Life-course Genome-Wide Association Study Meta-analysis of Total Body BMD and Assessment of Age-specific Effects

Carolina Medina-Gomez [1,2,3,63], John P. Kemp[4,5,63], Katerina Trajanoska[1,3], Jian'an Luan[6], Alessandra Chesi[7], Tarunveer S. Ahluwalia[8,9], Dennis O. Mook-Kanamori[10.11], Annelies Ham[1], Fernando P. Hartwig[12], Daniel S. Evans[13], Raimo Joro[14], Ivana Nedeljkovic[3], Hou-Feng Zheng[15,16,17], Kun Zhu[18,19], Mustafa Atalay[14], Ching-Ti Liu[20], Maria Nethander[21], Linda Broer[1], Gudmar Porleifsson[22], Benjamin H. Mullin[18,19], Samuel K. Handelman[23], Mike A. Nalls[24,25], Leon E. Jessen[8], Denise H.M. Heppe[2,3], J. Brent Richards[15,16], Carol Wang[26], Bo Chawes[8], Katharina E Schraut[27,28], Najaf Amin[2], Nick Wareham[6], David Karasik[29,30], Nathalie Van der Velde[1,31], M. Arfan Ikram[3], Babette S. Zemel[32], Yanhua Zhou[20], Christian J. Carlsson[8], Yongmei Liu[33] , Fiona E. McGuigan[34], Cindy G. Boer[1], Klaus Bønnelykke[8], Stuart H Ralston[35], John A. Robbins[36], John P. Walsh[18,19], M. Carola Zillikens[1], Claudia Langenberg[6], Ruifang Li-Gao[10], Frances M.K. Williams[37], Tamara B. Harris[38], Kristina Akesson[34,39], Rebecca D. Jackson[40], Gunnar Sigurdsson[41,42], Martin den Heijer[10,43], Bram C.J. van der Eerden[1], Jeroen van de Peppel[1],Timothy D. Spector[37], Craig Pennell[26], Bernardo L Horta[12], Janine F. Felix[2,3,44], Jing Hua Zhao[6], Scott G. Wilson[18,19,37], Renée de Mutsert[10], Hans Bisgaard[8], Unnur Styrkársdóttir[22], Vincent W. Jaddoe[2,3,44], Eric Orwoll[45], Timo A. Lakka[14,46,47], Robert Scott[6], Struan F.A. Grant[7,48], Mattias Lorentzon[49], Cornelia M van Duijn[3], James F. Wilson[27,50], Kari Stefansson[22,41], Bruce M Psaty[51,52], Douglas P Kiel[28,53,54], Claes Ohlsson[55], Andre J. van Wijnen[57], Vincenzo Forgetta[15,16], Mohsen Ghanbari[3,58], Graham R. Williams[59], J.H. Duncan Bassett[59], Evangelos Evangelou[56,60], Andre G. Uitterlinden[1,2,3], Cheryl L Ackert-Bicknell[61], Jonathan H. Tobias[62], David M. Evans[4,5,64], Fernando Rivadeneira[1,2,3,64]

1) Department of Internal Medicine, Erasmus MC, 3000 CA Rotterdam, The Netherlands; 2) The Generation R Study Group, Erasmus MC, 3000 CA Rotterdam, The Netherlands; 3) Department of Epidemiology, Erasmus MC, 3000 CA Rotterdam, The Netherlands; 4) University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Queensland 4102, Australia; 5) MRC Integrative Epidemiology Unit, University of Bristol, Bristol BS8 2BN, UK; 6) MRC Epidemiology Unit, University of Cambridge, Cambridge CB2 0QQ, UK; 7) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; 8) COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen 2820, Denmark; 9) Steno Diabetes Center Copenhagen 2820, Denmark; 10) Department of Clinical Epidemiology, Leiden University Medical Centre, 2333 ZA Leiden, The Netherlands; 11) Department of Public Health and Primary Care, Leiden University Medical Centre, 2333 ZA Leiden, The Netherlands; 12) Postgraduate Program in Epidemiology, Federal University of Pelotas, 96020220 Pelotas, Brazil; 13) California Pacific Medical Center Research Institute, San Francisco, CA 94107, USA; 14) Institute of Biomedicine, Physiology, University of Eastern Finland, Kuopio 70211, Finland; 15) Departments of Medicine, Human Genetics, Epidemiology and Biostatistics, McGill University, Montreal H3A 1A2, Canada; 16) Department ofMedicine, Lady Davis Institute for Medical Research, Jewish General Hospital,McGill University,Montreal H3T 1E2, Canada; 17) Hangzhou Normal University, 311121 Zhejiang, China; 18) Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands 6009, Australia; 19) School of Medicine and Pharmacology, University of Western Australia, Crawley 6009, Australia; 20) Department of Biostatistics, Boston University School of Public Health, Boston, MA 02131, USA; 21) Bioinformatics Core Facility, Sahlgrenska Academy, University of Gothenburg, 41390 Gothenburg, Sweden; 22) deCODE Genetics/Amgen, Reykjavik, IC-101, Iceland; 23) Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI 48201, USA; 24) Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA; 25) Data Tecnica International, Glen Echo, MD 20812, USA; 26) School of Women's and Infants' Health, University of Western Australia, Crawley 6009, Australia; 27) Centre for Global Health Research, Usher Institute for Population Health Sciences and Informatics, University of Edinburgh, Edinburgh EH16 4UX, Scotland; 28) Centre for Cardiovascular Sciences, Queen's Medical Research

Institute, University of Edinburgh, Edinburgh EH16 4TJ, Scotland; 29) Hebrew SeniorLife, Institute for Aging Research, Roslindale, MA 02131, USA; 30) Faculty of Medicine in the Galilee, Bar-Ilan University, Safed 1311502, Israel; 31) Department of Internal Medicine, Section of Geriatrics, Academic Medical Center, 1105 AZ Amsterdam, The Netherlands; 32) Division of GI, Hepatology, and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; 33) Wake Forest School of Medicine Winston-Salem, NC 27101, USA; 34) Lund University, Department of Clinical Sciences Malmö, Clinical and Molecular Osteoporosis Research Unit, 20502 Malmö, Sweden; 35) Centre for Genomic and Experimental Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, Scotland; 36) Department of Medicine, University of California, Davis; Sacramento, CA 95817, USA; 37) Department of Twin Research & Genetic Epidemiology, King's College London, London SE1 7EH, UK; 38) Laboratory of Epidemiology and Population Sciences, National Institute on Aging, Bethesda, MD 20892, USA; 39) Skåne University Hospital, Department of Orthopedics, 21428 Malmö, Sweden; 40) Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, USA; 41) Department of Endocrinology and Metabolism, Landspitali, The National University Hospital of Iceland, Reykjavik, IC-101, Iceland; 42) Faculty of Medicine, University of Iceland, Reykjavik, IC-101, Iceland; 43) Department of Clinical Epidemiology and Department of Internal Medicine, VU Medical Center, 1081 HV Amsterdam, The Netherlands; 44) Department of Pediatrics, Erasmus MC, 3000 CA Rotterdam, The Netherlands; 45) Department of Public Health & Preventive Medicine, Oregon Health & Science University, Oregon, Portland, OR 97239, USA; 46) Kuopio Research Institute of Exercise Medicine, Kuopio, 70100, Finland; 47) Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, 70210, Finland; 48) Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; 49) Geriatric Medicine, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, University of Gothenburg, 40530 Gothenburg, Sweden; 50) MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, Scotland; 51) Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA 98101, USA; 52) Kaiser Permanente Washington Health Research Institute, Seattle, WA 98101, USA; 53) Department of Medicine Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA; 54) Broad Institute of MIT and Harvard, Boston, MA 02115, USA; 55) Center for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, 41345 Gothenburg, Sweden; 56) Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, 45110, Greece; 57) Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN 55905, USA; 58) Department of Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; 59) Molecular Endocrinology Laboratory, Department of Medicine, Imperial College London, London W12 0NN, UK; 60) Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London W2 1PG, UK. 61) Center forMusculoskeletal Research, University of Rochester, Rochester, NY 14642,USA; 62) School of Clinical Sciences, University of Bristol, Bristol BS10 5NB, UK.

63)These authors contributed equally

64)These authors contributed equally

Corresponding author:

Dr. Fernando Rivadeneira MD PhD
Associate Professor Musculoskeletal Genomics
Genetic Laboratory - Department of Internal Medicine
Erasmus University Medical Center Rotterdam
The Netherlands
Email: f.rivadeneira@erasmusmc.nl

Abstract

Bone mineral density (BMD) assessed by DXA is used to evaluate bone health. In children, total body (TB) measurements are commonly used; in older individuals, BMD at the lumbar spine (LS) and femoral neck (FN) is used to diagnose osteoporosis. To date, genetic variants in more than 60 loci have been identified as associated with BMD. To investigate the genetic determinants of TB-BMD variation along the life course and test for age-specific effects, we performed a meta-analysis of 30 genome-wide association studies (GWAS) of TB-BMD including 66,628 individuals overall and divided across five age-strata each spanning 15 years. We identified variants associated with TB-BMD at 80 loci, of which 36 have not been previously identified; overall they explain approximately 10% of the TB-BMD variance when combining all age groups and influence the risk of fracture. Pathway and enrichment analysis of the association signals showed clustering within gene-sets implicated in the regulation of cell growth and SMAD proteins; overexpressed in the musculoskeletal system; and enrichment in enhancer and promoter regions. These findings reveal TB-BMD as a relevant trait for genetic studies of osteoporosis, enabling the identification of variants and pathways influencing different bone compartments. Only variants in *ESR1* and close proximity to *RANKL* showed a clear effect dependency on age. This most likely indicate that the majority of genetic variants identified influence BMD early in life and their effect can be captured throughout the life course.

Introduction

Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to increased risk of fracture[1]. It is diagnosed through the measurement

of bone mineral density (BMD) utilizing dual-energy X-ray absorptiometry (DXA), which is the single best predictor of fracture[1].

Bone is a dynamic tissue constantly undergoing resorption and formation. Bone mass increases steadily during childhood and markedly during adolescent growth[2]. Peak bone mass is attained at approximately the third decade of life. Thereafter, until about 50 years of age, BMD remains fairly stable, by virtue of the coupling between bone formation and resorption (e.g., bone remodeling). Subsequently, bone resorption exceeds the rate of bone formation, resulting in a decrease in BMD, particularly in women after the onset of menopause[3].

The International Society for Clinical Densitometry recommends performing DXA measurements at the lumbar spine, femoral neck and total hip to diagnose osteoporosis in postmenopausal women and men who are 50 years or older[4]. Consequently, studies of BMD determinants are frequently based on measurements at these skeletal sites. By contrast, for the assessment of bone health in children and adolescents, total body (excluding head) and lumbar spine are the preferred sites to minimize measurement artifacts resulting from changing areas in growing bones[4]. Nevertheless, in elderly individuals degenerative changes in the spine can give elevated BMD readings[5]. Moreover, total body DXA scans have been obtained in a number of adult research cohorts, primarily to assess body composition. Therefore, the total body BMD (TB-BMD) measurement is the most appropriate method for an unbiased assessment of BMD variation in the same skeletal site from childhood to old age.

To date, nearly 80 independent genetic variants have been shown to be robustly associated with variability in bone parameters[6-18]. Most of these markers have been identified in studies

134 comprising tens of thousands of adult and elderly individuals with DXA-derived BMD
135 measurements, although a few of them have been associated with BMD specifically in studies
136 of pediatric cohorts[8]. Furthermore, several of the associated variants display significant site-
137 specific effects, possibly reflecting differences in bone composition across skeletal sites (e.g.,
138 cortical bone vs. trabecular bone) or differential response to mechanical loading[8]. Moreover,
139 genetic studies on measures from peripheral quantitative computed tomography (pQCT) and
140 bone quantitative ultrasound, which provide additional information regarding bone size,
141 geometry and (micro) architecture identified genetic variants that may have specific effects on
142 bone properties that are poorly captured by conventional DXA measurements [9-10].

143 Given the complex physiological processes underlying age-related changes in BMD across the
144 life course, it is possible that genetic studies in more refined age groups will reveal variants in
145 unreported loci as well as age-specific genetic effects. Thus, the purpose of this study was to
146 identify gene variants associated with TB-BMD across the life span and investigate possible
147 differences of genetic effects across age periods.

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Methods

TB-BMD GWAS meta-analyses

Study Populations

Subjects

This study comprised 30 epidemiological studies comprising ~66,628 individuals from populations across America, Europe, and Australia, with a variety of designs (**Supplemental Data; Table S1**) and participant characteristics (**Table S2**). In summary, most participants came from population-based cohorts of European ancestry (86%), two cohorts comprising African-American individuals (2%) and other four studies holding a fraction of individuals from admixed background (14%). All research aims and the specific measurements have been approved by the correspondent Medical Ethical Committee of each participating study. Written informed consent was provided by all subjects or their parents in the case of children.

BMD measurement

Total body BMD (g/cm^2) was measured by DXA following standard manufacturer protocols. As recommended by the International Society for Clinical Densitometry total body less head (TBLH) was the measurement used in pediatric cohorts[4] (e.g., 0-15 years). Detailed information on the assessments performed by each study can be found in **Table S1**.

GWAS data and imputation

All individuals included in this study had genome-wide array data. Quality control of genotypes is summarized in **Table S1**. To enable meta-analysis, each study performed genotype

imputation using the cosmopolitan (all ethnicities combined) 1000 genomes phase 1 version 3 (March 2012) reference panel, yielding ~ 30,000,000 SNPs for analysis. Three studies used the combined 1000 genomes and the UK10K reference panels as presented in **Table S1.**

Association Analysis

TB(LH)-BMD was corrected for age, weight, height and genomic principal components (derived from GWAS data), as well as any additional study-specific covariates (e.g. recruiting center), in a linear regression model. For studies with non-related individuals, residuals were computed separately by sex, whereas for family-based studies sex was included as a covariate in the model. Finally, residuals were inverse normal transformed. The analyses were performed in each study for the overall population as well as in subgroups of individuals by age-strata, defined by bins of 15 years (i.e., 0-15 years, 15-30 years, 30-45 years, 45-60 years, and 60 or more years). SNP association was tested for autosomal variants, in which the additive effect of each SNP on the normalized BMD-residuals was estimated via linear regression.

Quality control of TB-BMD association summary statistics

A centralized quality-control procedure implemented in EasyQC[19] was applied to all study-specific files of association results to identify cohort-specific issues. We excluded variants if they had missing information (e.g., missing association P-value, beta estimate, alleles, allele frequency), or nonsensical values (e.g., absolute beta estimates or standard errors >10, association P-values >1 or <0; or imputation quality < 0; infinite beta estimates or standard errors); minor allele frequency (MAF) less than 0.5%; imputation quality scores <0.4 (Impute2) or <0.3 (Minimac). Moreover, variants were flagged if they had large allele frequency

deviations from reference populations (>0.6 for admixed studies and >0.3 for ancestry-homogeneous studies).

GWAS meta-analyses

In the first instance, no exclusion criteria based on ancestry were applied for the meta-analysis (N=66,628).In addition, meta-analyses were carried out across age strata (minimum sample size per bin N=200 for each study) comprising: 1) 0-15 years (N=11,807), 15-30 years (N=4,180), 30-45 years (N=10,062), 45-60 years (N=18,805), and 60 or more years (N=22,504). Further, summary data from cohorts of European ancestry only were meta-analyzed and used in subsequent analyses. We discarded variants present in less than three studies. Approximately 23,700,000 markers (including SNPs and INDELS) were assessed for association. We applied the conventional genome-wide significance level (GWS, $P<5x10^{-8}$) for SNP discovery.

Assessment of Age-dependent effects

We selected SNPs which were suggestively (12,567 SNPs, P<5x10-6) associated with BMD in the overall meta-analysis, present in at least 2 studies per age-bin and with MAF differences across these meta-analyses lower than 0.5. We clumped this dataset with an $r^2 \geq 0.8$, using as reference the most strongly associated SNPs with BMD and, pruning remaining SNPs within 0.7 Mb of each other. Age-dependent effects were assessed using a meta-regression approach for 1,464 SNPs obtained after this selection procedure. We ran a linear regression of the SNP effect estimates onto an intercept and the median age of each subgroup (e.g., each study stratified in age-bins). As proposed previously[20], standard errors of the effect estimates of each subgroup were multiplied by the square root of the genomic inflation factor when it was greater than 1.

We performed the meta-regression using the Metafor package[21], and any statistical evidence of linear association was corrected for multiple testing (Bonferroni correction; 0.05/1,464= $3.4x10^{-5}$). The difference between beta-estimates in children vs. elderly meta-analyses (Pdiff) was tested using Easy-strata[22].

Approximate conditional meta-analyses

Conditional analyses were undertaken based on the meta-analysis of the studies of European ancestry only (N=56,284). Only variants in the loci that reached GWS in this meta-analysis were assessed. The Rotterdam Study I (n=6,291) was used as reference for precise calculation of the linkage disequilibrium (LD) between the analyzed markers. We used an iterative strategy as implemented in GCTA[23] to determine: 1) independence of association signals within loci discovered in our study, by means of stepwise model selection procedure per chromosome (--massoc-slct routine); and 2) the novelty of the association signals discovered by our meta-analysis with regard to variants reported in previous well-powered GWAS of different bone traits (**Table S3**). To this end, we performed the association analysis conditional on 78 variants present in our data and associated with different bone-traits (--massoc-cond routine). These 78 SNPs were selected from different GWAS publications[6-10;12-14] , assuring their independence to avoid collinearity issues.

231 ***Shared Genetic architecture of TB-BMD fracture and other traits***

232 **LD score regression analyses**

233 We used the LD score regression package to estimate the heritability of TB-BMD and rule out
234 that our results were a product of bias (e.g., residual population stratification or cryptic
235 relatedness). LD score regression uses GWAS summary statistics and assesses the SNP-
236 heritability based on the expected relationship between linkage disequilibrium (LD) of
237 neighboring SNPs and strength of association under a polygenic model[24]. As this methodology
238 relies on the LD structure throughout the genome, we restricted this analysis to summary
239 statistics from the meta-analysis of cohorts comprising only individuals from European
240 ancestry. We used the publicly available, pre-computed LD structure data files specific to
241 European populations of the HAPMAP 3 reference panel. An extension of this method allows
242 estimating the genetic correlation between two traits[25]. This can be performed in the LDhub
243 pipeline, a web utility which gathers data from many different GWAS meta-analysis[26]. From the
244 199 traits, currently available in the website, we have restricted our analysis to those traits
245 whose heritability z-scores were larger than 4 and were analyzed only in European ancestry
246 individuals (following the recommendations in the LD score software website (**Web**
247 **Resources**)). Additionally, we incorporated data from a recent GWAS meta-analysis of any-type
248 of fracture in individuals from European ancestry (N= 264,267; 37,778 cases) (K.T, unpublished
249 data). In total, we assessed the genetic correlation between TB-BMD and 74 traits.

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251

Mendelian randomization analysis

We undertook a two-sample Mendelian randomization approach[27] to estimate the causal effect of TB-BMD on any-type of fracture in the Europeans samples. In short, we constructed a score based on the independent genetic variants from the TB-BMD meta-analysis (European set and excluding secondary signals), whenever the selected variant was not present in the fracture meta-analysis, the second variant with the lowest p-value in the locus ($P<5x10^{-8}$) and $r^2 > 0.8$ was used as proxy. Thereafter, estimates derived from the TB-BMD summary statistics were pooled using methods similar to inverse-variance weighted fixed meta-analysis using the meta R-package (**Web Resources**).

Search for biological and functional knowledge of the identified association regions

For all those SNPs outside a 500Kb window from previously known bone associated SNPs we did a literature search in PubMed and Web of Science to evaluate if nearby genes (within 500Kb) were known to play a role in bone metabolism. Also, we determined if the annotated genes underlie any human Mendelian disorder with a skeletal manifestation, had knockout mouse models with a skeletal phenotype or were annotated to pathways critical to bone metabolism. Genomic annotation for all SNPs was made based on UCSC hg19.

DEPICT analyses

We used DEPICT[28], a recently developed tool to prioritize genes at the associated regions, define possible pathways by enrichment testing, and identify tissue and cell types in which genes from loci associated with TB-BMD. The methodology first selects all lead SNPs below a certain threshold with respect to a target P-value. We tested both the complete set of GWS

SNPs and the subset of those mapping only to loci not previously reported. Enriched gene-set were group based on the degree of gene overlap into 'meta gene-sets' as proposed earlier[29], and their correlation visualized using Cytoscape 3.4 (**Web Resources**).

