TRAITS

TABLE 4
Top 10 BMD-related SNPs, direction of effect, and level of significance for association with type 2 diabetes and glycemic traits

<table>
<thead>
<tr>
<th>Type 2 diabetes</th>
<th>HbA1c</th>
<th>Fasting insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E/O</td>
<td>E/O</td>
<td>E/O</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs17208683</td>
<td>a/g</td>
<td>t/c</td>
</tr>
<tr>
<td>rs11745801</td>
<td>a/g</td>
<td>t/c</td>
</tr>
<tr>
<td>rs17274300</td>
<td>g/t</td>
<td>t/c</td>
</tr>
<tr>
<td>TNFRSF11B (Chr 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs9642843</td>
<td>a/c</td>
<td>t/c</td>
</tr>
<tr>
<td>rs7829123</td>
<td>a/c</td>
<td>t/c</td>
</tr>
<tr>
<td>rs8335846</td>
<td>c/t</td>
<td>t/c</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11573849</td>
<td>g/t</td>
<td>t/c</td>
</tr>
<tr>
<td>rs11573828</td>
<td>a/c</td>
<td>t/c</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs13448</td>
<td>c/t</td>
<td>t/c</td>
</tr>
</tbody>
</table>

HOMA-B |

<table>
<thead>
<tr>
<th>E/O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs1466445</td>
<td>t/c</td>
</tr>
<tr>
<td>rs2452864</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2934215</td>
<td>t/g</td>
</tr>
<tr>
<td>rs2934216</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2456216</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2047067</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2452869</td>
<td>t/c</td>
</tr>
<tr>
<td>rs2447860</td>
<td>t/c</td>
</tr>
<tr>
<td>rs10038398</td>
<td>a/c</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs10038838</td>
<td>a/c</td>
</tr>
</tbody>
</table>

HOMA-IR |

<table>
<thead>
<tr>
<th>E/O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs1466445</td>
<td>t/c</td>
</tr>
<tr>
<td>rs2452864</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2934215</td>
<td>t/g</td>
</tr>
<tr>
<td>rs2934216</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2456216</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2047067</td>
<td>a/g</td>
</tr>
<tr>
<td>rs2452869</td>
<td>t/c</td>
</tr>
<tr>
<td>rs2447860</td>
<td>t/c</td>
</tr>
<tr>
<td>rs10038398</td>
<td>a/c</td>
</tr>
</tbody>
</table>

2-h glucose |

<table>
<thead>
<tr>
<th>E/O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs827420</td>
<td>a/g</td>
</tr>
<tr>
<td>rs712221</td>
<td>a/t</td>
</tr>
<tr>
<td>rs1514345</td>
<td>t/g</td>
</tr>
<tr>
<td>rs827419</td>
<td>a/c</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs1709184</td>
<td>t/c</td>
</tr>
<tr>
<td>rs1709182</td>
<td>t/c</td>
</tr>
</tbody>
</table>

2-h insulin |

<table>
<thead>
<tr>
<th>E/O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs17274300</td>
<td>t/g</td>
</tr>
<tr>
<td>rs17208683</td>
<td>a/g</td>
</tr>
<tr>
<td>rs11745801</td>
<td>a/g</td>
</tr>
<tr>
<td>ESR1 (Chr 6)</td>
<td></td>
</tr>
<tr>
<td>rs9642843</td>
<td>a/g</td>
</tr>
<tr>
<td>rs9341052</td>
<td>a/g</td>
</tr>
<tr>
<td>rs9371564</td>
<td>a/g</td>
</tr>
<tr>
<td>TNFRSF11B (Chr 8)</td>
<td></td>
</tr>
<tr>
<td>rs1761052</td>
<td>t/g</td>
</tr>
<tr>
<td>ITGA1 (Chr 5)</td>
<td></td>
</tr>
<tr>
<td>rs1761052</td>
<td>a/t</td>
</tr>
</tbody>
</table>

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.

