

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

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91

92 **Abstract**

93 Bone mineral density (BMD) assessed by DXA is used to evaluate bone health. In children, total
94 body (TB) measurements are commonly used; in older individuals, BMD at the lumbar spine (LS)
95 and femoral neck (FN) is used to diagnose osteoporosis. To date, genetic variants in more than
96 60 loci have been identified as associated with BMD. To investigate the genetic determinants of
97 TB-BMD variation along the life course and test for age-specific effects, we performed a meta-
98 analysis of 30 genome-wide association studies (GWAS) of TB-BMD including 66,628 individuals
99 overall and divided across five age-strata each spanning 15 years. We identified variants
100 associated with TB-BMD at 80 loci, of which 36 have not been previously identified; overall they
101 explain approximately 10% of the TB-BMD variance when combining all age groups and
102 influence the risk of fracture. Pathway and enrichment analysis of the association signals
103 showed clustering within gene-sets implicated in the regulation of cell growth and SMAD
104 proteins; overexpressed in the musculoskeletal system; and enrichment in enhancer and
105 promoter regions. These findings reveal TB-BMD as a relevant trait for genetic studies of
106 osteoporosis, enabling the identification of variants and pathways influencing different bone
107 compartments. Only variants in *ESR1* and close proximity to *RANKL* showed a clear effect
108 dependency on age. This most likely indicate that the majority of genetic variants identified
109 influence BMD early in life and their effect can be captured throughout the life course.

110 **Introduction**

111 Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration
112 of bone tissue leading to increased risk of fracture¹. It is diagnosed through the measurement

113 of bone mineral density (BMD) utilizing dual-energy X-ray absorptiometry (DXA), which is the
114 single best predictor of fracture¹.

115 Bone is a dynamic tissue constantly undergoing resorption and formation. Bone mass increases
116 steadily during childhood and markedly during adolescent growth². Peak bone mass is attained
117 at approximately the third decade of life. Thereafter, until about 50 years of age, BMD remains
118 fairly stable, by virtue of the coupling between bone formation and resorption (e.g., bone
119 remodeling). Subsequently, bone resorption exceeds the rate of bone formation, resulting in a
120 decrease in BMD, particularly in women after the onset of menopause³.

121 The International Society for Clinical Densitometry recommends performing DXA
122 measurements at the lumbar spine, femoral neck and total hip to diagnose osteoporosis in
123 postmenopausal women and men who are 50 years or older⁴. Consequently, studies of BMD
124 determinants are frequently based on measurements at these skeletal sites. By contrast, for the
125 assessment of bone health in children and adolescents, total body (excluding head) and lumbar
126 spine are the preferred sites to minimize measurement artifacts resulting from changing areas
127 in growing bones⁴. Nevertheless, in elderly individuals degenerative changes in the spine can
128 give elevated BMD readings⁵. Moreover, total body DXA scans have been obtained in a number
129 of adult research cohorts, primarily to assess body composition. Therefore, the total body BMD
130 (TB-BMD) measurement is the most appropriate method for an unbiased assessment of BMD
131 variation in the same skeletal site from childhood to old age.

132 To date, nearly 80 independent genetic variants have been shown to be robustly associated
133 with variability in bone parameters⁶⁻¹⁸. 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 cohorts⁸. 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 loading⁸. 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 measurements⁹⁻¹⁰.

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.

148

149 **Methods**

150 ***TB-BMD GWAS meta-analyses***

151 **Study Populations**

152 Subjects

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

161 BMD measurement

162 Total body BMD (g/cm²) was measured by DXA following standard manufacturer protocols. As
163 recommended by the International Society for Clinical Densitometry total body less head (TBLH)
164 was the measurement used in pediatric cohorts⁴ (e.g., 0-15 years). Detailed information on the
165 assessments performed by each study can be found in **Table S1**.