Functional annotation to microRNA binding sites

We used the PolymiRTS[29], miRdSNP[30], and microSNiPer[31] databases to obtain a list of variants located in predicted microRNA binding sites on the 3'UTRs of genes, as described in detail elsewhere[32]. In summary, index SNPs (most associated variant) of the GWS loci were submitted to SNAP (**Web Resources**) to retrieve their high LD proxy SNPs (with $r^2 > 0.8$, limit distance 500 kb, and CEU panel) in the 1000 genomes project. The resulting list of SNPs was annotated to the list of microRNA binding site variants obtained from the above mentioned publicly available databases.

Functional enrichment analysis of trait-associated variants

GWAS Analysis of Regulatory or Functional Information Enrichment with LD correction (GARFIELD)[33] was used to characterize the putative functional contribution of TB-BMD associated variants mapping to non-coding regions. GARFIELD employs a non-parametric analysis to calculate fold enrichment values for regulatory marks, at given significance thresholds and then tests them for significance via permutation testing while accounting for LD, MAF and local gene density[33]. We used data regarding DNase I hypersensitive sites, transcription factor binding sites, histone modifications and chromatin states (ENCODE and Roadmap Epigenomics) from 424 cell types and tissues to capture and characterize possible cell-type-specific patterns of enrichment, as provided in the GARFIELD software (**Web**

Resources). Fold enrichment statistics were tested at the four different significance thresholds (i.e., 1×10^{-8}, 1×10^{-7}, 1×10^{-6} and 1×10^{-5}). Multiple-testing correction was performed on the effective number of annotations used, using the default P-value threshold of 1×10^{-4}.

Knockout animal models and gene expression in bone cells

Animal models survey

We surveyed databases from *The International Mouse Phenotyping Consortium*[34] together with *The International Knockout Mouse Consortium*[35] to identify knockout models of candidate genes resulting in skeletal phenotypes. Furthermore we mined data from *The Origins of Bone and Cartilage Disease* (OBCD) project[36], specialized in murine skeletal phenotypes including Digital X-ray microradiography on femurs and tail vertebrae, Micro-CT analysis, femur three-point bend test load–displacement curves and tail vertebrae compression testing from knockout mice and wild-type controls at 16 weeks of age.

Gene expression in murine bone cells

Gene expression profiles of candidate genes were examined in primary mouse osteoblasts undergoing differentiation and bone marrow derived osteoclasts. To study murine osteoblasts, pre-osteoblast-like cells were obtained from neonatal calvaria collected from C57BL/6J. Next Generation RNA sequencing using an Illumina HiSeq 2000 was used to evaluate the transcriptome every two days from day 2 to 18 days post osteoblast differentiation[7]. Expression of genes in murine osteoclasts was determined using publicly available data obtained using Next-Gen RNA-sequencing applied to bone marrow derived osteoclasts obtained from 6-8 week old C57BL/6 mice[37].

315 Gene expression in human bone cells

316 Gene expression profiles of candidate genes were examined in human bone marrow derived
317 mesenchymal stem cells differentiated into osteoblast. Total RNA (n=3) was isolated at day 0
318 (MSCs) and day 4 of osteoblast differentiation[38]. Also, RNA was isolated during osteoclast
319 differentiation. Peripheral blood mononuclear cells derived from buffy coats (Sanquin,
320 Amsterdam, the Netherlands) were seeded in 96-well plates ($5x10^5$ cells per well) as
321 previously described[39] Total RNA (n=3) was isolated using Trizol at day 0 (PBMCs) and at day
322 7 of osteoclast differentiation. Illumina HumanHT-12 v3 BeadChip human whole-genome
323 expression arrays were used for expression profiling. The quality of isolated RNA was
324 assessed on a 2100 Bioanalyzer (Agilent Technologies). Data were analyzed as described in
325 detail previously[38]. Genes were designated as being expressed when at least one probe
326 coding for the gene was significantly present in at least 2 of the 3 biological replicates.

327 ## Results

328 ***TB-BMD GWAS meta-analyses***

329 **Analyses including all age-strata**

330 Our meta-analysis of TB-BMD GWAS summary statistics (N=66,628) identified variants in 76
331 independent loci associated with TB-BMD at a genome-wide significant (GWS, $P<=5x10^{-8}$) level
332 (**Figure 1, Table S4**). Overall, there was no evidence of a strong inflation (genomic inflation
333 factor (λ) of 1.08, **Figure S1**). Yet, inflation was observed in the range of common variants
334 (0.2>MAF<0.5, λ=1.19) due to polygenicity (LD score regression intercept = 1.007). In our
335 results, one of the signals mapping to *LDLRAD3* was driven entirely by individuals of African

background (MAF=0.043 in YRI panel) since the two associated variants are monomorphic in all other populations. The low allele frequency of this variant in our study (MAF= 0.025) and our limited statistical power (N=6,748) in non-European samples warrants independent replication efforts to exclude the possibility of a false-positive association.

In addition, a meta-analysis comprising 56,284 individuals of European ancestry (~84% of the study population) identified variants in two additional GWS loci (**Figures S1-S2, Table S5**). Association signals mapping to these loci were close to the GWS threshold in the overall meta-analysis ($P=1x10^{-7}$) and showed no evidence of heterogeneity ($P_{het}>0.1$). One of them, in 12q24.21 (*MED13L*), has not been previously associated with bone parameters (**Table 1, Figure S3**), while the other in 21q22.13 (*CLDN14*), is not fully independent from the previously reported hip-BMD association signal[13] (**Table S5**).

Of the 78 identified loci, variants in 35 (45%) were not located within 500 kb of known association signals nor in regions of extended LD with them (**Table 1, Figure S4**). Index SNPs at these 35 loci were, in general, common non-coding variants. Twenty-two of these, are located in close proximity to genes likely to influence bone metabolism as shown by previous functional studies (**Table 1, Figure S3**), including *CSF1* ([MIM 120420] important for osteoclast differentiation[40]) and *SMAD3* ([MIM 603109] a critical component of the TGF-beta signaling pathway[41]). Across these 35 signals, 31 of the index SNPs were nominally associated ($P<0.05$) with either lumbar spine or femoral neck BMD in the same direction as in the previously published GEFOS GWAS meta-analysis[7] (**Table 1**). This comparison was not possble for the rs113964474 variant, because it was not available in the GEFOS study. Moreover, we found directionally-concordant effect estimates ($P < 0.05$) for 73 of the 78 index SNPs of known bone

association signals (**Table S3**). The markers which failed to replicate in our study were either previously associated with lumbar spine BMD but not femoral neck BMD (rs3905706 [*MPP7*, 10p12.1] and rs1878526 [*INSIG2*, 2q14.2]), associated specifically with the hip trochanter and intertrochanteric subregions (rs1949542 [*RP11-384F7.1*, 3q13.32]), or associated with BMD only in women (rs7017914 [*XKR9*, 8q13.3]) or only in children (rs754388 [*RIN3*, 14q32.12]).

Age-dependent effects

Meta-analyses across age strata resulted in the identification of variants mapping to 2 additional loci that were not detected in the overall meta-analysis (**Figure S5**; **Table S6**). In children (age group 0-15 years), the previously known 14q32.12 locus[8], harboring *RIN3* (rs72699866, $P=1x10^{-8}$); and in the middle-aged (age group 45-60 years), a signal in the 19q12 locus mapping in the vicinity of *TSHZ3* (rs6510186, $P=3.1x10^{-8}$) were identified. The rs72699866 variant leading the *RIN3* signal in the youngest age stratum showed no evidence of association (P=0.16) and high heterogeneity ($P_{het}=6.6x10^{-5}$) in the overall meta-analysis. In fact, the effect of rs72699866 decreased significantly with age ($P_{trend}=1.69x10^{-9}$) (**Figure S6**) and showed a significant difference between the two extreme groups, i.e. children vs elderly ($\beta_{0-15}=0.099$ [0.066, 0.134]; $\beta_{>60}=-0.035$ [-0.060, -0.010]; $P_{diff}=4.32x10^{-10}$). In contrast, the rs6510186 variant [19q12] showed nominal evidence of association and heterogeneity in the overall meta-analysis (P=0.02; $P_{het}=0.03$). Nevertheless, no clear pattern of age-dependency was identified (P=0.2) for this SNP (**Figure S6**).

We also applied meta-regression analysis and found that variants mapping to 42 different loci showed nominally significant age dependent effect (P<0.05) (**Table S7, Figure S7**). In summary,

27 (64%) of the loci showed stronger effects in the older age groups. Of these, variants in the 6q25.1 *(ESR1*) and 13q14.11 (*RANKL*) loci remained significant after multiple-testing correction ($P<3.4x10^{-5}$) (**Figure 2**); while variants in 6p21.1 (*RUNX2*, rs148460475), 15q21.2 (*CYP19A1*, rs2414098), 17q21.31 (*MEOX1*, rs74835612) and 11p15.1 (*SOX6*, rs11822790) were only suggestive at $P<1x10^{-3}$.

Conditional association analyses

The step-wise conditional approach included studies comprising only individuals of European ancestry, as the method used relies on appropriate representability of the LD reference. Of the 76 GWS loci identified in the overall analysis, variants in 57 (19 previously unreported) loci were also GWS in the European-only analysis (**Figure S2**), likely a consequence of the lower power in this subgroup. We identified 81 SNPs independently associated with TB-BMD mapping to 58 different loci (one European-specific), 18 of which depicted multiple distinct signals attaining GWS (**Table S8**). These independent variants together explained 10.2% of TB-BMD variance. This proportion is slightly higher than the 7.4% TB-BMD variance explained by the 78 known variants associated with bone traits. Moreover, we identified independent signals in 13 of the 78 known bone loci after conditional analyses. (**Figure S2**; **Table S8**).

Shared Genetic architecture of TB-BMD, fracture and other traits

SNP-heritability of TB-BMD in the European samples was estimated to be 0.259 (SE 0.017). TB-BMD was highly genetically correlated with BMD measured at other skeletal sites ($\rho>0.9$). Among the non-BMD traits, all-type of fracture showed the highest correlation [$\rho=-0.61$ ($P=1.6x10^{-27}$)]. The MR approach indicated a strong causal relation where per 1 standard

deviation decrease in genetically determined TB-BMD there is 56% increase in the risk of fracture (Odds ratio 1.56 [1.50-1.62]). Other anthropometric, metabolic and disease traits showed significant (yet weak) correlation with TB-BMD (**Table S9, Figure 3**). In contrast, other established risk factors for osteoporosis such as menopause or age of menarche showed no significant genetic correlation with TB-BMD.

Biological and functional knowledge of the genes in BMD-associated loci

Loci not previously reported and their potential role in bone metabolism are summarized in **Table 1**. Several loci harbor genes implicated directly in bone metabolism (*SLC8A1* [MIM 182305], *PLCL1* [MIM 600597]*, ADAMTS5* [MIM 605007]), affecting osteoblast or osteoclast differentiation and activity (*CSF1* [MIM 120420]*,, DUSP5* [MIM 603069], *SMAD3* [MIM 603109], *SMAD9* [MIM 603295], *CD44* [MIM 107269]), participating in Wnt signaling (*FZD7* [MIM 603410], *TCF7L1* [MIM 604652]), or regulating processes such as manganese or calcium absorption (*GCKR* [MIM 600842], *DGKD* [MIM 601826], *SLC30A10* [MIM 611146]) among others [40-61]; while genes in at least 14 loci exert a potential novel role in bone biology. Rodent knockout models of several genes in the implicated loci, show an altered skeletal phenotype (e.g., ostoepetrosis [*Csf1*[40]], increased bone resorption [*Aqp1*[50], *Cyp19a1*[57], *Cd44*[53]], impaired skeletogenesis [*Apc*[49], *Runx1*[60], *Smad3*[41]], deformities in the axial skeleton [*Btg1*[62], *Atpaf2*[63]]). Whereas an effect on bone can be inferred for genes in other associated loci, for example, *CYP19A1* [MIM 107910] in 15q21.2 is an estrogen synthesis gene, being estrogen a key compound for bone maturation and maintenance, and *ZKSCAN5* [MIM 611272] in 7q22.1 is associated with circulating dehydroepiandrosterone sulphate (DHEAS) levels[51]. DHEAS levels are positively correlated with BMD in adults and post-menopausal women[64]. Across these loci,

not previously reported as associated with BMD variation, we identified six exonic variants associated with TB-BMD, three of which were nonsynonymous variants all cataloged as benign both by SIFT and polyphen2. We also identified 53 GWS coding variants in known loci, of which 33 are non-synonymous (**Table S10**). Only a low-frequency variant in *LRP5* [MIM 603506], rs4988321/A (11:68174189, MAF=0.04), has a clinical annotation, constituting a homozygous G-to-A transition variant identified in a person with osteoporosis-pseudoglioma syndrome (OPPG [MIM 259770])[65].

DEPICT analyses

Based on the overall meta-analysis, 53 genes were prioritized (FDR<0.05), 15 of them mapping to loci not previously described (**Table S11**). Cells and tissues from the musculoskeletal system presented the largest enrichment of gene expression within the associated loci (**Figure 4**). These genes were overrepresented in 182 pathways clustered in 25 'meta gene-sets' (**Table S12**). The large majority of the clusters are involved in musculoskeletal development and bone homeostasis (**Figure 4**). The most significant of these implicated the regulation of cell growth, and the TGFB signaling pathway and its mediating SMAD proteins.

Restricting the DEPICT analysis to the subset of not previously reported associated regions resulted in significant enrichment of genes expressed in the musculoskeletal and immunological systems (**Figure S8**). Genes mapping to these loci were overrepresented in the SMAD binding pathway and TGFBR2 PPI (protein-protein interaction) subnetwork (FDR<0.05).

Functional annotation to microRNA binding sites

We then assessed if the index SNPs of the 80 GWS loci detected in the main and subsequent GWAS (or their proxies in strong LD; r^2>0.8) were located in predicted microRNA binding sites within the genes' 3'UTRs and thus, were expected to disrupt the regulation of gene expression (**Table S13**)**.** The index SNP within the 3'UTR of *ZKSCAN5* (mapping to a locus not previously identified)*,* rs34670419 (MAF=0.04), is predicted to create a binding site for miR-382-3p, a microRNA which is expressed in osteocytes and has been recently shown to be involved in osteogenic differentiation[66]. In addition, eight proxy SNPs (mapping to *PSMD13, ABCF2, GALNT3, PKDCC, REEP5, PPP6R3, AAGAB* and *TOM1L2*) are predicted to influence the binding of microRNAs to transcripts of their host gene.

Functional enrichment analysis of trait-associated variants

As typically found in GWAS, the great majority of identified associations emerged from non-coding common variants and hold no direct annotation to molecular mechanisms. To assess if there is relative enrichment of regulatory genomic marks underlying the associated variants in a cell-specific context, we used GARFIELD[33]. We found relative ubiquitous enrichment for TB-BMD variants (Empirical P<2.4×10^{-4}) in DNase I hypersensitive sites across the different cell types (**Figure S9**). Further, we found higher levels of fold-enrichment for enhancers (median 3.6, range [2.7, 4.4]) and promotors (median 3.2, range [2.9, 3.5]) than for transcribed regions (median 1.8, range [1.5, 2.2]).

Gene expression in bone cells and knockout animal models

From the 53 genes prioritized by DEPICT only 49 had a mouse orthologue (**Table S14**). From these genes, only *Mepe* (osteocyte-specific) and *Foxl1* were not expressed in murine osteoblast

or osteoclast. Moreover, 61% of the prioritized genes were expressed in human cells *in vitro* during osteoblast or osteoclast differentiation (**Table S14**). *AQP1* was the only prioritized gene mapping to a locus not previously reported showing no expression in the human bone cells differentiation experiments.

Knockout models were widely available in at least one of the different databases assessed. Nevertheless in-depth bone phenotyping performed under the OBCD project was only available for four knockout models (**Table S15**). Two of these, *DUSP5* and *CD300LG* showed no significant bone phenotype. The *TCF7L1* knockout model only showed lower cortical diameter in the femur without other clear bone phenotype. Nevertheless, *TCF7L1* was shown to be expressed during osteoblastogenesis. Conversely, homozygous knockout for *CREB3L1* showed a clear bone phenotype consisting of low BMC both at the vertebrae and femur together with a strong trabecular and cortical phenotype affecting bone strength (**Figure S10**). *CREB3L1* maps to 11p11.2, a previously identified BMD locus[6] harboring *ARHGAP1* and *LRP4* as candidates to underlie the GWAS signal in a region of extended LD.

Discussion

This meta-analysis of TB-BMD comprising up to 66,000 individuals identified variants in 36 loci not previously reported and replicated at GWS level several association signals identified by GWAS of diverse bone phenotypes. Bioinformatics analyses suggest enrichment of these 36 loci for genes expressed in the musculoskeletal system, and solidly represented in the SMAD binding pathway and the TGFBR2 PPI subnetwork. We also demonstrate that for variants in few loci the size of the effect is age dependent; variants in two loci (*RIN3* and *TSHZ3*) were

identified only by the age-stratified analyses despite less power (smaller sample size); while for variants in two other loci (*ESR1* and *RANKL*) there was significant evidence of age heterogeneity derived from a meta-regression of the genetic effects with age. Our results strengthen the evidence that genetic variants influence BMD from a young age and support the value of peak bone mass as an important determinant of bone health later in life.

Traditionally, DXA-BMD measurements performed at sites of high fracture risk (i.e., femoral neck, lumbar spine and forearm) have been used in genetic epidemiological investigations of bone health in adults. Instead, we have used BMD measurements derived from total body scans. Not only do we show a high overlap of association signals with previous GWAS of different bone traits, including DXA, pQCT and ultrasound measurements, but we have also identified unreported loci. Five known associations failed to replicate in our studies, even though we cannot discard these associations constitute false-positives, these results might also indicate that variants whose effect is highly specific to skeletal sites, skeletal properties, sex or age groups cannot be detected in our TB-BMD meta-analysis. It is plausible that more variants of this type exist and will be discovered as site-specific BMD meta-analyses are performed in increasingly powered settings. Furthermore, the genetic correlation of TB-BMD with BMD measured at other sites was close to one. Whilst, we found that a decrease of one standard deviation in the genetically determined TB-BMD resulted in at least 50% higher odds of suffering a fracture. Significant genetic correlations with other traits (i.e., BMI, IGF1 and ulcerative colitis) reflect the systemic context of skeletal biology and merit further study by future efforts to elucidate the underlying mechanisms.

505 Genes in the associated loci were highly expressed in the musculoskeletal system and
506 overrepresented in gene-sets related to bone development. The prioritized gene *CREB3L1* [MIM
507 616215] in 11p11.2 observed a clear bone phenotype in our mouse knockout model, which
508 corroborates the findings of previous work showing substantial rescue of *CREB3L1* deficiency
509 with bisphosphonates and its critical role for bone formation[67]. This locus characterized by
510 extended LD, also harbors *LRP4* [MIM 604270] whose knockout model presents with increased
511 trabecular and cortical bone mass[68]. This is in line with our conditional analysis identifying
512 multiple independent signals in the region making it likely that both genes are influencing bone
513 biology. Altogether, we demonstrated that TB-BMD offers a powerful alternative to identify
514 genetic variants associated with bone metabolism.

515 Variants mapping to 14q32 harboring *RIN3* [MIM 610223] were only associated at a GWS level
516 in children (i.e., <15 years), and were only nominally significant in the elderly group (i.e., >60
517 years). This age-related heterogeneity may explain why this locus has not been detected in
518 BMD meta-analyses in adults, although being identified in relation to pediatric BMD[8] and
519 Paget`s disease (PDB [602080]) GWAS[69]. In addition, another signal mapping to 19q12
520 harboring *TSHZ3* [MIM 614119] was significant in adults aged 45-60 years but not in other age
521 groups analyzed or in previous studies, alluding to a false-positive association, thus replication
522 of this finding is necessary.

523 Our analyses revealed variants in the 6q25.1 (*ESR1*) and 13q14.11 (*RANKL*) loci demonstrating
524 the most compelling evidence for age-modulation effects. The 6q25.1 locus harboring *ESR1*
525 [MIM 133430], an important genetic factor in normal BMD variability, was not associated with
526 BMD in children below 15 years of age, where the largest cohorts (i.e., Avon Longitudinal Study

of Parents and Children (ALSPAC) and the Generation R Study) comprise predominantly pre-pubertal children. As levels of estradiol before puberty are low[70], a negligible effect of *ESR1* variants on BMD is expected. Likewise, in mouse models the expression of *RANKL* [MIM 602642] in bone is markedly increased with advancing age from young to adult and related to bone loss[71]. Accordingly, variants influencing *RANKL* expression show a larger effect later in life. In general, a substantial heterogeneity of the genetic effects in the overall meta-analysis was explained by age, nevertheless, the inclusion of larger sample sizes (avoiding age exclusion criteria and incrementing statistical power) leveled off the loss of power due to the heterogeneity of the genetic effects.