BONE-RELATED GENES AND GLYCEMIC TRAITS

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 α1β1-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 ITGA1 gene in humans, which overlaps the PELO sequence identity. 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 intrinsic SNP, rs13179969,
whose G major allele had been associated with lower lumbar spine BMD at levels of study-wide significance ($P = 9.6 \times 10^{-5}$) (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 αβ1-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 increased type 2 diabetes pathogenesis and bone-related SNP selection from recently published GWAS ($P = 9.7 \times 10^{-10}$). 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 rs7723938 ($r^2 < 0.3$ per CEU with the SNPs followed up in this study) has been found to be associated with another anthropometric trait, brachial circumference ($P = 9.7 \times 10^{-5}$), 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

### Table 5

<table>
<thead>
<tr>
<th>SNP</th>
<th>Effect/other allele</th>
<th>Association of SNPs with ITGA1 eQTL</th>
<th>Association of SNPs with BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>P</td>
<td>β (SE)</td>
</tr>
<tr>
<td>rs6876040*</td>
<td>c/t</td>
<td>0.073 (0.043)</td>
<td>0.09</td>
</tr>
<tr>
<td>rs7716758*</td>
<td>a/t</td>
<td>0.064 (0.043)</td>
<td>0.15</td>
</tr>
<tr>
<td>rs12188019*</td>
<td>t/c</td>
<td>0.071 (0.043)</td>
<td>0.10</td>
</tr>
<tr>
<td>rs17209725*</td>
<td>c/t</td>
<td>0.084 (0.043)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs6875212*</td>
<td>t/a</td>
<td>0.077 (0.043)</td>
<td>0.11</td>
</tr>
<tr>
<td>rs17209760*</td>
<td>c/g</td>
<td>0.084 (0.043)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs6450088</td>
<td>a/g</td>
<td>0.094 (0.043)</td>
<td>0.03</td>
</tr>
<tr>
<td>rs11886*</td>
<td>t/g</td>
<td>0.089 (0.043)</td>
<td>0.04</td>
</tr>
<tr>
<td>rs10940273</td>
<td>c/a</td>
<td>0.078 (0.044)</td>
<td>0.08</td>
</tr>
<tr>
<td>rs10512998</td>
<td>a/t</td>
<td>0.107 (0.043)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs12153381</td>
<td>c/t</td>
<td>0.1 (0.043)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs6881900</td>
<td>a/g</td>
<td>0.099 (0.043)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs10512997</td>
<td>c/t</td>
<td>0.095 (0.043)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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

ACKNOWLEDGMENTS
L.K.B. has received support from National Research Service Award Institutional Training Grant T32-DK-007028-35 to the Massachusetts General Hospital, National Institutes of Health (NIH) Loan Repayment Award National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) 1L30-DK-089944-01, the Endocrine Society Lilly Endocrine Scholars Award, and a Doris Duke Charitable Foundation Distinguished Scientist Clinical Award to David Altshuler. Y.-H.H. was supported by NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Grant R21AR-056405. J.D. has received support from NIDDK R01-DK-078616. J.B.M. was supported by NIDDK R01-DK-078616 and NIDDK K24-DK-080140. D.P.K. has received support from NIAMS and National Institute on Aging Grant R01AR/AG-41398. J.C.F. was supported by NIDDK R01-DK-078616 and a Doris Duke Charitable Foundation Clinical Scientist Development Award. J.C.F. has received consulting honoraria from Novartis, Eli Lilly, and Pfizer. No other potential conflicts of interest relevant to this article were reported.

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. Parts of this study were presented in abstract form at the 12th International Congress of Human Genetics Meeting, Montreal, Quebec, Canada, 11–15 October 2011.