166 GWAS data and imputation

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

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

172 **Association Analysis**

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

182 **Quality control of TB-BMD association summary statistics**

183 A centralized quality-control procedure implemented in EasyQC¹⁹ was applied to all study-
184 specific files of association results to identify cohort-specific issues. We excluded variants if
185 they had missing information (e.g., missing association P-value, beta estimate, alleles, allele
186 frequency), or nonsensical values (e.g., absolute beta estimates or standard errors >10,
187 association P-values >1 or <0; or imputation quality < 0; infinite beta estimates or standard
188 errors); minor allele frequency (MAF) less than 0.5%; imputation quality scores <0.4 (Impute2)
189 or <0.3 (Minimac). Moreover, variants were flagged if they had large allele frequency

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

192 GWAS meta-analyses

193 In the first instance, no exclusion criteria based on ancestry were applied for the meta-analysis
194 (N=66,628). In addition, meta-analyses were carried out across age strata (minimum sample size
195 per bin N=200 for each study) comprising: 1) 0-15 years (N=11,807), 15-30 years (N=4,180), 30-
196 45 years (N=10,062), 45-60 years (N=18,805), and 60 or more years (N=22,504). Further,
197 summary data from cohorts of European ancestry only were meta-analyzed and used in
198 subsequent analyses. We discarded variants present in less than three studies. Approximately
199 23,700,000 markers (including SNPs and INDELS) were assessed for association. We applied the
200 conventional genome-wide significance level (GWS, $P < 5 \times 10^{-8}$) for SNP discovery.

201 Assessment of Age-dependent effects

202 We selected SNPs which were suggestively (12,567 SNPs, $P < 5 \times 10^{-6}$) associated with BMD in the
203 overall meta-analysis, present in at least 2 studies per age-bin and with MAF differences across
204 these meta-analyses lower than 0.5. We clumped this dataset with an $r^2 \geq 0.8$, using as
205 reference the most strongly associated SNPs with BMD and, pruning remaining SNPs within 0.7
206 Mb of each other. Age-dependent effects were assessed using a meta-regression approach for
207 1,464 SNPs obtained after this selection procedure. We ran a linear regression of the SNP effect
208 estimates onto an intercept and the median age of each subgroup (e.g., each study stratified in
209 age-bins). As proposed previously²⁰, standard errors of the effect estimates of each subgroup
210 were multiplied by the square root of the genomic inflation factor when it was greater than 1.

211 We performed the meta-regression using the Metafor package²¹, and any statistical evidence of
212 linear association was corrected for multiple testing (Bonferroni correction; $0.05/1,464 = 3.4 \times 10^{-5}$).
213 The difference between beta-estimates in children vs. elderly meta-analyses (Pdiff) was
214 tested using Easy-strata²².

215 Approximate conditional meta-analyses

216 Conditional analyses were undertaken based on the meta-analysis of the studies of European
217 ancestry only (N=56,284). Only variants in the loci that reached GWS in this meta-analysis were
218 assessed. The Rotterdam Study I (n=6,291) was used as reference for precise calculation of the
219 linkage disequilibrium (LD) between the analyzed markers. We used an iterative strategy as
220 implemented in GCTA²³ to determine: 1) independence of association signals within loci
221 discovered in our study, by means of stepwise model selection procedure per chromosome (--
222 massoc-slct routine); and 2) the novelty of the association signals discovered by our meta-
223 analysis with regard to variants reported in previous well-powered GWAS of different bone
224 traits (**Table S3**). To this end, we performed the association analysis conditional on 78 variants
225 present in our data and associated with different bone-traits (--massoc-cond routine). These 78
226 SNPs were selected from different GWAS publications^{6-10;12-14}, assuring their independence to
227 avoid collinearity issues.

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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 model²⁴. 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 traits²⁵. This can be performed in the LDhub
243 pipeline, a web utility which gathers data from many different GWAS meta-analysis²⁶. 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.