In brief, variants with evidence of age-specific effects were exceptional in our study. These results might reflect a lack of statistical power as only SNPs showing suggestive evidence ($P<5x10^{-6}$) of association with TB-BMD in the overall meta-analysis were tested for age-specific effects. This selection criteria aimed to include SNPs whose heterogeneity might have hampered their statistical significance in the overall meta-analysis, and at the same time maximize the power to discover variants with real age-dependet effects. Alternatively, these results indicate that most of the genetic variants identified so far, by us and others, influence BMD from early ages onwards, and their effect persist throughout the life course. However, variants in 27 of the 42 loci (64%) showing nominal evidence for age dependent effects had larger effects in the older groups. Nonetheless, this requires careful interpretation given the uneven sample sizes between the age groups and the criteria to select markers for the meta-regression based on significance in the overall meta-analysis. Collectively, this argues in favor of

548 enlarging studies focused on younger populations –where the statistical power is still restricted
549 – to discover additional genetic variants influencing BMD.

550 Our study has some limitations. A key disadvantage of our design is that we group the data
551 based on age spans rather than life stages. Crucial information for this assesment, such as
552 puberty onset in children and adolecents or menopausal status in the adults, was not available
553 across the majority of the cohorts. Other strategies like using shorter age spans will resulted in
554 even less statistical power of the discovery setting. Similarly, despite the large sample size of
555 our study, we identified very few variants in the low-frequency spectrum (MAF <5%) indicating
556 that comprehensive surveys of rare variation influencing BMD still require even larger sample
557 sizes, on top of better resources for imputation of the rarer variants, possibly needing
558 population-specific references. Such strategies will be key to explain a larger fraction of the
559 genetic variability of BMD phenotypes, as illustrated for other traits such as height or BMI[72].
560 Moreover, the identified SNPs are in their vast majority, non-coding variants, raising the
561 possibility that the causal genes are different from the candidate genes we have prioritized
562 based on the current biological knowledge and bioinformatic prediction tools. Additional
563 functional studies are required to determine the potential role of the genes in the identified
564 loci.

565 In conclusion, we performed a genome-wide survey for association with DXA derived TB-BMD,
566 combining data from five age groups including children and older individuals. In contrast to
567 previous large-scale meta-analyses[6;7], we used DXA derived TB-BMD rather than measurements
568 on specific skeletal sites prone to fracture to identify genetic factors influencing BMD variation.
569 We demonstrate that TB-BMD is a valid phenotype for this purpose, as we replicated more than

570 90% of the previously reported signals. Most importantly, we identify variants in 36 loci
571 associated with TB-BMD not previously reported by previous GWAS of bone phenotypes. Our
572 results show steadiness in the magnitude of the genetic effects on BMD for most of the BMD-
573 associated variants. While the contrasting skeletal physiology across different age periods is
574 well established (i.e. endochondral ossification, linear growth, modelling,
575 remodeling, etc.), peak bone mass acquisition remains the major determinant of variability at
576 any age. These findings strongly support the importance of the bone accrual process in the
577 definition of BMD status and fracture susceptibility throughout the life course.

578 **Accession Numbers**

579 GWAS Summary data for the main and age-strata meta-analyses together with the
580 corresponding regional plots of GWS signals have been deposited in the GEFOS website (**Web**
581 **Resources**). Gene expression data presented in this paper can be retrieved from the Gene
582 Expression Omnibus (GEO) as follows: Murine osteoclasts (GSM1873361) and osteoblasts
583 (GSE54461); human osteoblast differentiation (GSE54461).

584 **Supplemental Data**

585 Supplemental data include a full list of acknowledgements, cohort short descriptions, 15
586 tables and 10 figures.

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589 phenotypic characterization of the clinical samples, as well as genotyping and analysis of the

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591 **Conflict of interests**

592 Psaty serves on the DSMB of a clinical trial for the manufacturer (Zoll LifeCor) and on the
593 Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson.

594

595 **Web Resources**

596 GARFIELD, http://www.ebi.ac.uk/birney-srv/GARFIELD/GEFOS, http://www.gefos.org/

597 LDhub, http://ldsc.broadinstitute.org/

598 Meta R-package, https://github.com/guido-s/meta)

599 OBCD, http://www.boneandcartilage.com/

600 OMIM, http://www.omim.org/

601 SNAP, http://archive.broadinstitute.org/mpg/snap/

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Figure Titles and Legends

Figure 1. Manhattan plot of association statistics (-log10(P-values)) for TB-BMD overall meta-analysis. Each dot represents a SNP and the x-axis indicates its chromosomal position (built 37 NCBI). Red dots represent SNPs at GWS loci that are not within ±500Kb of leading SNPs in previous GWAS with different bone traits. Dashed horizontal red and yellow lines mark the GWS threshold ($P<5x10^{-8}$) and suggestive threshold ($P<1x10^{-6}$), respectively. Novel loci in the only-CEU analysis are not shown.

Figure 2. Age dependence of the genetic variant effect in the meta-regression. The panels display leading SNPs from two loci exhibiting significant evidence for age influences. Heterogeneity P-values (P_{het}) are reported for the overall meta-analysis. In the left panels, each circle represents a study subgroup (i.e., study divided in age strata), with the circle size proportional to the inverse variance of the SNP main effect. In the right panels, forest plots display estimates obtained from each age-bin meta-analysis, with the symbol size proportional to the inverse variance of the SNP main effect.

Figure 3. Genetic correlations between TB-BMD and other traits and diseases. Calculation was based on the summary statistics of the only-European meta-analysis (N=56,284) and estimated by LD score regression implemented in LDHub. The diagram only show traits whose correlation with TB-BMD was significant (P<0.05).

Figure 4. Depict results for gene-set and cell/tissue enrichment analyses. **Top panel**: 25 Meta gene-sets were defined from similarity clustering of significantly enriched gene sets (FDR<5%). Each Meta gene-set was named after one of its member gene sets. The color of the Meta gene-sets represents the P-value of the member set. Interconnection line width represents the Pearson correlation ρ between the gene membership scores for each Meta gene-set ($\rho < 0.3$, no line; $0.3 \leq \rho < 0.5$,narrow width; $0.5 \leq \rho < 0.7$, medium width; $\rho \geq 0.7$, thick width). **Bottom panel**: Bars represent the level of evidence for genes in the associated loci to be expressed in any of the 209 Medical Subject Heading (MeSH) tissue and cell type annotations. Highlighted in orange are these cell/tissue types significantly (FDR<5%) enriched for the expression of the genes in the associated loci.

Tables

Table 1. Index SNPs of loci not previously associated with BMD. Variants associated with TB-BMD in the all-ages combined meta-analysis that map outside +/- 500 Kb of known index SNPs of genetic associations with different bone traits. Genomic coordinates are on build 37 of the human genome. Notes refer to annotation based on the closest gene. Associations with Lumbar Spine (LS) and Femoral Neck (FN)-BMD [10]. Beta coefficients and allele frequencies (EAF) are reported for the A1 allele

CHR	BP	rsnumber	Locus	A1	A2	EAF	Effect	P	N	annotation	closest gene	Notes	LS-beta	LS-P	FN-beta	FN-P
1	8422676	rs2252865	1p36.23	T	C	0.32	-0.033	4.72E-08	66075	intronic	*RERE*	Novel biology	-0.019	0.043	-0.025	0.002
1	110475971	rs7548588	1p13.3	T	C	0.61	-0.037	9.29E-09	66240	intergenic	*CSF1*	Osteoclast differentiation[40]	-0.030	0.001	-0.022	0.005
1	220038825	rs185048405	1q41	T	C	0.54	0.042	3.07E-09	66540	intronic	*SLC30A10*	Manganese transport[42]	-0.035	0.076	-0.003	0.878
2	27741072	rs780096	2p23.3	C	G	0.44	-0.031	4.58E-08	66578	intronic	*GCKR*	Calcium regulation[43], hepatic traits[44]	-0.014	0.129	-0.017	0.029
2	40630678	rs10490046	2p22.1	A	C	0.76	0.043	1.43E-10	65961	intronic	*SLC8A1*	Bone mineralization[45]	0.015	0.162	0.021	0.025
2	68962137	rs10048745	2p13.3	A	G	0.25	-0.039	6.44E-09	66565	5'-UTR	*ARHGAP25*	Novel biology	-0.050	1.03E-06	-0.036	5.21E-05
2	85484818	rs11904127	2p11.2	A	G	0.55	-0.032	2.65E-08	66561	intronic	*TCF7L1*	Factors in Wnt signaling[46]	-0.021	0.023	-0.015	0.054
2	198874006	rs1595824	2q33.1	T	C	0.47	0.034	2.65E-08	60171	intronic	*PLCL1*	Negative regulation of bone formation[47]	0.022	0.201	0.052	2.20E-04
2	202799604	rs2350085	2q33.2	T	C	0.87	-0.064	3.80E-14	66412	intergenic	*FZD7*	Factors in Wnt signaling[48]	-0.042	0.002	-0.044	1.96E-04
2	234303405	rs838721	2q37.1	A	G	0.44	-0.031	4.48E-09	65516	intronic	*DGKD*	Calcium regulation[43]	-0.016	0.070	-0.014	0.068
5	112221869	rs818427	5q22.2	T	C	0.31	0.034	2.37E-08	66592	intronic	*APC*	Bone metabolism[49]	0.004	0.645	0.008	0.327
5	122847622	rs11745493	5q23.2	A	G	0.75	0.044	7.75E-12	66597	promoter	*CSNK1G3*	Novel Biology	0.010	0.326	0.025	0.005
7	27989403	rs757138	7p15.1	T	G	0.69	-0.035	3.33E-08	66043	intronic	*JAZF1*	Novel Biology	-0.016	0.126	-0.025	0.004
7	30957702	rs28362721	7p14.3	T	C	0.18	-0.059	6.71E-14	66274	intronic	*AQP1*	Bone metabolism[50]	-0.037	0.002	-0.049	1.39E-06
7	50901491	rs1548607	7p12.1	A	G	0.69	0.036	4.18E-08	66564	intergenic	*GRB10*	Novel biology	0.034	5.59E-04	0.005	0.517
7	99130834	rs34670419	7q22.1	T	G	0.04	-0.088	1.09E-08	66336	3'-UTR	*ZKSCAN5*	DHEAS and aging mechanisms[51]	-0.127	9.28E-08	-0.080	8.19E-05
10	112245400	rs73349318	10q25.2	A	T	0.87	-0.047	2.68E-08	66341	intronic	*DUSP5*	Osteoclast differentiation[52]	-0.042	0.001	-0.051	8.76E-06
10	124015986	rs10788264	10q26.13	A	G	0.48	-0.034	2.61E-09	66565	intergenic	*TACC2*	Novel Biology	-0.030	9.64E-04	-0.029	1.29E-04
11	242859	rs55781332	11p15.5	A	G	0.78	-0.055	8.07E-16	66198	intronic	*PSMD13*	Novel Biology	-0.046	1.76E-05	-0.026	0.005
11	35083633	rs2553773	11p13	C	G	0.41	-0.037	1.49E-10	66619	intergenic	*CD44*	Osteoclast activity[53]	-0.015	0.101	-0.015	0.054
11	35981346	rs113964474*	11p.13*	A	G	0.03	0.485	1.41E-08	6748	intronic	*LDLRAD3*	Novel Biology	.	.	.	.
11	69299537	rs4980659	11q13.3	C	G	0.52	0.033	1.16E-08	66537	intergenic	*CCND1*	Target of Wnt signalling[54]	0.039	1.58E-05	0.023	0.003
11	121913230	rs725670	11q24.1	A	G	0.38	-0.032	3.61E-08	66565	intergenic	*BLID*	Novel Biology	-0.020	0.028	-0.011	0.172
12	90334829	rs10777212	12q21.33	T	G	0.35	0.045	5.05E-14	66619	intergenic	*ATP2B1*	Calcium absorption[55]	0.028	0.003	0.021	0.010
12	116555786	rs73200209**	12q24.21	A	T	0.80	0.045	2.51E-08	51240	intronic	*MED13L*	Novel biology	0.030	0.167	0.036	0.044
13	37487021	rs556429	13q13.3	A	C	0.23	0.039	1.46E-08	66504	intronic	*SMAD9*	Osteoblast differentiation[56]	0.023	0.027	0.013	0.135
15	38340874	rs12442242	15q14	A	G	0.85	-0.051	4.94E-10	66403	intergenic	*TMCO5A*	Novel Biology	-0.046	3.03E-04	-0.047	2.26E-05
15	51537806	rs2414098	15q21.2	T	C	0.39	-0.033	1.99E-08	66562	intronic	*CYP19A1*	Estrogen byosynthesis[57]	-0.034	0.007	-0.038	0.001
15	67420680	rs1545161	15q22.33	A	G	0.56	0.041	1.06E-12	66004	intronic	*SMAD3*	Osteoblast differentiation[41]	0.034	1.27E-04	0.035	5.78E-06
17	17804725	rs8070128	17p11.2	T	C	0.58	-0.039	1.98E-11	66625	intronic	*TOM1L2*	Novel biology	-0.033	4.80E-04	-0.015	0.052
17	63771079	rs9972944	17q24.1	A	G	0.41	0.036	6.87E-10	66595	intronic	*CEP112*	Novel Biology	0.028	0.003	0.004	0.576
19	31654615	rs6510186***	19q12	T	C	0.26	0.068	3.11E-08	18782	intergenic	*TSHZ3*	Novel Biology	0.004	0.713	0.006	0.492

20	39103882	rs6029130	20q12	T	C	0.30	0.035	3.50E-08	66497	intergenic	*MAFB*	Osteoclast differentiation[58]	0.027	0.007	0.015	0.083
21	28773868	rs1452102	21q21.3	T	G	0.59	-0.035	1.74E-09	66489	intergenic	*ADAMTS5*	Endochondral Ossification[59]	-0.029	0.001	-0.015	0.056
21	36970350	rs9976876	21q22.12	T	G	0.45	-0.038	8.01E-11	66514	intronic	*RUNX1*	Osteoclast differentiation[60]	-0.019	0.031	-0.016	0.041
21	40350744	rs11910328	21q22.2	A	G	0.84	-0.043	2.99E-08	66298	intergenic	*ETS2*	Osteoblast maturation[61]	-0.028	0.020	-0.028	0.007

* Monomorphic in European cohorts. ** Reported statistics from the in the meta-analysis of European populations. *** Reported statistics from the meta-analysis in the 30-45 age-strata.

Supplemental data

COHORTS SHORT DESCRIPTION

1982 Pelotas Birth Cohort Study:

The 1982 Pelotas (Brazil) Birth Cohort Study is a longitudinal population-based birth cohort. The maternity hospitals in Pelotas, a southern Brazilian city (current population ~330,000), were visited daily in the year of 1982. The 5,914 live-borns whose families lived in the urban area were examined and their mothers interviewed. Information was obtained for more than 99% of the livebirths. These subjects have been followed-up at the following mean ages: 11.3 months (all children born from January to Abril 1982; n=1457), 19.4 months (entire cohort; n=4934), 43.1 months (entire cohort; n=4742), 13.1 years (random subsample; n=715), 14.7 years (systematic subsample; n=1076); 18.2 (male cohorts attending to compulsory Army recruitment examination; n=2250), 18.9 (systematic subsample; n=1031), 22.8 years (entire cohort; n=4297) and 30.2 years (entire cohort; n=3701). Details about follow-up visits and available data can be found in the two Cohort Profile papers (1, 2). DNA samples (collected at the mean age of 22.8 years) were genotyped for ~2.5 million of SNPs using the Illumina HumanOmni2.5-8v1 array (which includes autosomal, X and Y chromosomes, and mitochondrial variants). After quality control, the data were pre-phased using SHAPEIT and imputed using IMPUTE2 based on 1000 Genomes haplotypes.

Avon Longitudinal Study of Parents and their Children (ALSPAC):

The Avon Longitudinal Study of Parents and their Children (ALSPAC) is a longitudinal population-based birth cohort that recruited pregnant women residing in Avon, UK, with an expected delivery date between 1st April 1991 and 31st December 1992. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. This cohort is described in detail on the website (http://www.alspac.bris.ac.uk) and elsewhere (3) and the total body DXA measures and cohort analyzed in the present paper are described in Kemp et al. (2014) (4). Please note that the study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

Bone Mineral Density in Childhood Study (BMDCS):

The Bone Mineral Density in Childhood Study is an ongoing longitudinal study in which boys and girls aged 6-16 year old were recruited between 2002-2003, and whose DXA measurements are obtained annually at five clinical centers in the United States (5, 6).

BPROOF:

B-PROOF is a trial investigating the effect of 2-year supplementation with 400 mcg folic acid and 500 mcg vitamin B12 on fracture incidence in hyperhomoycsteinemic persons aged 65y and older.

CHS:

The Cardiovascular Health Study (CHS) is a prospective investigation of risk factors for CVD in community-dwelling adults aged 65 and older. Participants were identified from Medicare-eligibility lists at four field centers in the U.S. (California, Maryland, North Carolina, and Pennsylvania). Recruitment of an original cohort of 5,201 participants occurred in 1989-90, followed by a supplemental cohort of 697 predominantly African-American individuals in 1992-93. At the exam in 1994-5 1,563 participants underwent DXA using the array beam mode QDR 2000 or 2000+ bone densitometers (Hologic, Inc., Bedford, MA) according to a standardized protocol. See, (7), for a description of the cohort.

Copenhagen Prospective Studies on Asthma (COPSAC) cohort:

The Copenhagen Prospective Studies on Asthma in Childhood is a clinical study. All mothers had a history of a doctor's diagnosis of asthma after 7 years of age. Newborns were enrolled in the first month of life, as previously described in detail (8). The Ethics Committee for Copenhagen and the Danish Data Protection Agency approved this study.

deCODE genetics BMD study:

The deCODE genetics BMD study is an ongoing population based study of all subjects who have undergone a DEXA-Hologic bone mineral density scan at the Landspitali University Hospital, Reykjavik, Iceland. The study samples have been previously described in detail (9). All participants gave informed consent and the study was approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland.

EPIC NorFolk:

The European Prospective Investigation of Cancer (EPIC) began as a large multi-centre cohort study primarily looking at the connection between diet, lifestyle factors and cancer, although the study was broadened from the outset to include other conditions. EPIC-Norfolk is part of a Europe-wide programme (http://www.srl.cam.ac.uk/epic/international/index.shtml). With the help of over 30,000 people living in Norfolk, the aim of the study is to provide data-based evidence for health policies to prevent or delay disease onset and maintain health and independence in older people. EPIC-Norfolk participants are men and women who were aged between 40 and 79 when they joined the study and who lived in Norwich and the surrounding towns and rural areas. They have been contributing information about their diet, lifestyle and health through questionnaires and health checks over two decades.

ERF:

Erasmus Rucphen Family study (ERF) is a family-based cohort study that includes inhabitants of a genetically isolated community in the South-West of the Netherlands, studied as part of the Genetic Research in Isolated Population (GRIP) program. ERF includes over 3,000 individuals who are living descendants of 22 couples, who had at least six children baptized in the community church, and their spouses. All data were collected between June 2002 and February 2005. The population shows minimal

immigration and high inbreeding, therefore frequency of rare alleles is increased in this population. All participants gave informed consent, and the Medical Ethics Committee of the Erasmus University Medical Centre, approved the study.

FENLAND:

The Fenland study is a population-based cohort study that uses objective measures of disease exposure to investigate the influence of diet, lifestyle and genetic factors on the development of diabetes and obesity. The volunteers are recruited from general practice lists in and around Cambridgeshire (Cambridge, Ely, and Wisbech) in the United Kingdom from birth cohorts from 1950–1975 (10).

FHS:

The Framingham Osteoporosis Study (FOS) / Framingham Heart Study (FHS) is a family-based, multigenerational cohort study initiated originally to study the risk factors for cardiovascular disease. (11). The FHS was initiated in 1948 to study determinants of cardiovascular disease and other major illnesses. The Original Cohort included 5,209 men and women, aged 28-62 years at enrolment who have undergone routine biennial examinations (12, 13). In 1971, Offspring of the Original Cohort participants and Offspring spouses including 5,124 men and women, aged 5 to 70 years, were enrolled into the Framingham Offspring Study. Offspring participants have been examined approximately every 4 years (14, 15). In the 1990s, DNA was obtained for genetic studies from surviving Original Cohort and Offspring participants. The body composition measurements used in this analysis have been previously

The Generation R Study:

The Generation R Study is a multiethnic prospective cohort study in which 9,778 pregnant women living in Rotterdam and with delivery date from April 2002 until January 2006 were enrolled. Details of study design and data collection can be found elsewhere (16). Genotype and imputation of this cohort are described elsewhere (17).