The authors would like to thank Denis Rybin at Boston University School of Public Health for creating Fig. 2. The authors acknowledge the contribution of the GIANT Consortium, which provided summary statistics for the association between selected SNPs and BMI. Individual cohort acknowledgments are as follows: GEFOS Consortium (www.gefos.org) is funded by the European Commission (HEALTH-2008-201865-GEFOS). ARIC is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute (NHLBI) contracts (HSN20820110005C, HSN208201100006C, HSN208201100007C, HSN208201100008C, HHSN268201100009C, HSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01-HL-058641, R01-HL-50567, and R01-HL-086604, National Human Genome Research Institute contract U01-HG-004102, and NIH contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions.
Infrastructure of the ARIC study was partly supported by Grant UL1-RR-025005, a component of the NIH and NIH Roadmap for Medical Research. The Fenland Study is funded by the Wellcome Trust and the Medical Research Council. The authors are grateful to all the volunteers for their time and help and to the general practitioners and practice staff for help with recruitment. The authors thank the Fenland Study Investigators, Fenland Study Co-ordination Team and the Epidemiology Field, Data, and Technical Teams. Biochemical assays were performed by the National Institute for Health Research, Cambridge Biomedical Research Centre, Core Biochemistry Assay Laboratory, and the Cambridge University Hospitals National Health Service Foundation Trust, Department of Clinical Biochemistry. The BHS acknowledges the generous support for the 1994/1995 follow-up study from Healthway, Western Australia, and the numerous Busselton community volunteers who assisted with data collection and the study participants from the Shire of Busselton. The BHS is supported by the Great Wine Estates of the Margaret River region of Western Australia. The AMISH Cohort was supported by NIH research grants U01-HL-72515, R01-DK-04261, and R01-AG-18728; University of Maryand General Clinical Research Center Grant M01-RR-16500; the Mid-Atlantic Nutrition and Obesity Research Center (P30-DK-072488); the Baltimore Diabetes Research and Training Center (P60-DK-079637); and the Baltimore Veterans Administration Medical Center Geriatric Research and Education Clinical Center. French genetic studies (DESIR and French Obese) were supported in part by the Conseil Re- gional Nord-Pas-de-Calais: Fonds européen de développement économique et regional, Genome Quebec—Genome Canada and the British Medical Research Council. The authors acknowledge INSERM (employer of N.B.-N.). FamHS work was supported in part by NIH grants 5- R01-HL-08770003 and 5-R01-HL-08821502 from the NHLBI (to M.A.P.) and 5-R01-DK-07568102 and 5-R01-DK-0833603 from NIDDK (to I.B.B.). The GenomEUtwin project is supported by the European Commission under the program Quality of Life and Management of the Living Resources of Fifth Framework Programme (no. QLG2-CT-2002-01254) and the European Community’s Seventh Framework Prog- rame (FP7/2007-2013), ENGAGE Consortium, Grant Agreement HEALTH-F4-2007-201413. The Swedish Twin Cohort would like to acknowledge the Swedish Research Council and the Swedish Foundation for Strategic Research. The Finnish Twin Cohort would like to acknowl- edge the Center of Excellence in Complex Disease Genetics of the Academy of Finland. The GEMINAKAR study was supported by the Danish Medical Research Council, the Danish Heart Association, the Danish Diabetes Asso- ciation, and GenomEUtwin. The FHS component of this work was supported by the NHLBI’s FHS (Contract N01-HC-25155), its contract with Affymetrix, Inc. for genotyping services (Contract N02-HL-6-4278), and the resources of the FHS SNP Health Association Resource (SHARE) project, the Boston University Linux Cluster for Genetic Analysis (LinGA) funded by the NIH National Center for Research Resources Shared Instrumentation Grant 1S10-RR-163736-01A1, and the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Cen- ter. The ASAP study liver eQTL data were supported by the Swedish Research Council (12660), the Swedish Heart-Lung Foundation, the European Commission (FAD, Health-F2-2008-200647), and a donation by Fredrik Lundberg.

REFERENCES


Bone-related genes and glycemic traits