250

251

252 **Mendelian randomization analysis**

253 We undertook a two-sample Mendelian randomization approach²⁷ to estimate the causal effect
254 of TB-BMD on any-type of fracture in the Europeans samples. In short, we constructed a score
255 based on the independent genetic variants from the TB-BMD meta-analysis (European set and
256 excluding secondary signals), whenever the selected variant was not present in the fracture
257 meta-analysis, the second variant with the lowest p-value in the locus ($P < 5 \times 10^{-8}$) and $r^2 > 0.8$
258 was used as proxy. Thereafter, estimates derived from the TB-BMD summary statistics were
259 pooled using methods similar to inverse-variance weighted fixed meta-analysis using the meta
260 R-package (**Web Resources**).

261 ***Search for biological and functional knowledge of the identified association regions***

262 For all those SNPs outside a 500Kb window from previously known bone associated SNPs we
263 did a literature search in PubMed and Web of Science to evaluate if nearby genes (within
264 500Kb) were known to play a role in bone metabolism. Also, we determined if the annotated
265 genes underlie any human Mendelian disorder with a skeletal manifestation, had knockout
266 mouse models with a skeletal phenotype or were annotated to pathways critical to bone
267 metabolism. Genomic annotation for all SNPs was made based on UCSC hg19.

268 **DEPICT analyses**

269 We used DEPICT²⁸, a recently developed tool to prioritize genes at the associated regions,
270 define possible pathways by enrichment testing, and identify tissue and cell types in which
271 genes from loci associated with TB-BMD. The methodology first selects all lead SNPs below a
272 certain threshold with respect to a target P-value. We tested both the complete set of GWS

273 SNPs and the subset of those mapping only to loci not previously reported. Enriched gene-set
274 were group based on the degree of gene overlap into 'meta gene-sets' as proposed earlier²⁹,
275 and their correlation visualized using Cytoscape 3.4 (**Web Resources**).

276 **Functional annotation to microRNA binding sites**

277 We used the PolymiRTS²⁹, miRdSNP³⁰, and microSNIPer³¹ databases to obtain a list of variants
278 located in predicted microRNA binding sites on the 3'UTRs of genes, as described in detail
279 elsewhere³². In summary, index SNPs (most associated variant) of the GWS loci were submitted
280 to SNAP (**Web Resources**) to retrieve their high LD proxy SNPs (with $r^2 > 0.8$, limit distance 500
281 kb, and CEU panel) in the 1000 genomes project. The resulting list of SNPs was annotated to the
282 list of microRNA binding site variants obtained from the above mentioned publicly available
283 databases.

284 **Functional enrichment analysis of trait-associated variants**

285 GWAS Analysis of Regulatory or Functional Information Enrichment with LD correction
286 (GARFIELD)³³ was used to characterize the putative functional contribution of TB-BMD
287 associated variants mapping to non-coding regions. GARFIELD employs a non-parametric
288 analysis to calculate fold enrichment values for regulatory marks, at given significance
289 thresholds and then tests them for significance via permutation testing while accounting for LD,
290 MAF and local gene density³³. We used data regarding DNase I hypersensitive sites,
291 transcription factor binding sites, histone modifications and chromatin states (ENCODE and
292 Roadmap Epigenomics) from 424 cell types and tissues to capture and characterize possible
293 cell-type-specific patterns of enrichment, as provided in the GARFIELD software (**Web**

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

297 **Knockout animal models and gene expression in bone cells**

298 Animal models survey

299 We surveyed databases from *The International Mouse Phenotyping Consortium*³⁴ together
300 with *The International Knockout Mouse Consortium*³⁵ to identify knockout models of
301 candidate genes resulting in skeletal phenotypes. Furthermore we mined data from *The*
302 *Origins of Bone and Cartilage Disease* (OBCD) project³⁶, specialized in murine skeletal
303 phenotypes including Digital X-ray microradiography on femurs and tail vertebrae, Micro-CT
304 analysis, femur three-point bend test load–displacement curves and tail vertebrae
305 compression testing from knockout mice and wild-type controls at 16 weeks of age.