GOOD Study:

The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study was initiated to determine both environmental and genetic factors involved in the regulation of bone and fat mass. The GOOD study is a population-based cohort in which male subjects from between 18 and 20 years of age in the Gothenburg area in Sweden were randomly selected using national population registers and invited to participate in this initiative by phone. From the selected candidates 1,068 agreed to participate providing oral and written informed consent. The GOOD study was approved by the local ethics committee at Gothenburg University (18).

HABC:

A population based, prospective cohort study of well-functioning, unrelated men and women aged 70 and older. It was initiated to assess changes in body composition. A detailed description of this cohor can be found elsewhere (19-21).

MROS USA:

The Osteoporotic Fractures in Men (MrOS) Study is a multi-center prospective, longitudinal, observational study of risk factors for vertebral and all non-vertebral fractures in older men, and of the sequelae of fractures in men (22, 23). The original specific aims of the study include: (1) to define the skeletal determinants of fracture risk in older men, (2) to define lifestyle and medical factors related to fracture risk, (3) to establish the contribution of fall frequency to fracture risk in older men, (4) to determine to what extent androgen and estrogen concentrations influence fracture risk, (5) to examine the effects of fractures on quality of life, (6) to identify sex differences in the predictors and outcomes of fracture, (7) to collect and store serum, urine and DNA for future analyses as directed by emerging evidence in the fields of aging and skeletal health, and (8) define the extent to which bone mass/fracture risk and prostate diseases are linked. The MrOS Study enrolled 5,994 community dwelling, ambulatory men aged 65 years or older from six communities in the United States (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley near Pittsburgh, PA; Portland, OR; and San Diego, CA) between 2000 and 2002. Inclusion criteria were designed to provide a study cohort that is representative of the broad population of older men. The inclusion criteria were: (1) ability to walk without the assistance of another, (2) absence of bilateral hip replacements, (3) ability to provide self-reported data, (4) residence near a clinical site for the duration of the study, (5) absence of a medical condition that (in the judgment of the investigator) would result in imminent death, and (6) ability to understand and sign an informed consent. To qualify as an enrollee, the participant had to provide written informed consent, complete the self-administered questionnaire (SAQ), attend the clinic visit, and complete at least the anthropometric, DXA, and vertebral X-ray procedures. There were no other exclusion criteria. Written informed consent was obtained from all participants, and the Institutional Review Board at each study site approved the study.

Whole body total BMD (g/cm^2) and head BMD (g/cm^2) was measured using dual energy x-ray absorptiometry (DXA) (Hologic, Inc., MA) using Hologic QDR 4500 workstations at the baseline clinic visit. A central quality control lab, certification of DXA operators, and standardized procedures for scanning were used to insure reproducibility of DXA measurements. At baseline, a Hologic whole body phantom was circulated and measured at the 6 clinical sites. The variability across clinics was within acceptable limits, and cross-calibration correction factors were not required.

NEO:

The NEO was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected.

OPRA:

The Osteoporosis Risk Assessment Cohort (OPRA) cohort recruited Swedish women aged 75, at which time age-related bone loss is already obvious and fractures prevalent. The study was designed to investigate genetic and lifestyle factors contributing to osteoporosis and fracture risk. Of 1604 women invited between December 1995 and May 1999, 1044 (65%) attended at baseline. No exclusion criteria were applied. All participants answered a detailed questionnaire regarding their general health; BMD and body composition was assessed by DXA. All participants gave written informed consent and the Lund University Ethics Committee approved the study.

ORCADES:

The Orkney Complex Disease Study is an ongoing family-based genetic epidemiology collection in the isolated Scottish archipelago of Orkney. Genetic diversity in this population is decreased compared to Mainland Scotland, consistent with the high levels of endogamy historically. Fasting blood samples were collected and over 300 health-related phenotypes and environmental exposures were measured in each individual. All participants gave informed consent and the study was approved by Research Ethics Committees in Orkney and Aberdeen.

PANIC:

The Physical Activity and Nutrition in Children (PANIC) study is a controlled physical activity and dietary intervention study in a population sample of 506 Finnish children aged 6-8 years at baseline in 2007-2009. Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Northern Savo. All children and their parents gave their written informed consent (24). (http://www.uef.fi/en/web/physical-activity-and-nutrition-in-children/home)

RAINE:

The Raine (West Australian Pregnancy Cohort) Study is a longitudinal population-based pregnancy cohort study, which recruited 2,900 pregnant women from the public antenatal clinic at King Edward

Memorial Hospital and surrounding private clinics in Perth, Western Australia between May 1989 and November 1991 (25). Of the 2868 live births, 1183 had a whole body DXA at 20 years (26).

Rotterdam Study:

The Rotterdam Study is a prospective cohort study of chronic disabling conditions in Dutch elderly individuals that started in 1990 in Ommoord, a suburb of Rotterdam, among 10,994, men and women aged 55 and over (27).

SOF:

The Study of Osteoporotic Fractures (SOF) is a prospective multicenter study of risk factors for vertebral and non-vertebral fractures (28). The cohort is comprised of 9,704 community-dwelling women 65 years old or older recruited from populations-based listings in four U.S. areas: Baltimore, Maryland; Minneapolis, Minnesota; Portland, Oregon; and the Monongahela Valley, Pennsylvania. The SOF participants were followed up every four months by postcard or telephone to ascertain the occurrence of falls, fractures and changes in address. To date, follow-up rates have exceeded 95% for vital status and fractures. All fractures are validated by x-ray reports or, in the case of most hip fractures, a review of pre-operative radiographs. The inclusion criteria were: 1) 65 years or older, (2) ability to walk without the assistance of another, (3) absence of bilateral hip replacements, (4) ability to provide self-reported data, (5) residence near a clinical site for the duration of the study, (6) absence of a medical condition that (in the judgment of the investigator) would result in imminent death, and (7) ability to understand and sign an informed consent.

This study used whole body total BMD (g/cm^2) and head BMD (g/cm^2) measured using dual energy x-ray absorptiometry (DXA) (Hologic, Inc., MA) using Hologic QDR 2000 workstations at the sixth clinic visit. Scans were performed and analyzed at each clinic. Review of scans was done at the UCSF Coordinating Center on random subsets of scans and on problematic scans identified by technicians at the clinic. Some scans were deemed unacceptable and are not included in the data or are set to a special missing value code.

TwinsUK:

The UK Adult Twin Registry (TwinsUK) (www.twinsuk.ac.uk/) was started in 1993 and is comprised of ~12,000 monozygotic and dizygotic twins (83% female) aged 16-85 years recruited by successive media campaigns from all over the UK without selection for any particular disease or trait. The cohort is from Northern European/UK ancestry and has been shown to be representative of singleton populations and the UK population in general (26). All twins received a series of detailed disease and environmental questionnaires and the majority have been assessed in detail clinically at several time points for several hundred phenotypes related to common diseases or intermediate traits. The primary focus of the study has been the genetic basis of healthy aging process and complex diseases, including cardiovascular, metabolic, musculoskeletal, and ophthalmologic disorders.

UKBB:

In 2006-2010, the UK Biobank recruited 502,647 individuals aged between 37-76 years (99.5% were 40-69 years) from across the country. Each participant provided information regarding their health and lifestyle using touch screen questionnaires, physical measurements and agreement to have their health followed and they also provided blood, urine and saliva samples for future analysis. UK Biobank has ethical approval from the Northwest Multi-centre Research Ethics Committee (MREC) and informed consent was obtained from all participants.

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1982 Pelotas Birth Cohort:

The 1982 Pelotas Birth Cohort Study is conducted by the Postgraduate Program in Epidemiology at Universidade Federal de Pelotas with the collaboration of the Brazilian Public Health Association (ABRASCO). From 2004 to 2013, the Wellcome Trust supported the study. The International Development Research Center, World Health Organization, Overseas Development Administration, European Union, National Support Program for Centers of Excellence (PRONEX), the Brazilian National Research Council (CNPq), and the Brazilian Ministry of Health supported previous phases of the study.

Genotyping of 1982 Pelotas Birth Cohort Study participants was supported by the Department of Science and Technology (DECIT, Ministry of Health) and National Fund for Scientific and Technological Development (FNDCT, Ministry of Science and Technology), Funding of Studies and Projects (FINEP, Ministry of Science and Technology, Brazil), Coordination of Improvement of Higher Education Personnel (CAPES, Ministry of Education, Brazil).

ALSPAC Study:

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. GWAS data was generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors, and JPK and DME will serve as guarantors for the contents of this paper.. This work is supported by a Medical Research Council program grant (MC_UU_12013/4 to D.M.E). D.M.E is supported by an Australian Research Council Future Fellowship (FT130101709).

The Bone Mineral Density in Childhood Study:

BMDCS is extremely grateful to all the families who participated in this study, and the whole team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. This work was funded by the National Institute of Child Health and Human Development (NICHD) contracts NO1-HD-1-3228, -3329, -3330, -3331, -3332 and -3333, R01 HD058886 and the Clinical and Translational Research Center (5-MO1-RR-000240 and UL1 RR-026314).

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deCODE:

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ERF:

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The Generation R Study:

We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam. The generation and management of GWAS genotype data for the Generation R Study was done at the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, The Netherlands. We thank Pascal Arp, Mila Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters for their help in creating, managing and QC of the GWAS database. The musculoskeletal research of the Generation R Study is partly supported by the European Commission grant HEALTH-F2-2008-201865-GEFOS. The general design of Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families. Additionally, the Netherlands Organization for Health Research and Development supported authors of this manuscript (ZonMw 907.00303, ZonMw 916.10159, ZonMw VIDI 016.136.361 to V.W.J., and ZonMw VIDI 016.136.367 to F.R.). V.W.J. received a Consolidator Grant from the European Research Council (ERC-2014-CoG-648916). J.F.F. has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 633595 (DynaHEALTH). The study was also supported by funding from the European Union's Horizon 2020 research and innovation programme (733206, LIFECYCLE).

GOOD Study:

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HABC:

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MROS-USA:

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ORCADES:

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Rotterdam Study:

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Twins UK:

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Functional Group:

C Ackert-Bicknell: National Institute of Health /National Institute of Arthritis Musculoskeletal and Skin Diseases grant number AR060981.

JH Duncan Bassett: Molecular Endocrinology Laboratory, Department of Medicine, Imperial College London, London, UK/ Wellcome Trust Strategic Award 101123/Z/13/A

Graham R Williams: Molecular Endocrinology Laboratory, Department of Medicine, Imperial College London, London, UK/ Wellcome Trust Strategic Award 101123/Z/13/A

SUPPLEMENTAL FIGURES

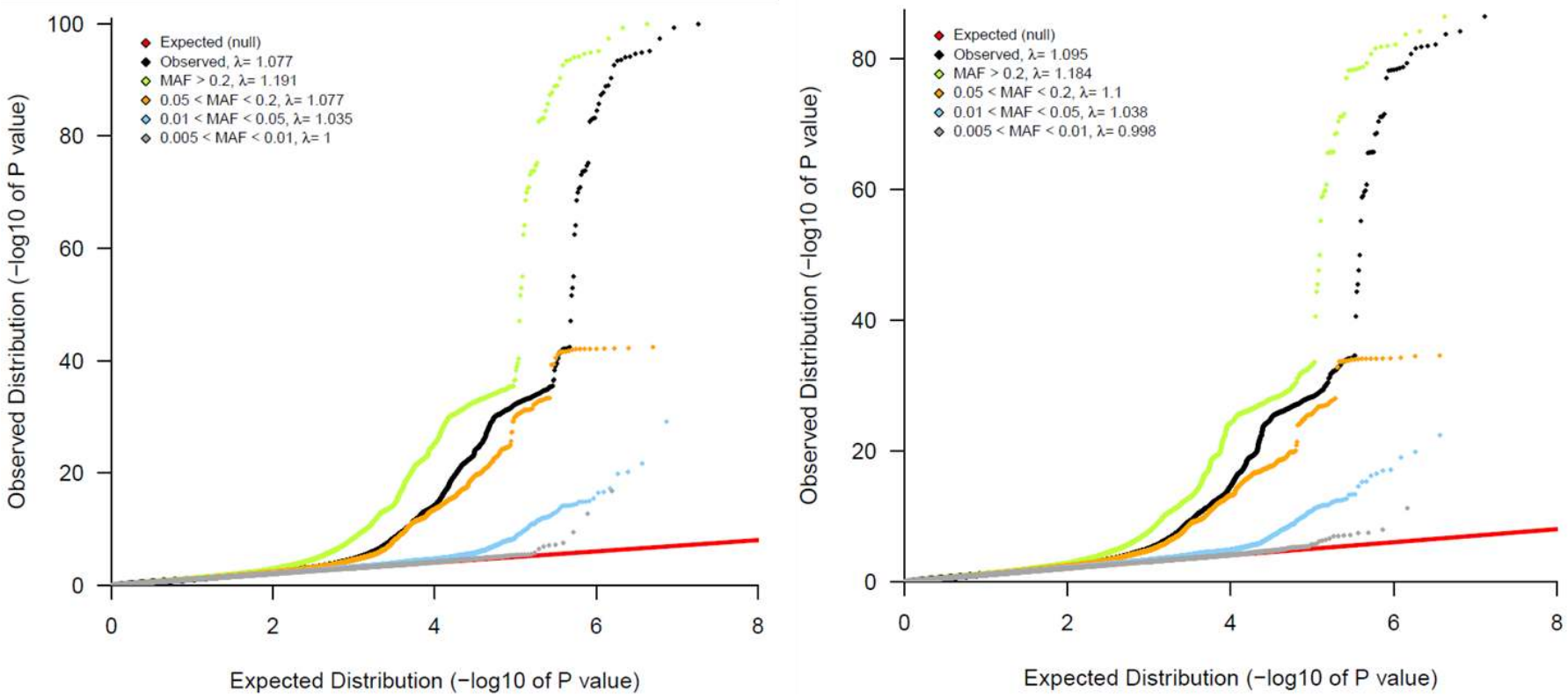


Figure S1. QQ-Plots for the genome-wide association study of TB-BMD. Left panel: Including studies regardless the ethnic background of the participants (N=66, 628). **Right panel:** Including only studies of European ancestry (N=56,284).

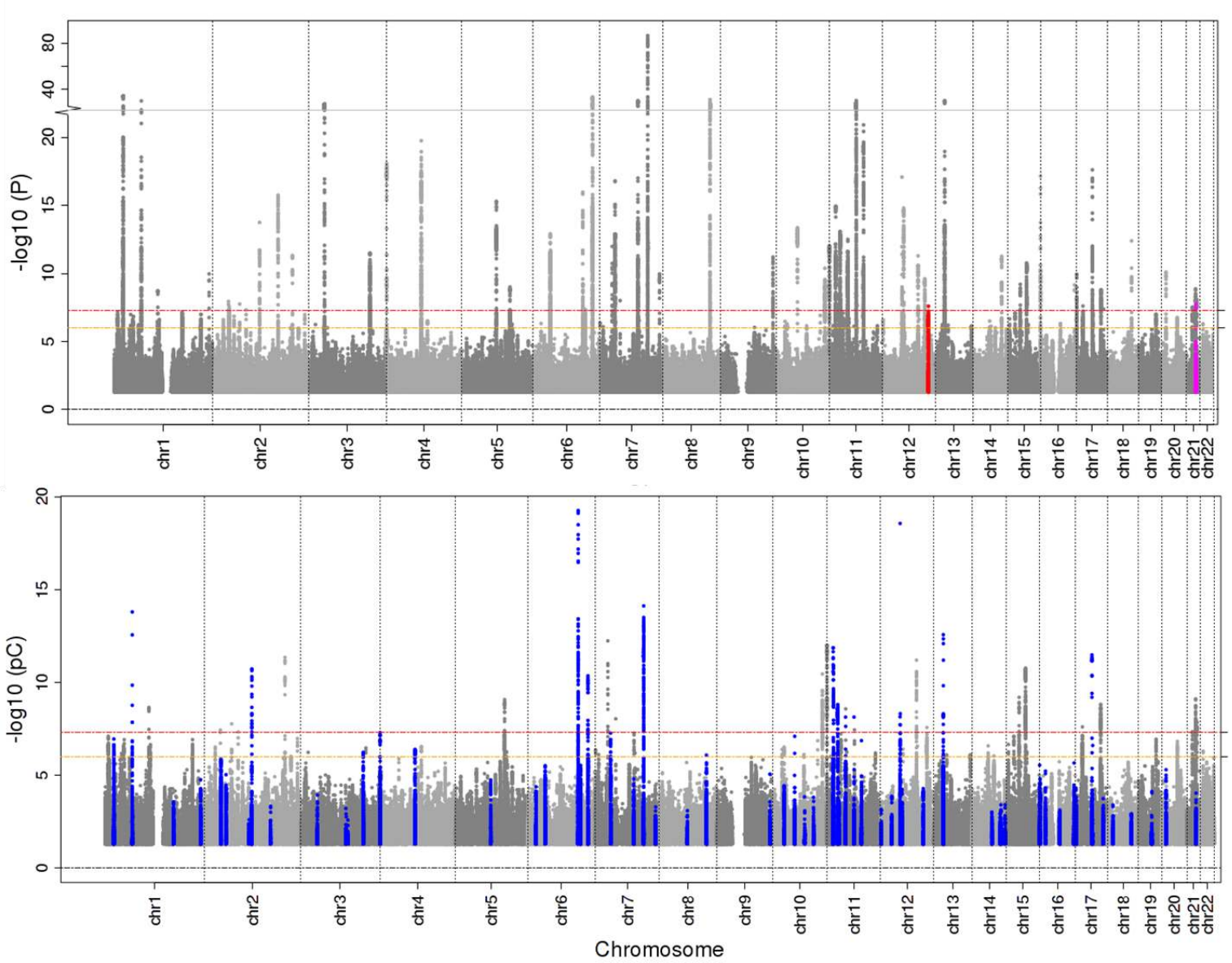
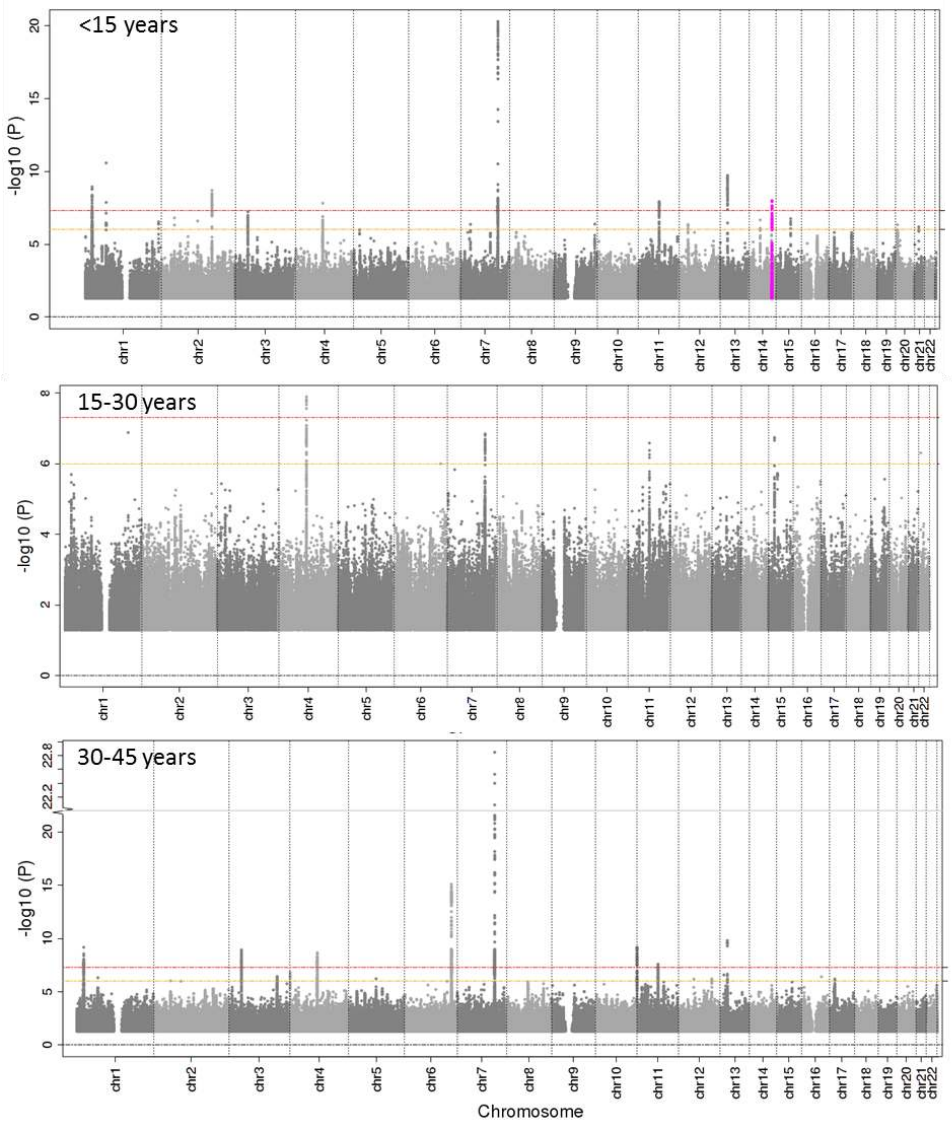


Figure S2. Manhattan plots of association statistics (-log10(P values)) for TB-BMD only-European meta-analysis. Each dot represents a SNP and the x-axis indicates its chromosomal position (Build 37 NCBI). Dashed horizontal red and yellow lines mark the GWS threshold ($P<5x10^{-8}$) and suggestive threshold ($P<1x10^{-6}$), respectively. **Top**: The association P-value (on $-\log_{10}$ scale) in the meta-analysis including only studies comprising individuals of European ancestry. Loci only reaching significance in this analysis are highlighted: the novel 19q12 in red and the known 21q22.13 in magenta. **Bottom:** The association P-value (on $-\log_{10}$ scale) after conditional analysis on all variants. Highlighted in blue previously reported loci (SNPs within ±500Kb of leading SNPs in previous GWAS with different bone traits).

Figure S3. Regional Plots for all novel loci associated with TB-BMD ($P<5x10^{-8}$). Circles show GWAS meta-analysis P-values and position of SNPs for the overall meta-analysis (N=66,628) unless stated otherwise. Different colors indicate varying degrees of pair-wise linkage disequilibrium with the top marker (1000 Genomes – CEU population, except for 11p13 in which AFR was the reference population). Locus 11p.13 (chr11:35481152−36481152) association is driven by association in non-European populations [S]. Locus 12q24.21 reached significance in the only-European meta-analysis (N=56,284) [Y]. Locus 19q12 reached significance only in the 45-60 age-bin meta-analysis (N=18,805)[Ff]. **Attached file.**

Figure S4. Forest Plots for all novel loci associated with TB-BMD ($P<5x10^{-8}$). Effect estimates for the leading SNPs of the 36 novel BMD loci in the overall meta-analysis. Novel loci detected in the overall and subgroup meta-analyses are displayed. Symbol size proportional to the inverse variance of the SNP main effect. **Attached file**



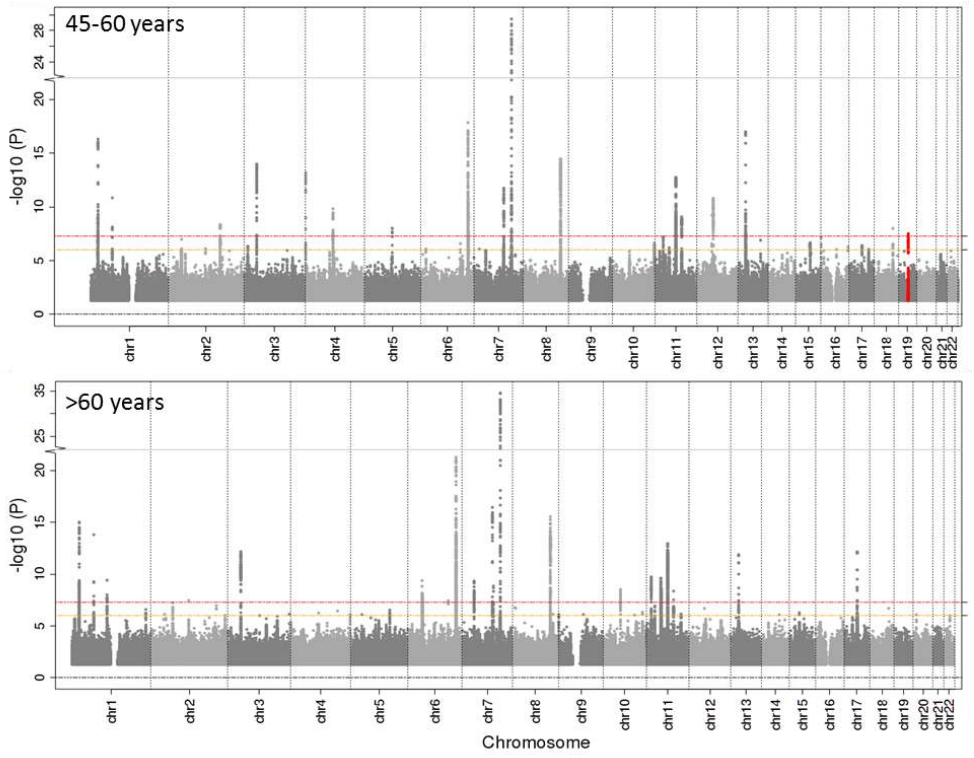


Figure S5. Manhattan plots of association statistics (-log10(P values)) for TB-BMD meta-analyses per age bin. Each dot represents an SNP and the x-axis indicates its chromosomal position (Build 37 NCBI). Dashed horizontal red and yellow lines mark the GWS threshold ($P<5x10^{-8}$) and suggestive threshold ($P<1x10^{-6}$), respectively. Sample sizes vary across the different age bins. <15 years; N= 1,870. 15-30 years; N=4,180. 30-45 years; N=10,062. 45-60 years; N= 18,805. >60 years N=22,504. Highlighted in red the age-specific signals: In red the novel locus 19q2 (45-60 years) and in magenta the known 14q32.12 locus (<15 years).

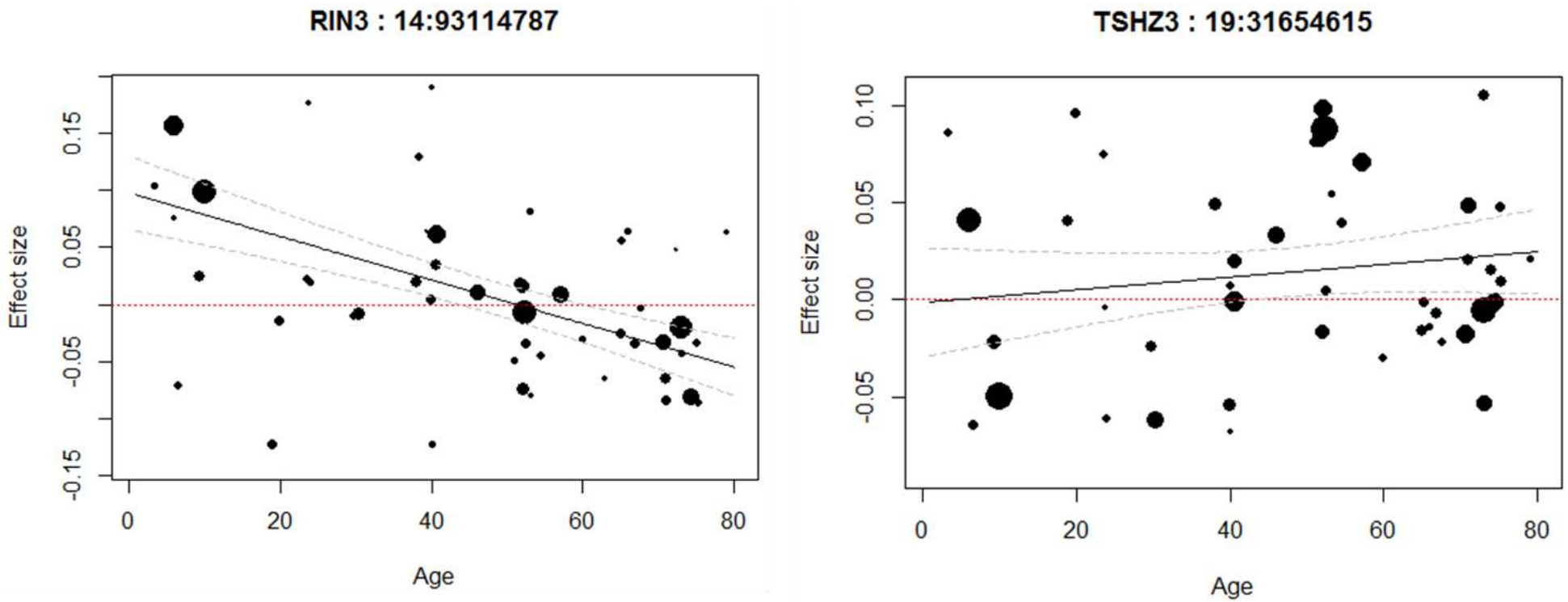


Figure S6. Meta-regression for GWS signals rising exclusively from an age-bin analysis. Left panel: Leading SNP of the signal mapping to 14q32.12 TB-BMD GWS associated only in the <15 years bin (N=11,870). **Right panel:** Leading SNP of the signal mapping to 19q12 TB-BMD GWS associated only in the 45-60 years bin (N=18,805). Each circle represents a study subgroup (i.e., study divided in age strata), with the circle size proportional to the inverse variance of the SNP main effect. At the left, estimates from each age-bin meta-analysis, with the symbol size proportional to the inverse variance of the SNP main effect.

Figure S7. Meta-regression for nominally significant signals in the meta- regression. Left panel: In total for 42 suggestive signals in the overall meta-analysis ($P<5x10^{-6}$) we found nominal evidence of an age-dependent effect of the associated variants. Meta-regression plots for each of the leading SNPs are shown. Each circle represents a study subgroup (i.e., study divided in age strata), with the circle size proportional to the inverse variance of the SNP main effect. At the left, estimates from each age-bin meta-analysis, with the symbol size proportional to the inverse variance of the SNP main effect. **Attached file**.

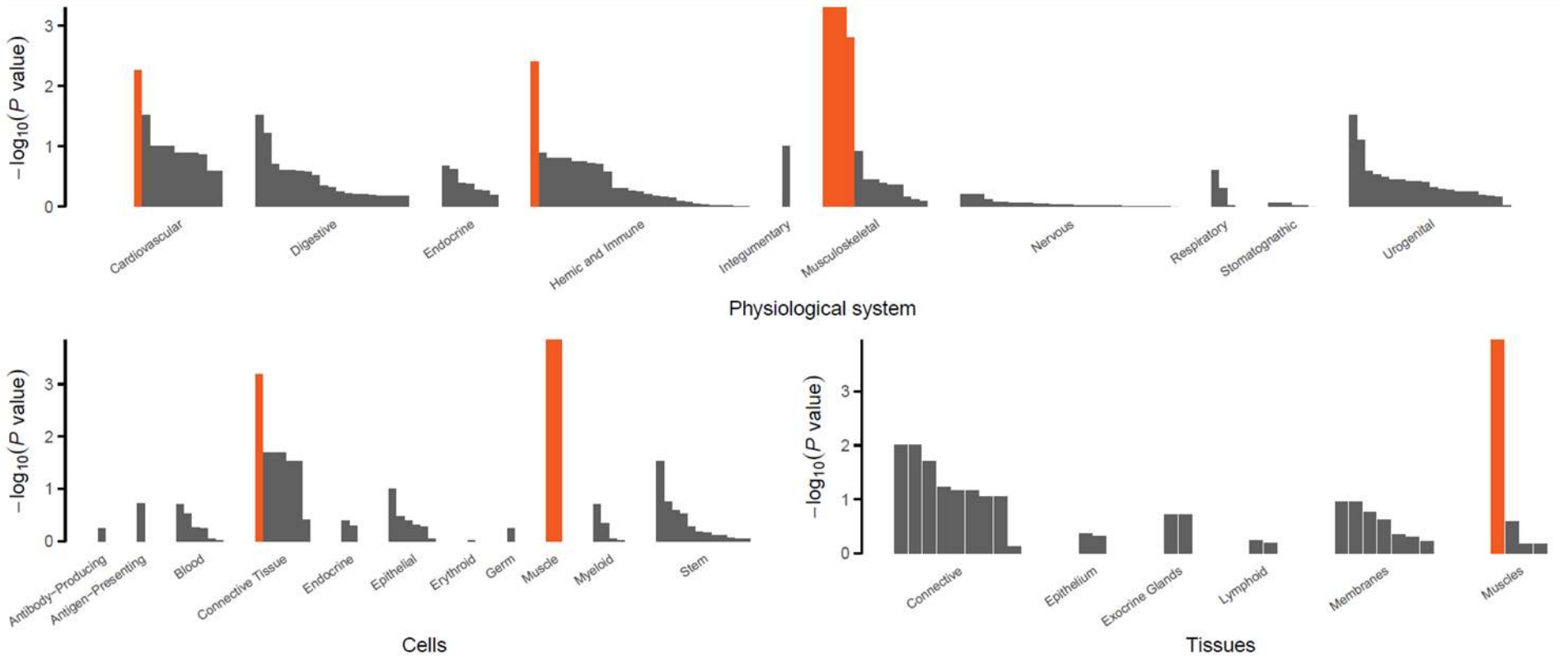


Figure S8. Depict results for cell/tissue enrichment analysis of novel TB-BMD associated regions. Bars represent the level of evidence for genes in the associated loci to be expressed in any of the 209 Medical Subject Heading (MeSH) tissue and cell type annotations. Highlighted in orange are these cell/tissue types significantly (FDR<5%) enriched for the expression of the genes in the associated loci

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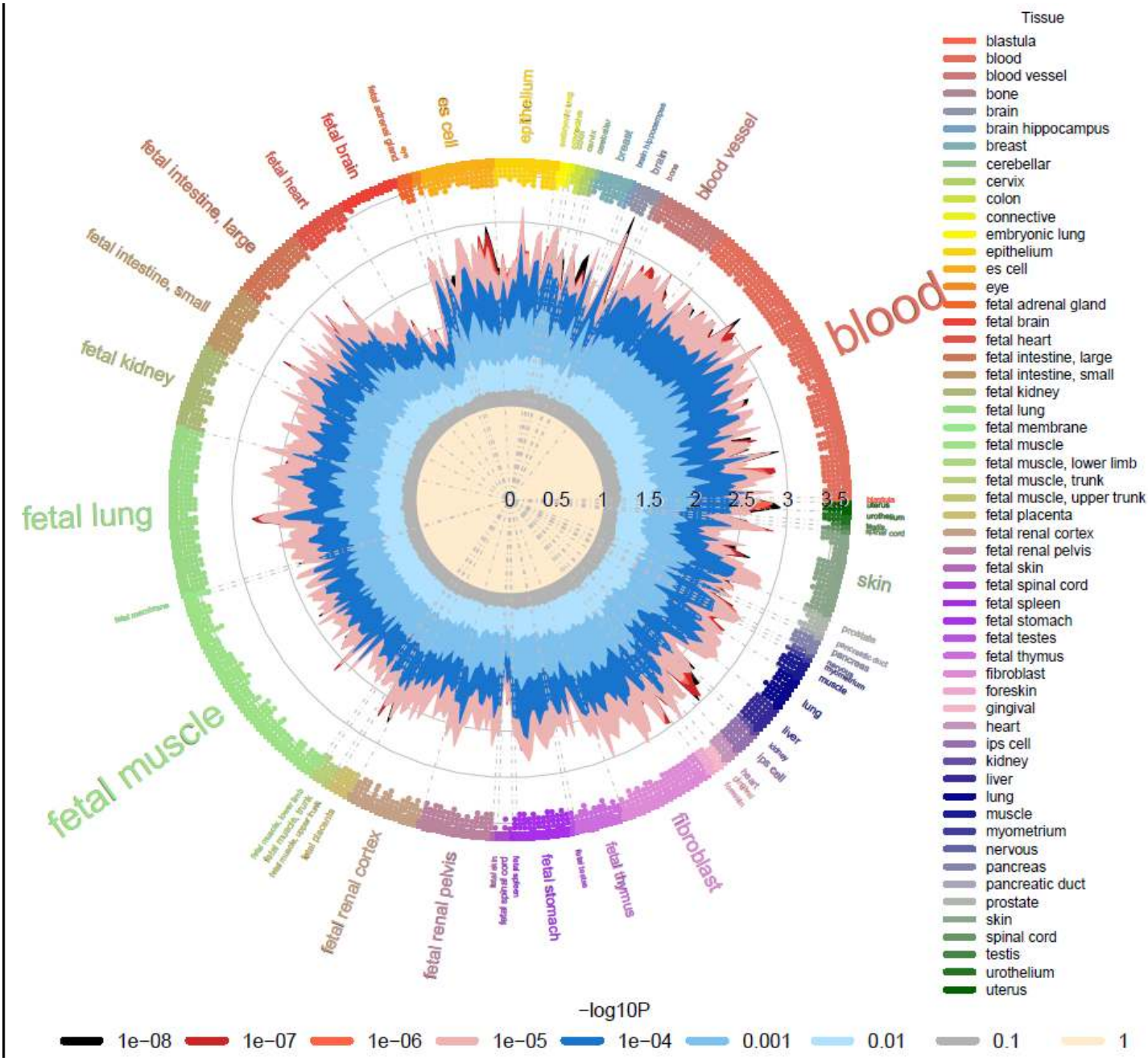

Figure S9. GARFIELD functional enrichment analyses. The wheel plot displays functional enrichment for associations with TB-BMD within DHS hotspot regions in ENCODE and Roadmap Epigenomics studies. The radial axis shows fold enrichment calculated at each of eight GWAS P-value thresholds ($P < 1\times10^{-1}$ to $P < 1\times10^{-8}$) for each of 424 cell types. Cell types are sorted by tissue, represented along the outside edge of the plot with font size proportional to the number of cell types from that tissue. Fold enrichment values at the different thresholds are plotted with different colors inside the plot (indicated at the bottom of the figure). Dots along the inside edge of the plot denote significant enrichment (if present; $P<1x10^{-4}$) for a given cell type at $P < 1\times10^{-5}$ (outermost dot) to $P < 1\times10^{-8}$ (innermost dot). Results show overall well-spread enrichment.