306 Gene expression in murine bone cells

307 Gene expression profiles of candidate genes were examined in primary mouse osteoblasts
308 undergoing differentiation and bone marrow derived osteoclasts. To study murine
309 osteoblasts, pre-osteoblast-like cells were obtained from neonatal calvaria collected from
310 C57BL/6J. Next Generation RNA sequencing using an Illumina HiSeq 2000 was used to
311 evaluate the transcriptome every two days from day 2 to 18 days post osteoblast
312 differentiation⁷. Expression of genes in murine osteoclasts was determined using publicly
313 available data obtained using Next-Gen RNA-sequencing applied to bone marrow derived
314 osteoclasts obtained from 6-8 week old C57BL/6 mice³⁷.

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 differentiation³⁸. 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 (5×10^5 cells per well) as
321 previously described³⁹ 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 previously³⁸. 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, $P \leq 5 \times 10^{-8}$) 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 ($0.2 > \text{MAF} < 0.5$, $\lambda = 1.19$) 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**

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

340 In addition, a meta-analysis comprising 56,284 individuals of European ancestry (~84% of the
341 study population) identified variants in two additional GWS loci (**Figures S1-S2, Table S5**).
342 Association signals mapping to these loci were close to the GWS threshold in the overall meta-
343 analysis ($P=1 \times 10^{-7}$) and showed no evidence of heterogeneity ($P_{\text{het}} > 0.1$). One of them, in
344 12q24.21 (*MED13L*), has not been previously associated with bone parameters (**Table 1, Figure**
345 **S3**), while the other in 21q22.13 (*CLDN14*), is not fully independent from the previously
346 reported hip-BMD association signal¹³ (**Table S5**).

347 Of the 78 identified loci, variants in 35 (45%) were not located within 500 kb of known
348 association signals nor in regions of extended LD with them (**Table 1, Figure S4**). Index SNPs at
349 these 35 loci were, in general, common non-coding variants. Twenty-two of these, are located
350 in close proximity to genes likely to influence bone metabolism as shown by previous functional
351 studies (**Table 1, Figure S3**), including *CSF1* ([MIM 120420] important for osteoclast
352 differentiation⁴⁰) and *SMAD3* ([MIM 603109] a critical component of the TGF-beta signaling
353 pathway⁴¹). Across these 35 signals, 31 of the index SNPs were nominally associated ($P < 0.05$)
354 with either lumbar spine or femoral neck BMD in the same direction as in the previously
355 published GEFOS GWAS meta-analysis⁷ (**Table 1**). This comparison was not possible for the
356 rs113964474 variant, because it was not available in the GEFOS study. Moreover, we found
357 directionally-concordant effect estimates ($P < 0.05$) for 73 of the 78 index SNPs of known bone

358 association signals (**Table S3**). The markers which failed to replicate in our study were either
359 previously associated with lumbar spine BMD but not femoral neck BMD (rs3905706 [*MPP7*,
360 10p12.1] and rs1878526 [*INSIG2*, 2q14.2]), associated specifically with the hip trochanter and
361 intertrochanteric subregions (rs1949542 [*RP11-384F7.1*, 3q13.32]), or associated with BMD
362 only in women (rs7017914 [*XKR9*, 8q13.3]) or only in children (rs754388 [*RIN3*, 14q32.12]).

363 Age-dependent effects

364 Meta-analyses across age strata resulted in the identification of variants mapping to 2
365 additional loci that were not detected in the overall meta-analysis (**Figure S5; Table S6**). In
366 children (age group 0-15 years), the previously known 14q32.12 locus⁸, harboring *RIN3*
367 (rs72699866, $P=1 \times 10^{-8}$); and in the middle-aged (age group 45-60 years), a signal in the 19q12
368 locus mapping in the vicinity of *TSHZ3* (rs6510186, $P=3.1 \times 10^{-8}$) were identified. The rs72699866
369 variant leading the *RIN3* signal in the youngest age stratum showed no evidence of association
370 ($P=0.16$) and high heterogeneity ($P_{\text{het}}=6.6 \times 10^{-5}$) in the overall meta-analysis. In fact, the effect of
371 rs72699866 decreased significantly with age ($P_{\text{trend}}=1.69 \times 10^{-9}$) (**Figure S6**) and showed a
372 significant difference between the two extreme groups, i.e. children vs elderly ($\beta_{0-15}=0.099$
373 [0.066, 0.134]; $\beta_{>60}=-0.035$ [-0.060, -0.010]; $P_{\text{diff}}=4.32 \times 10^{-10}$). In contrast, the rs6510186 variant
374 [19q12] showed nominal evidence of association and heterogeneity in the overall meta-analysis
375 ($P=0.02$; $P_{\text{het}}=0.03$). Nevertheless, no clear pattern of age-dependency was identified ($P=0.2$) for
376 this SNP (**Figure S6**).