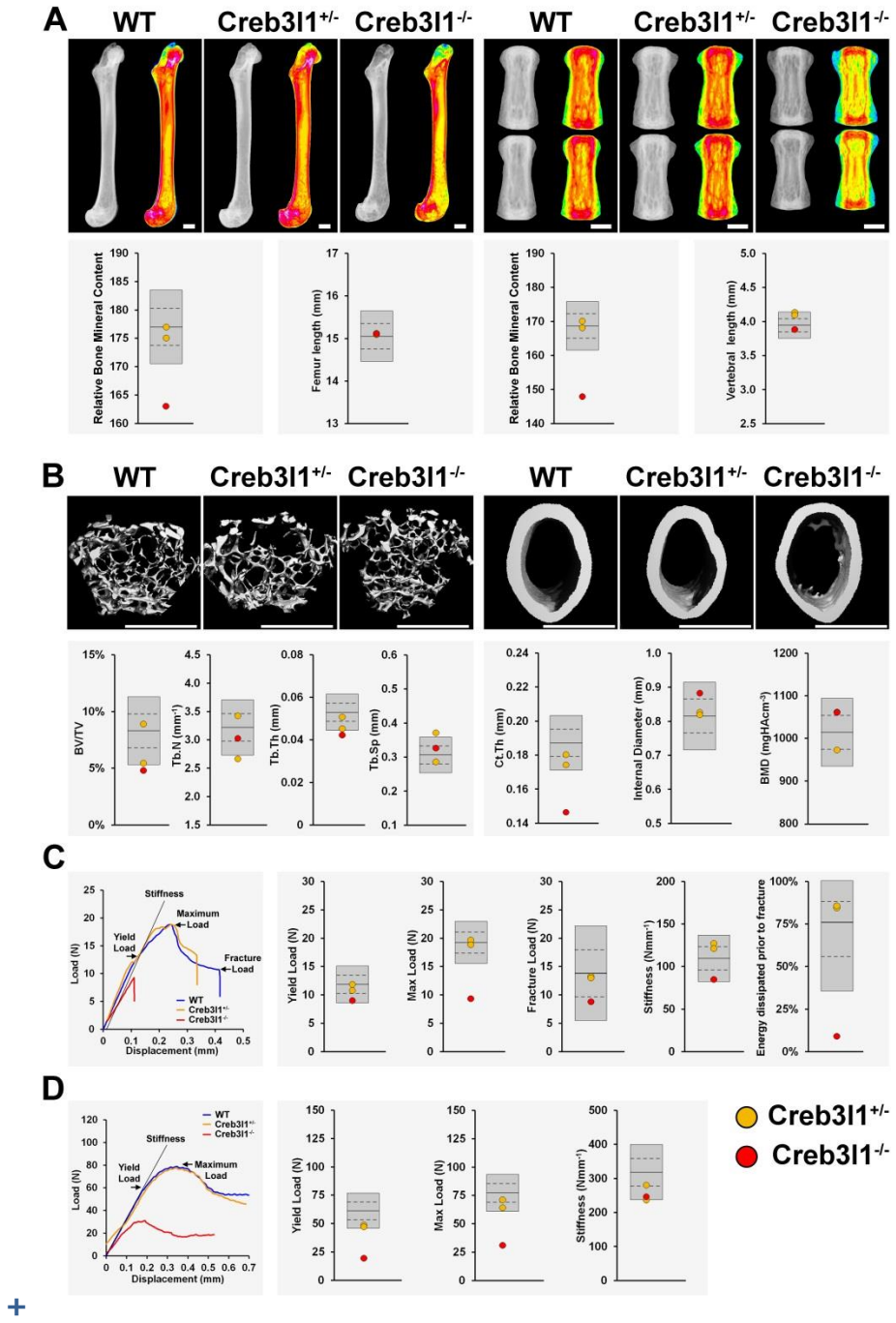

Figure S10. Skeletal phenotype screening of Cyclic AMP-responsive element-binding protein 3-like 1 (Crebl1) knockout mice. Decreased bone mass and strength in adult Creb3l1 knockout mice. **A.** X-ray microradiography (Faxitron MX20) of femur and caudal vertebrae from female wild-type (WT), heterozygous (*Creb3l1*$^{+/-}$) and homozygous (*Creb3l1*$^{-/-}$) knockout mice at postnatal day 112 (P112). Pseudocolored grey-scale images in which low bone mineral content (BMC) is blue/green and high BMC is pink. Reference ranges are derived from >300 WT mice of identical age, sex and genetic background (C57BL/6), mean (solid line), 1.0SD (dotted lines) and 2.0SD (grey box). Values for parameters from individual animals are shown as orange dots (*Creb3l1*$^{+/-}$ n=2) and red dots (*Creb3l1*$^{-/-}$ n=1). Scale bar: 1mm. **B.** Micro-CT images (Scanco MicroCT-50) of proximal femur trabecular bone (left) and mid-diaphysis cortical bone (right) from WT, *Creb3l1*$^{+/-}$ and *Creb3l1*$^{-/-}$ mice. Graphs showing trabecular bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), cortical thickness (Ct.Th), internal cortical diameter and cortical bone mineral density (BMD). Scale bar: 1mm. **C**. Representative load displacement curves from destructive 3-point bend testing (Instron 5543 load frame) of WT, *Creb3l1*$^{+/-}$ and *Creb3l1*$^{-/-}$ femurs. Yield load, maximum load, fracture load and gradient of the linear elastic phase (stiffness) are indicted for the WT curve. Graphs showing yield load, maximum load, fracture load, stiffness and energy dissipated prior to fracture (Toughness). **D.** Representative load displacement curves from destructive compression testing (Instron 5543 load frame) of WT, *Creb3l1*$^{+/-}$ and *Creb3l1*$^{-/-}$ caudal vertebra showing yield load, maximum load and stiffness.

SUPPLEMENTAL TABLES

Table S1. Descriptives per cohort of genotype data for analysis. Attached Excel file

Table S2. Participant description per cohort and age strata. Attached Excel file

CHR	BP	Rsid	locus	Phenotype	Reference	A1	EAF	beta_TB	P_TB	beta_LS	P_LS	beta_FN	P_FN
1	22490724	rs7521902	1p36.12	FN-BMD, LS-BMD	Estrada et al.	A	0.24	-0.066	3.32E-23	-0.034	0.002	-0.037	6.60E-05
1	22711473	rs6426749	1p36.12	FN-BMD, LS-BMD	Estrada et al.	C	0.18	0.101	2.73E-42	0.088	1.2E-13	0.082	5.9E-16
1	68639385	rs17482952	1p31.3	FN-BMD, LS-BMD	Estrada et al.	G	0.08	-0.065	8.21E-10	-0.045	0.008	-0.043	0.003
1	68647716	rs12407028	1p31.3	FN-BMD, LS-BMD	Estrada et al.	C	0.38	-0.051	1.10E-18	-0.063	4.3E-12	-0.046	5.07E-09
1	172199573	rs479336	1q24.3	FN-BMD	Estrada et al.	G	0.28	0.032	1.18E-06	0.034	9.74E-04	0.043	9.08E-07
1	240597214	rs9287237	1q43	Trabecular vBMD	Paternoster et al.	T	0.17	0.056	3.29E-12	0.037	0.002	0.039	1.47E-04
2	42250549	rs7584262	2p21	FN-BMD	Estrada et al.	T	0.24	0.033	5.41E-07	0.009	0.392	0.044	1.34E-06
2	54659707	rs4233949	2p16.2	LS-BMD	Estrada et al.	C	0.37	0.031	2.47E-07	0.055	1.49E-03	0.044	0.002
2	112500035	rs17040773	2q13	FN-BMD	Estrada et al.	C	0.22	-0.021	0.008	-0.004	0.714	-0.023	0.012
2	119038598	rs1878526	2q14.2	LS-BMD	Estrada et al.	A	0.22	0.012	0.092	0.038	4.29E-04	0.006	0.529
2	119154872	rs6542457	2q14.2	LS-BMD	Zheng et al.	C	0.09	0.048	2.19E-05	0.083	6.53E-06	-0.005	0.757
2	119545994	rs11692564	2q14.2	LS-BMD	Zheng et al.	T	0.01	0.229	7.09E-15	0.238	4.1E-09	0.116	7.23E-04
2	166601046	rs1346004	2q24.3	FN-BMD, LS-BMD	Estrada et al.	A	0.47	-0.051	3.62E-19	-0.052	8.7E-09	-0.058	7.15E-14
3	41128564	rs430727	3p22.1	FN-BMD, LS-BMD	Estrada et al.	T	0.45	-0.070	1.53E-34	-0.056	5.3E-10	-0.061	2.02E-15
3	113370010	rs1026364	3q13.2	FN-BMD	Estrada et al.	T	0.37	0.023	1.50E-04	0.013	0.171	0.024	0.003
3	118183783	rs1949542	3q13.2	INT-BMD, TRO-BMD	Pei et al.	A	0.40	0.006	0.282	-0.013	0.150	-0.019	0.015
3	156555984	rs344081	3q25.31	LS-BMD	Estrada et al.	C	0.16	-0.056	7.40E-12	-0.047	4.28E-04	-0.035	0.002
4	994414	rs3755955	4p16.3	FN-BMD, LS-BMD	Estrada et al.	A	0.16	-0.074	2.10E-19	-0.049	5.05E-05	-0.044	2.87E-05
4	88773849	rs6532023	4q22.1	FN-BMD, LS-BMD	Estrada et al.	T	0.34	0.060	1.31E-23	0.048	2.34E-07	0.034	2.78E-05
5	88376061	rs1366594	5q14.3	FN-BMD	Estrada et al.	C	0.49	-0.048	2.35E-17	-0.007	0.435	-0.079	5.44E-25
6	21384613	rs9466056	6p22.3	FN-BMD, LS-BMD	Estrada et al.	A	0.39	-0.028	1.90E-06	-0.039	1.81E-05	-0.038	8.86E-07
6	44639184	rs11755164	6p21.1	LS-BMD	Estrada et al.	T	0.41	-0.041	7.13E-12	-0.024	0.010	-0.013	0.108
6	127167072	rs13204965	6q22.33	FN-BMD, LS-BMD	Estrada et al.	C	0.23	-0.062	1.02E-18	-0.039	2.81E-04	-0.052	1.39E-08
6	133350936	rs3012465	6q23.2	Skull BMD	Kemp et al.	A	0.33	-0.022	2.61E-04	0.016	0.079	0.025	0.002
6	151895456	rs6909279	6q25.1	Cortical vBMD	Paternoster et al.	G	0.44	-0.072	2.52E-35	-0.055	1.3E-09	-0.051	8.07E-11
6	151907748	rs4869742	6q25.1	FN-BMD, LS-BMD	Estrada et al.	T	0.33	-0.070	5.20E-30	-0.061	2.8E-08	-0.052	1.75E-08
6	151946658	rs7751941	6q25.1	FN-BMD, LS-BMD	Estrada et al.	A	0.21	-0.044	1.45E-10	-0.059	9.8E-08	-0.031	0.001
7	37938422	rs10226308	7p14.1	LS-BMD	Estrada et al.	G	0.18	0.033	1.06E-05	0.059	2.48E-07	0.025	0.012
7	38128326	rs6959212	7p14.1	FN-BMD, LS-BMD	Estrada et al.	T	0.34	-0.043	7.34E-13	-0.066	2.6E-12	-0.033	5.12E-05
7	96120675	rs4727338	7q21.3	FN-BMD, LS-BMD	Estrada et al.	G	0.32	-0.073	6.07E-33	-0.059	5.8E-10	-0.063	5E-15
7	120742980	rs148771817	7q31.31	FA-BMD	Zheng et al.	T	0.01	0.154	7.88E-04	0.136	0.006	0.011	0.798
7	120785064	rs13245690	7q31.31	LS-BMD	Estrada et al.	G	0.37	-0.057	4.06E-22	-0.028	0.002	-0.023	0.003
7	120903815	rs4609139	7q31.31	TB-BMD	Medina-Gomez et al.	T	0.35	-0.046	1.17E-14	-0.015	0.117	-0.013	0.114
7	120974765	rs3801387	7q31.31	FN-BMD	Estrada et al.	G	0.27	0.135	1.15E-100	0.073	1.7E-13	0.054	3.26E-10
7	150919829	rs7812088	7q36.1	FN-BMD	Estrada et al.	A	0.12	0.058	3.54E-11	0.035	0.010	0.044	1.61E-04
8	71591203	rs7017914	8q13.3	Fem FN-BMD	Estrada et al.	G	0.48	-0.008	0.151	0.011	0.219	-0.016	0.045
8	120007420	rs2062377	8q24.12	FN-BMD, LS-BMD	Estrada et al.	T	0.41	0.064	1.47E-28	0.081	6.6E-19	0.060	1.64E-14
9	133478827	rs7851693	9q34.11	FN-BMD	Estrada et al.	G	0.35	-0.046	5.73E-14	-0.017	0.074	-0.040	9.62E-07
10	28479942	rs3905706	10p12.1	LS-BMD	Estrada et al.	T	0.23	0.009	0.176	0.055	7.69E-07	-0.014	0.157
10	54427825	rs1373004	10q21.1	FN-BMD, LS-BMD	Estrada et al.	T	0.15	-0.067	1.22E-14	-0.056	1.10E-04	-0.045	2.81E-04
10	79401316	rs7071206	10q22.3	LS-BMD	Estrada et al.	C	0.21	0.016	0.027	0.053	8.60E-07	-0.014	0.122
10	101813802	rs7084921	10q24.2	FN-BMD	Estrada et al.	T	0.41	0.024	3.94E-05	0.018	0.043	0.026	9.35E-04
11	15710084	rs7108738	11p15.1	FN-BMD	Estrada et al.	G	0.18	0.056	9.06E-14	0.043	2.05E-04	0.083	8.07E-17
11	16296412	rs1347677	11p15.1	Hip BMD	Yang TL et al.	C	0.21	0.045	5.41E-11	0.030	0.006	0.039	3.37E-05
11	27505677	rs10835187	11p14-p13	LS-BMD	Estrada et al.	C	0.48	0.044	4.38E-14	0.026	0.004	0.009	0.275
11	30951674	rs163879	11p14.1	FN-BMD, LS-BMD	Estrada et al.	C	0.34	0.031	3.02E-07	0.039	6.17E-05	0.018	0.026
11	46722221	rs7932354	11p11.2	FN-BMD, LS-BMD	Estrada et al.	T	0.34	0.043	9.33E-12	0.036	4.23E-04	0.041	1.64E-06
11	68201295	rs3736228	11q13.2	FN-BMD, LS-BMD	Estrada et al.	T	0.15	-0.102	5.03E-34	-0.078	2.9E-10	-0.049	4.79E-06
11	68263370	rs12272917	11q13.2	SK-BMD	Kemp et al.	C	0.25	-0.077	2.74E-31	-0.074	4.6E-13	-0.045	2.89E-07
11	86853997	rs597319	11q14.2	BUA and VOS	Moayyeri et al.	G	0.32	-0.055	1.72E-19	-0.042	1.25E-05	-0.026	0.002
12	1638171	rs2887571	12p13.33	FN-BMD, LS-BMD	Estrada et al.	G	0.24	0.038	6.83E-09	0.034	8.57E-04	0.022	0.012
12	28017159	rs7953528	12p11.22	FN-BMD	Estrada et al.	A	0.17	0.018	0.019	-0.011	0.349	0.038	1.35E-04
12	49474605	rs12821008	12q13.12	LS-BMD	Estrada et al.	T	0.38	0.034	1.94E-08	.	.	0.012	0.525
12	53727955	rs2016266	12q13.13	FN-BMD, LS-BMD	Estrada et al.	G	0.34	0.048	1.55E-15	0.056	6.7E-09	0.039	1.89E-06
12	54417576	rs736825	12q13.13	FN-BMD, LS-BMD	Estrada et al.	G	0.37	-0.017	4.09E-03	-0.062	2.7E-11	-0.043	8.70E-08
12	107367225	rs1053051	12q23.3	FN-BMD	Estrada et al.	C	0.49	0.032	2.46E-08	0.024	0.006	0.023	0.003
13	42951449	rs9533090	13q14.11	FN-BMD, LS-BMD	Estrada et al.	T	0.45	-0.060	1.09E-25	-0.082	5.2E-20	-0.035	6.19E-06
13	43116133	rs1021188	13q14.11	Cortical BMD	Paternoster et al.	C	0.19	-0.037	5.79E-07	-0.026	0.024	-0.020	0.051
14	70456699	rs227425	14q24.2	LS-BMD	Zhang et al.	G	0.49	-0.013	0.026	-0.035	1.13E-04	-0.010	0.185
14	91442779	rs1286083	14q32.11	FN-BMD, LS-BMD	Estrada et al.	C	0.20	0.053	1.95E-13	0.065	1E-08	0.038	7.66E-05
14	93114787	rs754388	14q32.12	TB-BMD, LL-BMD	Kemp et al.	A	0.17	-0.010	0.167	-0.007	0.543	-0.004	0.670
14	103883633	rs11623869	14q32.32	FN-BMD, LS-BMD	Estrada et al.	T	0.34	-0.029	1.11E-06	-0.020	0.029	-0.029	2.96E-04
16	375782	rs9921222	16p13.3	FN-BMD, LS-BMD	Estrada et al.	T	0.48	-0.048	1.76E-17	-0.053	3.2E-09	-0.050	6.36E-11
16	1532463	rs13336428	16p13.3	FN-BMD, LS-BMD	Estrada et al.	A	0.44	-0.025	1.56E-05	-0.027	0.003	-0.039	8.33E-07
16	15129459	rs4985155	16p13.11	FN-BMD, LS-BMD	Estrada et al.	G	0.34	0.021	4.35E-04	0.035	2.09E-04	0.022	0.007
16	50986308	rs1564981	16q12.1	LS-BMD	Estrada et al.	A	0.49	-0.025	6.86E-06	-0.044	7.95E-07	-0.032	2.38E-05
16	51021803	rs1566045	16q12.1	FN-BMD	Estrada et al.	C	0.20	0.027	8.05E-04	0.012	0.317	0.043	2.90E-05
16	86710660	rs10048146	16q24.1	FN-BMD, LS-BMD	Estrada et al.	G	0.18	-0.048	2.09E-10	-0.064	4.5E-08	-0.056	2.31E-08

CHR	BP	Rsid	locus	Phenotype	Reference	A1	EAF	beta_TB	P_TB	beta_LS	P_LS	beta_FN	P_FN
17	2068932	rs4790881	17p13.3	FN-BMD, LS-BMD	Estrada et al.	C	0.29	-0.038	1.04E-09	-0.035	3.41E-04	-0.050	2.92E-09
17	41798824	rs4792909	17q21.31	FN-BMD, LS-BMD	Estrada et al.	T	0.40	0.039	5.96E-11	0.047	3.07E-07	0.048	1.52E-09
17	42225547	rs227584	17q21.31	FN-BMD, LS-BMD	Estrada et al.	C	0.34	0.032	2.16E-07	0.043	4.05E-05	0.048	2.69E-08
17	43977827	rs1864325	17q21.31	LS-BMD	Estrada et al.	T	0.21	-0.023	0.008	-0.052	2.48E-04	-0.019	0.103
17	69949016	rs7217932	17q24.3	FN-BMD	Estrada et al.	A	0.48	0.025	1.14E-05	0.006	0.501	0.033	1.89E-05
18	13708574	rs4796995	18p11.21	FN-BMD	Estrada et al.	G	0.37	-0.022	2.23E-04	-0.025	0.006	-0.037	2.56E-06
18	60054857	rs884205	18q21.33	FN-BMD, LS-BMD	Estrada et al.	A	0.24	-0.053	4.39E-15	-0.062	2.8E-09	-0.042	2.71E-06
19	33599127	rs10416218	19q13.11	LS-BMD	Estrada et al.	C	0.29	0.028	1.19E-05	0.070	8.3E-09	0.042	2.93E-05
20	10639988	rs3790160	20p12.2	FN-BMD, LS-BMD	Estrada et al.	C	0.50	-0.035	7.99E-10	-0.051	1.50E-08	-0.029	1.94E-04
21	37848334	rs170183	21q22.13	Hip BMD-Female	Zhang et al.	G	0.50	0.026	6.80E-06	0.016	0.082	0.020	0.009

Table S3 Known independent markers associated with bone phenotypes. Index SNPs of the GWS association reported for the specific bone phenotype [fifth column] in the reference stated [sixth column]. All effect sizes (β) are reported for the minor allele (A1). EAF=Effect Allele Frequency, TB= total body BMD, assessed in this study, LS=lumbar spine BMD, assessed in Zheng et al. , FN=Femoral Neck BMD, assessed in Zheng at al. Phenotype for which association was previously reported and the correspondent reference are given.

Table S4. Genome-wide significant SNPs for the overall TB-BMD meta-analysis. Estimates were derived from the overall approach. Beta coefficients and allele frequency (EAF) are reported for the A1 allele. **Attached Excel file**.