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

379 27 (64%) of the loci showed stronger effects in the older age groups. Of these, variants in the
380 6q25.1 (*ESR1*) and 13q14.11 (*RANKL*) loci remained significant after multiple-testing correction
381 ($P < 3.4 \times 10^{-5}$) (**Figure 2**); while variants in 6p21.1 (*RUNX2*, rs148460475), 15q21.2 (*CYP19A1*,
382 rs2414098), 17q21.31 (*MEOX1*, rs74835612) and 11p15.1 (*SOX6*, rs11822790) were only
383 suggestive at $P < 1 \times 10^{-3}$.

384 **Conditional association analyses**

385 The step-wise conditional approach included studies comprising only individuals of European
386 ancestry, as the method used relies on appropriate representability of the LD reference. Of the
387 76 GWS loci identified in the overall analysis, variants in 57 (19 previously unreported) loci were
388 also GWS in the European-only analysis (**Figure S2**), likely a consequence of the lower power in
389 this subgroup. We identified 81 SNPs independently associated with TB-BMD mapping to 58
390 different loci (one European-specific), 18 of which depicted multiple distinct signals attaining
391 GWS (**Table S8**). These independent variants together explained 10.2% of TB-BMD variance.
392 This proportion is slightly higher than the 7.4% TB-BMD variance explained by the 78 known
393 variants associated with bone traits. Moreover, we identified independent signals in 13 of the
394 78 known bone loci after conditional analyses. (**Figure S2; Table S8**).

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

396 SNP-heritability of TB-BMD in the European samples was estimated to be 0.259 (SE 0.017). TB-
397 BMD was highly genetically correlated with BMD measured at other skeletal sites ($\rho > 0.9$).
398 Among the non-BMD traits, all-type of fracture showed the highest correlation [$\rho = -0.61$
399 ($P = 1.6 \times 10^{-27}$)]. The MR approach indicated a strong causal relation where per 1 standard

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

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

406 Loci not previously reported and their potential role in bone metabolism are summarized in
407 **Table 1**. Several loci harbor genes implicated directly in bone metabolism (*SLC8A1* [MIM
408 182305], *PLCL1* [MIM 600597], *ADAMTS5* [MIM 605007]), affecting osteoblast or osteoclast
409 differentiation and activity (*CSF1* [MIM 120420], *DUSP5* [MIM 603069], *SMAD3* [MIM 603109],
410 *SMAD9* [MIM 603295], *CD44* [MIM 107269]), participating in Wnt signaling (*FZD7* [MIM
411 603410], *TCF7L1* [MIM 604652]), or regulating processes such as manganese or calcium
412 absorption (*GCKR* [MIM 600842], *DGKD* [MIM 601826], *SLC30A10* [MIM 611146]) among others
413 ⁴⁰⁻⁶¹; while genes in at least 14 loci exert a potential novel role in bone biology. Rodent
414 knockout models of several genes in the implicated loci, show an altered skeletal phenotype
415 (e.g., osteopetrosis [*Csf1*⁴⁰], increased bone resorption [*Aqp1*⁵⁰, *Cyp19a1*⁵⁷, *Cd44*⁵³], impaired
416 skeletogenesis [*Apc*⁴⁹, *Runx1*⁶⁰, *Smad3*⁴¹], deformities in the axial skeleton [*Btg1*⁶², *Atpaf2*⁶³]).
417 Whereas an effect on bone can be inferred for genes in other associated loci, for example,
418 *CYP19A1* [MIM 107910] in 15q21.2 is an estrogen synthesis gene, being estrogen a key
419 compound for bone maturation and maintenance, and *ZKSCAN5* [MIM 611272] in 7q22.1 is
420 associated with circulating dehydroepiandrosterone sulphate (DHEAS) levels⁵¹. DHEAS levels
421 are positively correlated with BMD in adults and post-menopausal women⁶⁴. Across these loci,