Table S5. Genome-wide significant SNPs for the TB-BMD meta-analysis in European cohorts. Estimates were derived from the all-age combined approach. Beta coefficients and allele frequency (EAF) are reported for the A1 allele. **Attached Excel file.**

CHR	BP	Rsid	A1	A2	EAF	beta	P.value	HetISq	HetPVal	N	locus	unreported
1	22700351	rs34920465	a	g	0.7968	-0.1008	9.41E-16	0	0.6114	22467	1p36.12	no
1	68656697	rs2566752	t	c	0.6119	-0.0776	1.55E-14	0	0.841	22380	1p31.3	no
1	110475971	rs7548588	t	c	0.6075	-0.0617	3.80E-10	34.4	0.07127	22324	1p13.3	yes
2	119529829	rs55983207	t	c	0.9474	-0.1409	3.37E-08	36.1	0.06437	22187	2q14.2	no
3	41171177	rs2371447	t	g	0.4846	-0.0711	6.58E-13	0	0.5362	22460	3p22.1	no
6	45144224	rs184065563	a	g	0.3052	-0.0623	7.14E-09	7.9	0.3586	22491	6p21.1	no
6	127423055	rs1936792	a	g	0.2652	0.0607	3.72E-08	20	0.2109	22458	6q22.33	no
6	151910126	rs6557155	t	g	0.428	-0.0981	5.18E-22	0	0.9731	22490	6q25.1	no
7	38136277	rs1524058	t	c	0.4044	-0.0604	7.43E-10	10.7	0.3237	22479	7p14.1	no
7	96134115	rs6465511	c	g	0.3265	-0.0849	1.16E-16	0	0.5605	22493	7q21.3	no
7	99130834	rs34670419	t	g	0.0387	-0.1603	1.40E-09	0	0.7425	22223	7q22.1	yes
7	120974765	rs3801387	a	g	0.7266	-0.1337	2.82E-35	13.3	0.2911	22423	7q31.31	no
8	120012700	rs11995824	c	g	0.4289	0.0806	2.80E-16	0	0.6858	22476	8q24.12	no
10	54425325	rs10824760	t	c	0.8217	0.0886	3.05E-09	0	0.9745	22453	10q21.1	no
11	16348061	rs7131442	a	t	0.7918	-0.0762	1.81E-10	0	0.6208	22497	11p15.1	no
11	46783435	rs61884328	t	c	0.9013	-0.1063	2.46E-10	29.7	0.1088	22502	11p11.2	no
11	68218290	rs11228240	t	c	0.254	-0.0848	1.12E-13	16.7	0.2501	22483	11q13.2	no
11	86873599	11:86873599:I	d	i	0.7333	0.0768	4.34E-09	0	0.6918	18952	11q14.2	no
13	42951449	rs9533090	t	c	0.4555	-0.0663	8.41E-12	53.8	0.002882	22493	13q14.11	no
17	41826839	rs2741856	c	g	0.0764	0.1391	7.03E-13	2.6	0.4249	22392	17q21.31	no
1	22697860	rs6679981	a	g	0.1811	0.1162	4.81E-17	34.1	0.1257	18784	1p36.12	no
1	68656697	rs2566752	t	c	0.6056	-0.075	1.42E-11	0	0.7436	18734	1p31.3	no
2	166618262	rs1968294	t	c	0.4895	-0.0632	4.41E-09	0	0.9458	18786	2q24.3	no
3	41127606	rs444561	c	g	0.5642	0.0831	1.02E-14	0	0.9578	18782	3p22.1	no
4	1008386	rs56396408	t	c	0.1529	-0.1198	1.37E-13	16.2	0.2938	16206	4p16.3	no
4	88831249	rs11934731	a	g	0.6784	-0.0718	3.71E-10	0	0.7177	18802	4q22.1	no
5	88354675	rs10037512	t	c	0.5173	0.0604	2.10E-08	0	0.6152	18780	5q14.3	no
6	151910126	rs6557155	t	g	0.4255	-0.0968	1.42E-18	11.1	0.3381	18802	6q25.1	no
7	96133871	rs6465510	a	c	0.6498	0.0787	1.79E-12	0	0.464	18802	7q21.3	no
7	120974765	rs3801387	a	g	0.7348	-0.1359	3.49E-30	0	0.4875	18735	7q31.31	no
8	119946656	rs7010267	a	c	0.4438	0.0838	3.34E-15	0	0.7913	18728	8q24.12	no
11	68220905	rs57502260	a	g	0.8295	0.1088	1.81E-13	36.4	0.1075	18792	11q13.2	no
11	86880458	rs540403	a	g	0.3312	-0.0699	1.36E-09	0	0.859	18791	11q14.2	no
12	49379537	rs118115924	t	g	0.0143	-0.3132	6.10E-10	23.7	0.2178	18764	12q13.12	no
12	53659448	rs7398996	t	c	0.6855	-0.0762	1.56E-11	0	0.4527	18787	12q13.13	no
13	42969049	rs9533095	t	g	0.4647	-0.091	1.32E-17	0	0.4543	18797	13q14.11	no
18	60054857	rs884205	a	c	0.2479	-0.072	9.97E-09	0	0.532	18757	18q21.33	no
19	31654615	rs6510186	t	c	0.2602	0.0677	3.11E-08	0	0.614	18782	19q12	yes*
1	22682366	rs12742784	t	c	0.2193	0.1126	6.64E-10	0	0.6669	10049	1p36.12	no
3	41112656	rs62259232	a	g	0.4885	0.0899	1.21E-09	0	0.8597	10050	3p22.1	no
4	88852643	rs10005067	t	c	0.5212	0.0883	2.16E-09	0	0.545	10062	4q22.1	no
6	151874122	rs9478217	a	g	0.4637	-0.1173	4.33E-15	13.1	0.3249	10055	6q25.1	no
7	120983343	rs10242100	a	g	0.7296	-0.1614	8.22E-23	4.1	0.4007	10025	7q31.31	no
11	242859	rs55781332	a	g	0.7823	-0.1088	6.98E-10	8.7	0.3624	9965	11p15.5	yes
11	68218290	rs11228240	t	c	0.2576	-0.0974	2.58E-08	0	0.9399	10049	11q13.2	no
13	42965694	rs8001611	t	c	0.5404	0.0947	1.54E-10	0	0.8615	10059	13q14.11	no

4	88815986	rs77034375	t	c	0.3089	-0.1397	1.26E-08	0	0.5184	4180	4q22.1	no
1	22444975	rs10737462	t	c	0.2267	-0.0925	1.67E-09	22	0.2685	11807	1p36.12	no
1	68658266	1:68658266:I	d	i	0.4541	0.0947	2.60E-11	39.9	0.1553	11360	1p31.3	no
2	166573776	rs35969972	t	c	0.5148	0.0771	2.01E-09	26.3	0.2369	11807	2q24.3	no
4	88831249	rs11934731	a	g	0.6716	-0.0777	1.52E-08	0	0.6512	11807	4q22.1	no
7	121018857	rs917726	a	t	0.7269	-0.137	5.07E-21	36.4	0.1644	11807	7q31.31	no
11	68252123	rs12364620	t	g	0.7508	0.0848	1.29E-08	0	0.8874	11807	11q13.2	no
13	43128577	rs9525638	t	c	0.5826	-0.0844	1.90E-10	0	0.9245	11360	13q14.11	no
14	93114787	rs72699866	g	a	0.8247	0.0994	1.01E-08	54.3	0.05281	11807	14q32.12	no*

Table S6. Index Genome-wide significant SNPs in the age-bin meta-analyses. Genomic coordinates are on build 37 of the human genome. Beta coefficients and allele frequencies (EAF) are reported for the A1 allele. * Only GWS in the particular age-bin.

Table S7. Nominally significant variants after meta-regression analysis. Only suggestively associated variants ($P<5x10^{-6}$) in the TB-BMD overall meta-analysis were subjected to meta-regression assessment. Genomic coordinates are on build 37 of the human genome. Allele frequencies (EAF) are reported for the A1 allele. C.L-C.U, 95% Confidence interval lower and upper limit. **Attached Excel file**

CHR	BP	locus	rsnumber	A1	EAF	beta	P	N	betaJ	PJ
1	22484575	1p36.12	rs3971300	T	0.71	0.069	7.41E-23	57561	0.071	2.56E-24
1	22700351	1p36.12	rs34920465	A	0.82	-0.101	2.67E-35	59625	-0.103	7.68E-37
1	68635879	1p31.3	rs145119306	A	0.07	-0.026	0.03436	58593	-0.088	2.77E-11
1	68656697	1p31.3	rs2566752	T	0.61	-0.074	6.79E-31	59727	-0.091	7.06E-40
1	110480220	1p13.3	rs7364724	A	0.40	-0.038	1.84E-09	60973	-0.038	2.20E-09
1	240581653	1q43	rs12044944	T	0.19	0.052	1.06E-10	58925	0.052	8.86E-11
2	40630678	2p22.1	rs10490046	A	0.77	0.042	1.13E-08	59278	0.043	9.30E-09
2	42280066	2p21	rs78572108	A	0.13	-0.054	2.66E-08	55121	-0.054	2.35E-08
2	68962137	2p13.3	rs10048745	A	0.25	-0.041	1.76E-08	58660	-0.041	1.68E-08
2	85483350	2p11.2	rs2043230	A	0.44	0.034	4.77E-08	61631	0.034	4.59E-08
2	119507607	2q14.2	rs115242848	T	0.01	0.312	1.75E-14	35647	0.305	6.28E-14
2	119632724	2q14.2	rs12621139	A	0.20	-0.060	3.88E-12	48934	-0.058	1.12E-11
2	166577489	2q24.3	rs7586085	A	0.52	0.051	1.72E-16	60651	0.051	1.76E-16
2	202803881	2q33.2	rs6716216	A	0.88	-0.066	4.71E-12	61489	-0.066	4.40E-12
3	41129297	3p22.1	rs415997	A	0.53	0.068	2.64E-28	60766	0.068	3.61E-28
3	156474152	3q25.31	rs344024	A	0.77	0.050	3.11E-12	62621	0.050	2.61E-12
4	996165	4p16.3	rs6831280	A	0.16	-0.080	8.26E-19	53031	-0.080	1.06E-18
4	88831249	4q22.1	rs11934731	A	0.68	-0.062	1.69E-20	61124	-0.062	1.09E-20
5	88376061	5q14.3	rs1366594	A	0.52	0.051	5.03E-16	60698	0.051	3.01E-16
5	122847622	5q23.2	rs11745493	A	0.75	0.044	9.90E-10	61202	0.044	8.30E-10
6	44636919	6p21.1	rs7741085	T	0.59	0.047	1.19E-13	60657	0.047	1.61E-13
6	127167072	6q22.33	rs13204965	A	0.76	0.062	1.05E-16	57298	0.085	5.46E-27
6	127446790	6q22.33	rs9482772	T	0.55	-0.039	5.70E-10	59203	-0.061	3.17E-20
6	151910126	6q25.1	rs6557155	T	0.42	-0.079	2.97E-34	58412	-0.074	3.00E-30
6	151994910	6q25.1	rs7765040	A	0.84	0.059	5.37E-12	58188	0.054	7.30E-10
6	152008982	6q25.1	rs2941741	A	0.41	0.058	1.29E-20	62437	0.040	4.26E-10
7	30957702	7p14.3	rs28362721	T	0.18	-0.061	1.02E-12	53488	-0.062	6.40E-13
7	37965963	7p14.1	rs28457747	T	0.18	0.039	9.85E-07	61792	0.045	1.89E-08
7	38136277	7p14.1	rs1524058	T	0.40	-0.054	1.73E-17	60883	-0.057	2.72E-19
7	50901491	7p12.1	rs1548607	A	0.69	0.041	9.71E-09	52156	0.041	9.02E-09
7	96133319	7q21.3	rs6965122	A	0.68	0.077	4.64E-31	61668	0.076	1.52E-30
7	96656572	7q21.3	rs3757493	T	0.42	-0.036	1.28E-08	60079	-0.035	4.29E-08
7	120790559	7q31.31	rs56335989	T	0.55	-0.023	0.0001878	59259	-0.045	5.38E-09
7	120902676	7q31.31	rs4731006	T	0.35	-0.040	5.45E-10	60966	-0.060	2.85E-14
7	120959155	7q31.31	rs2536195	A	0.68	-0.089	1.11E-29	43891	-0.068	1.46E-14
7	120974765	7q31.31	rs3801387	A	0.73	-0.138	3.31E-87	60474	-0.163	7.27E-91
7	120985854	7q31.31	rs2041490	C	0.18	0.017	0.04031	59352	0.084	9.83E-21
7	121178195	7q31.31	rs73717393	T	0.93	-0.094	4.69E-13	55499	-0.088	8.04E-12
7	150933044	7q36.1	rs10233479	T	0.12	0.062	1.20E-10	63298	0.062	9.70E-11
8	120012700	8q24.12	rs11995824	C	0.44	0.074	5.21E-32	59397	0.074	8.68E-32
9	133471891	9q34.11	rs10901216	A	0.35	-0.045	6.20E-12	58789	-0.045	6.84E-12
10	54423853	10q21.1	rs12258451	C	0.88	0.075	4.32E-14	56851	0.075	5.63E-14

10	124015986	10q26.13	rs10788264	A	0.49	-0.041	4.10E-11	60632	-0.041	3.43E-11
11	243268	11p15.5	rs505404	T	0.76	-0.052	9.04E-13	60292	-0.052	9.75E-13
11	15708792	11p15.2	rs7926837	A	0.79	-0.052	7.46E-12	60590	-0.054	1.76E-12
11	15814794	11p15.2	rs11023718	T	0.04	0.128	7.30E-13	52490	0.118	5.96E-11
11	16248894	11p15.1	rs12800049	T	0.26	0.056	1.11E-15	61150	0.048	1.43E-11
11	16630779	11p15.1	rs35199438	T	0.31	-0.047	2.24E-12	60906	-0.040	4.72E-09
11	27308483	11p14-p13	rs10450586	C	0.62	-0.048	8.17E-14	60024	-0.051	2.26E-15
11	27593899	11p14-p13	rs1352479	A	0.27	0.039	6.88E-08	57320	0.042	5.05E-09
11	35083633	11p13	rs2553773	C	0.43	-0.034	8.09E-08	60038	-0.036	1.79E-08
11	46856536	11p11.2	rs10838622	T	0.36	0.049	3.04E-13	56422	0.045	2.54E-11
11	47252107	11p11.2	rs4647728	A	0.03	-0.124	5.88E-11	49763	-0.111	5.94E-09
11	68174189	11q13.2	rs4988321	A	0.04	-0.160	4.08E-23	54286	-0.114	1.63E-11
11	68218290	11q13.2	rs11228240	T	0.26	-0.084	3.57E-31	56682	-0.069	3.51E-19
11	86887931	11q14.2	rs634277	A	0.67	0.062	2.19E-20	58914	0.062	2.01E-20
12	49379537	12q13.12	rs118115924	T	0.01	-0.277	8.01E-18	39253	-0.304	6.84E-21
12	49385679	12q13.12	rs10875906	T	0.27	0.053	1.07E-12	53734	0.061	1.57E-16
12	53737840	12q13.13	rs12424778	A	0.28	0.054	2.23E-15	61906	0.054	1.16E-15
12	90334829	12q21.33	rs10777212	T	0.35	0.045	5.00E-12	58906	0.045	6.15E-12
12	107297862	12q23.3	rs6539288	A	0.50	-0.040	2.44E-10	60592	-0.040	1.86E-10
12	116555786	12q24.21	rs73200209	A	0.80	0.045	2.52E-08	56109	0.045	2.54E-08
13	42952145	13q14.11	rs9594738	T	0.47	-0.072	5.00E-31	60695	-0.069	2.43E-28
13	43153869	13q14.11	rs117543324	A	0.96	-0.162	3.13E-17	46599	-0.147	2.42E-14
14	91445162	14q32.11	rs1286079	T	0.19	0.055	5.42E-12	59543	0.055	5.30E-12
15	51126002	15q21.2	rs34293575	A	0.82	-0.025	0.002497	60821	-0.049	1.80E-08
15	51524292	15q21.2	rs2414095	A	0.35	-0.040	6.22E-10	60408	-0.054	7.30E-15
15	67420680	15q22.33	rs1545161	A	0.54	0.038	1.16E-09	61086	0.036	7.67E-09
15	67562214	15q22.33	rs12901789	A	0.76	-0.049	1.68E-11	59612	-0.047	1.22E-10
16	392318	16p13.3	rs8047501	A	0.49	0.056	6.83E-18	55097	0.056	8.38E-18
16	86714715	16q24.1	rs71390846	C	0.19	-0.050	6.95E-10	58067	-0.050	7.94E-10
17	2048713	17p13.3	rs7209460	T	0.70	0.044	1.15E-10	59907	0.044	1.20E-10
17	17843396	17p11.2	rs8070624	A	0.44	0.036	2.45E-08	57633	0.036	2.45E-08
17	41798621	17q21.31	rs66838809	A	0.08	0.109	2.35E-18	50133	0.110	1.59E-18
17	42283037	17q21.31	rs9910055	T	0.25	0.044	3.13E-09	57268	0.045	1.37E-09
17	63840961	17q24.1	rs9907056	A	0.32	0.041	1.61E-09	59188	0.041	1.38E-09
18	60054857	18q21.33	rs884205	A	0.25	-0.053	3.96E-13	58528	-0.053	3.96E-13
20	10640877	20p12.2	rs6040063	A	0.51	0.040	7.75E-11	60605	0.040	1.01E-10
21	36970350	21q22.12	rs9976876	T	0.46	-0.038	1.35E-09	59146	-0.038	1.48E-09
21*	37836973	21q22.13	rs7277076	T	0.43	0.036	1.82E-08	59889	0.036	1.21E-08
21	40350744	21q22.2	rs11910328	A	0.84	-0.049	8.51E-09	59137	-0.050	5.82E-09

Table S8. Independent variants associated with TB-BMD in the only –European meta-analysis. Genomic coordinates are on build 37 of the human genome. Beta coefficients and allele frequencies (EAF) are reported for the A1 allele. *J* suffix refers to the summary statistics in the join analysis fitting all variants together. * Only significant in the meta-analysis of European individuals.

Trait	PMID	year	rg	se	Z	P
Age.at.menarche	25231870	2014	-0.05	0.028	-1.801	0.072
Age.at.Menopause	26414677	2015	0.002	0.043	0.054	0.957
Anorexia.nervosa	24514567	2014	-0.027	0.034	-0.791	0.429
Asthma	17611496	2007	0.02	0.063	0.312	0.755
Autism.spectrum.disorder	www.med.unc.edu	2015	-0.059	0.064	-0.925	0.355
Bipolar.disorder	21926972	2011	0.035	0.052	0.669	0.504
Birth.length	25281659	2015	-0.109	0.059	-1.84	0.066
Birth.weight	23202124	2013	-0.023	0.059	-0.39	0.697
Body.mass.index.2010	20935630	2010	0.108	0.029	3.748	0.0002
Childhood.intelligence.quotient	23358156	2014	-0.012	0.065	-0.183	0.855
Childhood.obesity	22484627	2012	0.091	0.049	1.858	0.063
Cholesterol.esters.in.large.HDL	27005778	2016	-0.077	0.107	-0.721	0.471
Cholesterol.esters.in.large.LDL	27005778	2016	0.036	0.068	0.535	0.593
Cholesterol.esters.in.large.VLDL	27005778	2016	-0.078	0.106	-0.735	0.463
Cholesterol.esters.in.medium.HDL	27005778	2016	0.076	0.094	0.807	0.42
Cholesterol.esters.in.medium.LDL	27005778	2016	-0.062	0.104	-0.598	0.55
Cognitive.performance	25201988	2014	0.057	0.039	1.472	0.141
College.completion	23722424	2013	0.059	0.042	1.43	0.153
Creatinine	27005778	2016	0.093	0.069	1.354	0.176
Crohn's.disease	26192919	2015	-0.044	0.042	-1.042	0.297
Depressive.symptoms	27089181	2016	-0.051	0.039	-1.307	0.191
Ever.vs.never.smoked	20418890	2010	-0.015	0.044	-0.335	0.737
Extreme.body.mass.index	23563607	2013	0.094	0.044	2.122	0.034
Extreme.height	23563607	2013	-0.087	0.044	-1.978	0.048
Extreme.waist.to.hip.ratio	23563607	2013	-0.035	0.072	-0.486	0.627
Fasting.glucose	22581228	2012	0.065	0.047	1.388	0.165
Fasting.insulin	22581228	2012	0.027	0.058	0.459	0.647
Femoral.neck.bone.mineral.density	22504420	2012	0.923	0.035	26.03	2.25E-149
Forced.expiratory.volume.in.1.second	21946350	2011	0.054	0.062	0.877	0.381
forced.vital.capacity	21946350	2011	0.056	0.044	1.272	0.203
Former.vs.current.smoker	20418890	2010	-0.019	0.067	-0.289	0.772
Free.cholesterol.in.IDL	27005778	2016	-0.101	0.106	-0.952	0.341
Free.cholesterol.in.large.HDL	27005778	2016	0.117	0.114	1.02	0.308
Free.cholesterol.in.large.LDL	27005778	2016	0.04	0.068	0.59	0.555
Free.cholesterol.in.large.VLDL	27005778	2016	-0.049	0.108	-0.453	0.651
Free.cholesterol.in.medium.HDL	27005778	2016	0.073	0.085	0.861	0.389
Free.cholesterol.in.medium.VLDL	27005778	2016	-0.068	0.072	-0.952	0.341
Free.cholesterol.in.serum	27005778	2016	-0.162	0.129	-1.258	0.208
Free.cholesterol.in.small.VLDL	27005778	2016	-0.102	0.093	-1.091	0.275
Glucose	27005778	2016	0.079	0.069	1.145	0.252
Glycated hemoglobin.HbA1C	20858683	2010	0.122	0.06	2.033	0.042
HDL.cholesterol	20686565	2010	-0.07	0.033	-2.116	0.034

Height.2010	20881960	2010	-0.057	0.031	-1.846	0.065
Hip.circumference	25673412	2015	-0.038	0.032	-1.174	0.24
Homeostasis.model.assessment-B	20081858	2011	0.026	0.059	0.44	0.66
Homeostasis.model.assessment-IR	20081858	2011	0.057	0.069	0.825	0.409
Infant.head.circumference	22504419	2012	0.258	0.074	3.493	0.0005
Inflammatory.bowel.disease	26192919	2015	-0.112	0.038	-2.908	0.004
Insulin.like.growth.factor.1	27329260	0	0.174	0.062	2.798	0.005
Leptin	26833098	2016	-0.028	0.065	-0.424	0.671
Leptin.adjusted.for.body.mass.index	26833098	2016	-0.081	0.07	-1.163	0.245
Lumbar.spine.bone.mineral.density	22504420	2012	0.99	0.035	28.097	1.06E-173
Lung.cancer.all	24880342	2016	0.009	0.067	0.136	0.891
Major.depressive.disorder	22472876	2013	0.004	0.048	0.073	0.942
Mean.platelet.volume	22139419	2011	0.109	0.048	2.278	0.023
Neuroticism	27089181	2016	-0.107	0.036	-3.012	0.003
Obesity.class.1	23563607	2013	0.077	0.032	2.422	0.015
Obesity.class.2	23563607	2013	0.029	0.04	0.714	0.475
Obesity.class.3	23563607	2013	-0.008	0.055	-0.14	0.889
Overweight	23563607	2013	0.097	0.033	2.902	0.004
PGC.cross.disorder.analysis	23453885	2013	0.012	0.048	0.259	0.796
Platelet.count	22139419	2011	-0.056	0.041	-1.379	0.168
Rheumatoid.Arthritis	24390342	2014	-0.028	0.051	-0.548	0.584
Subjective well being	27089181	2016	0.124	0.045	2.759	0.006
Total.cholesterol	20686565	2010	-0.085	0.038	-2.278	0.023
Triglycerides	20686565	2010	-0.027	0.04	-0.68	0.497
Type.2.diabetes	22885922	2012	0.108	0.05	2.153	0.031
Ulcerative.colitis	26192919	2015	-0.145	0.043	-3.358	0.001
Urinary.albumin/creatinine	26631737	2015	0.065	0.063	1.038	0.299
Urinary.albumin/creatinine.non.diabetes	26631737	2015	0.087	0.084	1.038	0.299
Waist.circumference	25673412	2015	-0.012	0.027	-0.435	0.664
Waist.to.hip.ratio	25673412	2015	0.038	0.032	1.171	0.241
Years.of.schooling	23722424	2013	0.053	0.038	1.366	0.172

Table S9. Genetic correlation of TB-BMD with different traits. The genetic correlation was calculated based on the summary statistics of the only-Europeans meta-analysis in LD-Hub using its current dataset. Significant results are shown in **Figure 3**.