422 not previously reported as associated with BMD variation, we identified six exonic variants
423 associated with TB-BMD, three of which were nonsynonymous variants all cataloged as benign
424 both by SIFT and polyphen2. We also identified 53 GWS coding variants in known loci, of which
425 33 are non-synonymous (**Table S10**). Only a low-frequency variant in *LRP5* [MIM 603506],
426 rs4988321/A (11:68174189, MAF=0.04), has a clinical annotation, constituting a homozygous G-
427 to-A transition variant identified in a person with osteoporosis-pseudoglioma syndrome (OPPG
428 [MIM 259770])⁶⁵.

429 **DEPICT analyses**

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

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

441 **Functional annotation to microRNA binding sites**

442 We then assessed if the index SNPs of the 80 GWS loci detected in the main and subsequent
443 GWAS (or their proxies in strong LD; $r^2 > 0.8$) were located in predicted microRNA binding sites
444 within the genes' 3'UTRs and thus, were expected to disrupt the regulation of gene expression
445 (**Table S13**). The index SNP within the 3'UTR of *ZKSCAN5* (mapping to a locus not previously
446 identified), rs34670419 (MAF=0.04), is predicted to create a binding site for miR-382-3p, a
447 microRNA which is expressed in osteocytes and has been recently shown to be involved in
448 osteogenic differentiation⁶⁶. In addition, eight proxy SNPs (mapping to *PSMD13*, *ABCF2*,
449 *GALNT3*, *PKDCC*, *REEP5*, *PPP6R3*, *AAGAB* and *TOM1L2*) are predicted to influence the binding of
450 microRNAs to transcripts of their host gene.

451 **Functional enrichment analysis of trait-associated variants**

452 As typically found in GWAS, the great majority of identified associations emerged from non-
453 coding common variants and hold no direct annotation to molecular mechanisms.
454 To assess if there is relative enrichment of regulatory genomic marks underlying the associated
455 variants in a cell-specific context, we used GARFIELD³³. We found relative ubiquitous
456 enrichment for TB-BMD variants (Empirical $P < 2.4 \times 10^{-4}$) in DNase I hypersensitive sites across
457 the different cell types (**Figure S9**). Further, we found higher levels of fold-enrichment for
458 enhancers (median 3.6, range [2.7, 4.4]) and promoters (median 3.2, range [2.9, 3.5]) than for
459 transcribed regions (median 1.8, range [1.5, 2.2]).

460 **Gene expression in bone cells and knockout animal models**

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

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

467 Knockout models were widely available in at least one of the different databases assessed.
468 Nevertheless in-depth bone phenotyping performed under the OBCD project was only available
469 for four knockout models (**Table S15**). Two of these, *DUSP5* and *CD300LG* showed no significant
470 bone phenotype. The *TCF7L1* knockout model only showed lower cortical diameter in the femur
471 without other clear bone phenotype. Nevertheless, *TCF7L1* was shown to be expressed during
472 osteoblastogenesis. Conversely, homozygous knockout for *CREB3L1* showed a clear bone
473 phenotype consisting of low BMC both at the vertebrae and femur together with a strong
474 trabecular and cortical phenotype affecting bone strength (**Figure S10**). *CREB3L1* maps to
475 11p11.2, a previously identified BMD locus⁶ harboring *ARHGAP1* and *LRP4* as candidates to
476 underlie the GWAS signal in a region of extended LD.