CHR	BP	rsID	A1	A2	Freq1	P-value	Gene Name	Codons	SNP Type	SIFT	Polyphen2	OMIM Disease
1	68603586	rs983034	C	T	0.62	1.21E-08	*GPR177*	GTC-aTC	Nonsynonymous	Tolerated	benign	
1	68624878	rs3748705	C	T	0.66	3.77E-09	*GPR177*	GCG-GCa	Synonymous	.	.	
2	166535918	rs777346	C	T	0.52	5.08E-16	*CSRNP3*	ACC-ACt	Synonymous	.	.	
3	156570703	rs414683	A	G	0.79	1.88E-08	*AC117392.3*	CAA-CAg	Synonymous	.	.	
4	994414	rs3755955	A	G	0.16	2.10E-19	*IDUA*	CGG-CaG	Nonsynonymous	Tolerated	benign	Scheie Syndrome
4	995305	rs6815946	T	C	0.84	7.16E-18	*IDUA*	AAT-AAc	Synonymous	.	.	Scheie Syndrome
4	995868	rs114806891	C	T	0.93	1.21E-10	*IDUA*	AAC-AAt	Synonymous	.	.	Scheie Syndrome ; Hurler Syndrome
4	995919	rs6830825	C	G	0.16	3.63E-18	*IDUA*	GCG-GCc	Synonymous	.	.	Scheie Syndrome
4	995997	rs6811373	A	G	0.84	4.21E-18	*IDUA*	AGA-gGA	Nonsynonymous	Not Predicted	benign	
4	996012	rs6831021	C	G	0.16	5.57E-18	*IDUA*	GCG-cCG	Nonsynonymous	Not Predicted	benign	
4	996165	rs6831280	A	G	0.16	9.66E-19	*IDUA*	GCG-aCG	Nonsynonymous	Tolerated	benign	Scheie Syndrome
4	996248	rs6836258	C	G	0.16	2.56E-18	*IDUA*	ACG-ACc	Synonymous	.	.	Scheie Syndrome
4	996560	rs115790973	C	G	0.84	4.35E-18	*IDUA*	ACC-ACg	Synonymous	.	.	Scheie Syndrome
4	996690	rs73066479	A	G	0.16	2.97E-17	*IDUA*	GTC-aTC	Nonsynonymous	Tolerated	benign	Scheie Syndrome
4	996888	rs115929690	T	C	0.16	2.59E-18	*IDUA*	CGC-CGt	Synonymous	.	.	Scheie Syndrome
4	1019011	rs4647932	C	T	0.93	2.52E-11	*FGFRL1*	CCA-CtA	Nonsynonymous	Damaging*	benign	
4	88732692	rs1054627	A	G	0.29	7.98E-14	*IBSP*	GGA-GaA	Nonsynonymous	Tolerated	benign	
4	88732918	rs1054629	A	T	0.71	1.26E-13	*IBSP*	GAA-GAt	Nonsynonymous	Tolerated	benign	
6	151859314	rs4870034	A	G	0.32	7.04E-15	*C6orf97*	GAA-GAg	Synonymous	.	.	
6	151894340	rs12205837	T	C	0.11	6.63E-13	*C6orf97*	GCT-GtT	Nonsynonymous	Tolerated	benign	
6	151936677	rs6929137	A	G	0.33	1.28E-15	*C6orf97*	GTC-aTC	Nonsynonymous	Tolerated	benign	
6	151939181	rs3734804	A	G	0.52	2.88E-15	*C6orf97*	GTC-aTC	Nonsynonymous	Tolerated	benign	
7	120876835	rs35793694	A	G	0.93	2.26E-13	*C7orf58*	GAA-GgA	Nonsynonymous	Tolerated	benign	
7	120969769	rs2908004	A	G	0.46	1.43E-89	*WNT16*	GGG-aGG	Nonsynonymous	Tolerated	benign	
7	120979089	rs2707466	T	C	0.46	4.79E-88	*WNT16*	ACA-AtA	Nonsynonymous	Tolerated	benign	
7	150915948	rs7782699	T	C	0.12	5.93E-11	*ABCF2*	GCG-GCa	Synonymous	.	.	
8	119964052	rs2073618	C	G	0.48	1.54E-19	*TNFRSF11B*	AAC-AAg	Nonsynonymous	Tolerated	benign	Paget Disease, Juvenile
10	**124089036**	**rs2421013**	**G**	**A**	**0.48**	**3.33E-09**	***BTBD16***	**CGG-CaG**	**Nonsynonymous**	**Tolerated**	**benign**	
11	**198062**	**rs11605246**	**C**	**G**	**0.78**	**5.37E-14**	***ODF3***	**CCT-CgT**	**Nonsynonymous**	**Tolerated**	**benign**	
11	**280464**	**rs77447196**	**C**	**G**	**0.79**	**5.39E-11**	***NLRP6***	**CCG-gCG**	**Nonsynonymous**	**Tolerated**	**benign**	
11	46339011	rs35652107	A	G	0.08	1.15E-08	*CREB3L1*	GCA-aCA	Nonsynonymous	Tolerated	benign	
11	46387868	rs1317826	A	G	0.68	2.07E-09	*DGKZ*	CAG-CgG	Nonsynonymous	Tolerated	benign	
11	46406767	rs2067482	A	G	0.16	2.10E-12	*CHRM4*	ACC-ACt	Synonymous	.	.	
11	46886077	rs117936904	A	T	0.98	9.97E-10	*LRP4*	CTT-CaT	Nonsynonymous	Damaging*	prob. damaging	
11	46893108	rs2306029	T	C	0.48	6.99E-13	*LRP4*	AGC-gGC	Nonsynonymous	Tolerated	benign	
11	46898771	rs6485702	T	C	0.37	1.19E-13	*LRP4*	ATT-gTT	Nonsynonymous	Tolerated	benign	Cenani-Lenz Syndactyly Syndrome
11	46916179	rs72897663	T	G	0.96	3.44E-08	*LRP4*	AAC-cAC	Nonsynonymous	Tolerated	benign	
11	68174189	rs4988321	A	G	0.04	7.64E-30	*LRP5*	GTG-aTG	Nonsynonymous	Damaging	prob. damaging	Van Buchem Disease, Type 2; Osteopetrosis, Autosomal Dominant 1; Exudative Vitreoretinopathy 4; Osteoporosis-Pseudoglioma Syndrome; Osteoporosis; Hyperostosis Corticalis Generalisata, Benign Form Of Worth, With Torus

CHR	BP	rsID	A1	A2	Freq1	P-value	Gene Name	Codons	SNP Type	SIFT	Polyphen2	OMIM Disease
11	68177510	rs2306862	T	C	0.16	1.34E-33	*LRP5*	AAC-AAt	Synonymous	.	.	Van Buchem Disease, Type 2; Osteopetrosis, Autosomal Dominant 1; Exudative Vitreoretinopathy 4; Osteoporosis-Pseudoglioma Syndrome; Osteoporosis; Hyperostosis Corticalis Generalisata, Benign Form Of Worth, With Torus
11	68192690	rs556442	A	G	0.65	6.53E-25	*LRP5*	GTG-GTa	Synonymous	.	.	Van Buchem Disease, Type 2; Osteopetrosis, Autosomal Dominant 1; Exudative Vitreoretinopathy 4; Osteoporosis-Pseudoglioma Syndrome; Osteoporosis; Hyperostosis Corticalis Generalisata, Benign Form Of Worth, With Torus
11	68201295	rs3736228	T	C	0.15	5.03E-34	*LRP5*	GCG-GtG	Nonsynonymous	Not scored	benign	Van Buchem Disease, Type 2; Osteopetrosis, Autosomal Dominant 1; Exudative Vitreoretinopathy 4; Osteoporosis-Pseudoglioma Syndrome; Osteoporosis; Hyperostosis Corticalis Generalisata, Benign Form Of Worth, With Torus
12	49168798	rs3730071	A	C	0.03	6.95E-10	*ADCY6*	GCC-tCC	Nonsynonymous	Tolerated	benign	
12	53662624	rs6580942	C	A	0.30	2.56E-16	*ESPL1*	GCC-GaC	Nonsynonymous	Tolerated	benign	
12	53670545	rs1318648	A	C	0.63	3.32E-12	*ESPL1*	AGC-AGa	Nonsynonymous	Damaging	prob. damaging	
12	53682326	rs1110720	A	G	0.63	6.26E-12	*ESPL1*	GGG-GGa	Synonymous	.	.	
12	53682457	rs56358776	A	G	0.34	2.95E-13	*ESPL1*	CGG-CaG	Nonsynonymous	Tolerated	benign	
13	43148546	rs138818878	C	G	0.97	5.47E-14	*TNFSF11*	CCT-CgT	Nonsynonymous	Damaging*	prob. damaging	Osteopetrosis, Autosomal Recessive 2
15	**67528374**	**rs7173826**	**T**	**G**	**0.67**	**7.49E-09**	***AAGAB***	**ATC-cTC**	**Nonsynonymous**	**Tolerated**	**benign**	
16	396264	rs1805105	A	G	0.34	5.40E-10	*AXIN1*	GAT-GAc	Synonymous	.	.	Caudal Duplication Anomaly; Hepatocellular Carcinoma
17	**17698254**	**rs8067439**	**G**	**A**	**0.39**	**4.69E-08**	***RAI1***	**CCG-CCa**	**Synonymous**	**.**	**.**	
17	**17997209**	**rs2230316**	**G**	**A**	**0.44**	**3.23E-08**	***DRG2***	**TCG-TCa**	**Synonymous**	**.**	**.**	
17	42254417	rs7212854	A	G	0.71	3.53E-09	*C17orf65*	CGT-CGc	Synonymous	.	.	
17	42287519	rs2071167	T	C	0.27	5.00E-09	*UBTF*	AAG-AAa	Synonymous	.	.	

Table S10. Genome-wide Significant coding variants. P-values are derived from the overall meta-analysis including all ethnicities. Bold rows correspond to SNPs mapping to novel loci for the first time described in this GWAS analysis. * Low confidence

Locus	Ensembl gene ID	Gene symbol	P value	FDR
1p13.3	**ENSG00000184371**	***CSF1***	**1.91E-03**	**<0.05**
1p31.3	ENSG00000116729	*WLS*	4.84E-03	<0.05
1p36.12	ENSG00000162552	*WNT4*	2.79E-03	<0.05
1p36.23	**ENSG00000142599**	***RERE***	**1.44E-04**	**<=0.01**
2p11.2	**ENSG00000152284**	***TCF7L1***	**7.41E-04**	**<0.05**
2p21	ENSG00000162878	*PKDCC*	3.74E-04	<0.05
2q14.2	ENSG00000163064	*EN1*	2.23E-04	<=0.01
2q33.2	**ENSG00000155760**	***FZD7***	**1.19E-03**	**<0.05**
3q25.31	ENSG00000163659	*TIPARP*	3.41E-06	<=0.01
4p16.3	ENSG00000127418	*FGFRL1*	1.09E-03	<0.05
4q22.1	ENSG00000152595	*MEPE*	5.51E-06	<=0.01
5q14.3	ENSG00000248309	*MEF2C-AS1*	4.44E-03	<0.05
6p21.1	ENSG00000124813	*RUNX2*	2.80E-09	<=0.01
6q22.33	ENSG00000146374	*RSPO3*	9.63E-06	<=0.01
7p12.1	**ENSG00000106070**	***GRB10***	**6.19E-03**	**<0.05**
7p14.1	ENSG00000106483	*SFRP4*	1.39E-05	<=0.01
7p14.3	**ENSG00000240583**	***AQP1***	**1.18E-03**	**<0.05**
7q21.3	ENSG00000105880	*DLX5*	7.75E-05	<=0.01
7q22.1	**ENSG00000197037**	***ZSCAN25***	**5.15E-05**	**<=0.01**
7q31.31	ENSG00000106034	*CPED1*	2.71E-04	<=0.01
8q24.12	ENSG00000164761	*TNFRSF11B*	1.19E-03	<0.05
10q25.2	**ENSG00000138166**	***DUSP5***	**8.22E-03**	**<0.05**
11p11.2	ENSG00000157613	*CREB3L1*	2.66E-04	<=0.01
11p11.2	ENSG00000165917	*RAPSN*	3.09E-03	<0.05
11p11.2	ENSG00000165915	*SLC39A13*	3.50E-03	<0.05
11p14-p13	ENSG00000176697	*BDNF*	1.55E-03	<0.05
11p14-p13	ENSG00000245573	*BDNF-AS1*	2.37E-03	<0.05
11p14-p13	ENSG00000205213	*LGR4*	5.31E-04	<0.05
11p15.1	ENSG00000110693	*SOX6*	2.02E-04	<=0.01
11p15.2	ENSG00000188487	*INSC*	2.24E-03	<0.05
11q13.2	ENSG00000162337	*LRP5*	5.67E-04	<0.05
11q13.3	**ENSG00000110092**	***CCND1***	**4.08E-05**	**<=0.01**
11q24.1	**ENSG00000255248**	**-**	**1.81E-03**	**<0.05**
12p13.33	ENSG00000111186	*WNT5B*	1.45E-03	<0.05
12q13.12	ENSG00000167548	*MLL2*	5.32E-06	<=0.01
12q13.12	ENSG00000125084	*WNT1*	1.65E-03	<0.05
12q13.13	ENSG00000257194	-	5.05E-04	<0.05
12q13.13	ENSG00000185591	*SP1*	4.12E-03	<0.05
12q13.13	ENSG00000170374	*SP7*	1.64E-07	<=0.01
13q13.3	**ENSG00000120693**	***SMAD9***	**4.15E-03**	**<0.05**
15q22.33	**ENSG00000166949**	***SMAD3***	**4.05E-05**	**<=0.01**
16q24.1	ENSG00000176678	*FOXL1*	7.57E-04	<0.05

17p11.2	**ENSG00000108557**	***RAI1***	**3.50E-04**	**<0.05**
17p13.3	ENSG00000070366	*SMG6*	8.74E-03	<0.05
17q21.31	ENSG00000161664	*ASB16*	4.18E-03	<0.05
17q21.31	ENSG00000161649	*CD300LG*	2.73E-03	<0.05
17q21.31	ENSG00000108840	*HDAC5*	7.24E-03	<0.05
17q21.31	ENSG00000005102	*MEOX1*	7.38E-03	<0.05
17q21.31	ENSG00000167941	*SOST*	6.18E-05	<=0.01
17q21.31	ENSG00000108312	*UBTF*	7.66E-03	<0.05
20p12.2	ENSG00000101384	*JAG1*	3.66E-04	<0.05
20q12	**ENSG00000204103**	***MAFB***	**3.85E-04**	**<0.05**
21q22.12	**ENSG00000159216**	***RUNX1***	**2.24E-03**	**<0.05**

Table S11. DEPICT Gene prioritization (FDR<5%). Based on genome-wide significant variants in the overall TB-BMD meta-analysis. Bold rows correspond to genes mapping to novel loci not previously described in this GWAS analysis of bone phenotypes.

Table S12. DEPICT Gene-set enrichment analysis (FDR<5%). Based on genome-wide significant variants in the overall TB-BMD meta-analysis. These 182 gene-sets were further clustered in 25 'metagene-sets' shown in **Figure 4**. **Attached Excel file.**

Position	Lead SNP	*Proxy SNP*	LD	Host gene	Related miRNA	Ancestral A	Derived A	Conservation	Change contex score	Functional class
7q22.1	**rs34670419**	***rs34670419***	1	***ZKSCAN5***	**mir-382-3p**	**G**	**T**	**4**	**0.09**	**Create**
11p15.5	**rs11601356**	***rs6541***	0.86	***PSMD13***	**mir-942-5p**	**A**	**G**	**0**	**0.02**	**Create**
7q36.1	rs73169649	*rs73169654*	0.88	*ABCF2*	mir-140-3p	C	T	1	0.09	Create
2q24.3	rs7586085	*rs13429321*	0.84	*GALNT3*	mir-499-3p	T	A	6	0.2	Disrupte
2p21	rs78572108	*rs1044305*	0.93	*PKDCC*	mir-1470	T	C	9	0.25	Create
5q22.2	**rs818427**	***rs2545167***	1	***REEP5***	**mir-4444**	**C**	**A**	**0**	**0.2**	**Create**
11q13.2	rs11228240	*rs4988291*	0.95	*PPP6R3*	mir-138-3P	G	A	5	0.02	Disrupte
15q22.33	**rs3743347**	***rs10518716***	1	***AAGAB***	**mir-380/mir-424-3p**	**C**	**G**	**2&6**	**0.22/0.19**	**Disrupte/Create**
17p11.2	**rs8070128**	***rs1052299***	1	***TOM1L2***	**miR-133a, 138-3p**	**T**	**C**	**1**	**0.3**	**Create**

Table S13. Putative effect of the TB-BMD top associated variants in miRNA-binding sites. The effect of the derived allele in the creation/disruption of a binding site (functional class) of a specific miRNA (miRNA) is described (using PolymiRTS database v3.0). Shown are 9 SNPs, including the lead SNP rs34670419 *in ZKSCAN5* and proxy SNPs of other 8 lead SNPs, located in predicted miRNA binding sites. Loci not previously reported are in bold font. Proxy SNP, SNP with r2 > 0.8, limit distance 500 kb, population panel CEU and in 1000 Genome project; Conservation, Occurrence of the miRNA site in other vertebrate genomes in addition to the query genome; LD, linkage disequilibrium; Related miRNA, miRNA that the SNP is predicted to create/disrupt its binding site; Context score predicts the binding of a miRNA to the gene 3'UTR by summing over contributions made by individual sites within the 3'UTR that have perfect sequence complementarity to the miRNA seed region. Change contex score, A more negative value of the context score difference indicates an increased likelihood that the miRNA targeting is disrupted or newly created by the SNP in the target sites.

Table S14. Skeletal phenotype data from the International Mouse Phenotyping Consortium and Mouse Genome Informatics databases and expression data from murine osteoblasts, osteocytes and osteoclasts. Data was collected for the 55 genes prioritized by DEPICT. Detailed bone phenotyping from the Origins of Bone and Cartilage Disease (OCBD) is presented in Table S15. **Attached Excel file**

Table S15. Detailed bone phenotyping of knockout models from the Origins of Bone and Cartilage Disease (OCBD) initiative. Knockout lines with a skeletal parameter greater than 2 standard deviations from the reference mean are highlighted in orange. The standard deviation from the reference mean for each parameter is shown with those greater than 2 highlighted (Black above the mean: Red below the mean). **Attached Excel file.**

Supplemental references

1 Victora, C.G. and Barros, F.C. (2006) Cohort profile: the 1982 Pelotas (Brazil) birth cohort study. *Int J Epidemiol*, **35**, 237-242.

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