477 **Discussion**

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

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

489 Traditionally, DXA-BMD measurements performed at sites of high fracture risk (i.e., femoral
490 neck, lumbar spine and forearm) have been used in genetic epidemiological investigations of
491 bone health in adults. Instead, we have used BMD measurements derived from total body
492 scans. Not only do we show a high overlap of association signals with previous GWAS of
493 different bone traits, including DXA, pQCT and ultrasound measurements, but we have also
494 identified unreported loci. Five known associations failed to replicate in our studies, even
495 though we cannot discard these associations constitute false-positives, these results might also
496 indicate that variants whose effect is highly specific to skeletal sites, skeletal properties, sex or
497 age groups cannot be detected in our TB-BMD meta-analysis. It is plausible that more variants
498 of this type exist and will be discovered as site-specific BMD meta-analyses are performed in
499 increasingly powered settings. Furthermore, the genetic correlation of TB-BMD with BMD
500 measured at other sites was close to one. Whilst, we found that a decrease of one standard
501 deviation in the genetically determined TB-BMD resulted in at least 50% higher odds of
502 suffering a fracture. Significant genetic correlations with other traits (i.e., BMI, IGF1 and
503 ulcerative colitis) reflect the systemic context of skeletal biology and merit further study by
504 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 formation⁶⁷. This locus characterized by
510 extended LD, also harbors *LRP4* [MIM 604270] whose knockout model presents with increased
511 trabecular and cortical bone mass⁶⁸. 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 BMD⁸ and
519 Paget's disease (PDB [602080]) GWAS⁶⁹. 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

527 of Parents and Children (ALSPAC) and the Generation R Study) comprise predominantly pre-
528 pubertal children. As levels of estradiol before puberty are low⁷⁰, a negligible effect of *ESR1*
529 variants on BMD is expected. Likewise, in mouse models the expression of *RANKL* [MIM
530 602642] in bone is markedly increased with advancing age from young to adult and related to
531 bone loss⁷¹. Accordingly, variants influencing *RANKL* expression show a larger effect later in life.
532 In general, a substantial heterogeneity of the genetic effects in the overall meta-analysis was
533 explained by age, nevertheless, the inclusion of larger sample sizes (avoiding age exclusion
534 criteria and incrementing statistical power) leveled off the loss of power due to the
535 heterogeneity of the genetic effects.

536 In brief, variants with evidence of age-specific effects were exceptional in our study. These
537 results might reflect a lack of statistical power as only SNPs showing suggestive evidence
538 ($P < 5 \times 10^{-6}$) of association with TB-BMD in the overall meta-analysis were tested for age-specific
539 effects. This selection criteria aimed to include SNPs whose heterogeneity might have
540 hampered their statistical significance in the overall meta-analysis, and at the same time
541 maximize the power to discover variants with real age-dependent effects. Alternatively, these
542 results indicate that most of the genetic variants identified so far, by us and others, influence
543 BMD from early ages onwards, and their effect persist throughout the life course. However,
544 variants in 27 of the 42 loci (64%) showing nominal evidence for age dependent effects had
545 larger effects in the older groups. Nonetheless, this requires careful interpretation given the
546 uneven sample sizes between the age groups and the criteria to select markers for the meta-
547 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 adolescents 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 BMI⁷².
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

590 GWAS data. Part of this work was conducted using the UK Biobank resource.

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 ($P < 5 \times 10^{-8}$) and suggestive threshold ($P < 1 \times 10^{-6}$), 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)-BMD¹⁰. 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	<i>RERE</i>	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	<i>CSF1</i>	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	<i>SLC30A10</i>	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	<i>GCKR</i>	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	<i>SLC8A1</i>	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	<i>ARHGAP25</i>	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	<i>TCF7L1</i>	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	<i>PLCL1</i>	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	<i>FZD7</i>	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	<i>DGKD</i>	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	<i>APC</i>	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	<i>CSNK1G3</i>	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	<i>JAZF1</i>	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	<i>AQP1</i>	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	<i>GRB10</i>	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	<i>ZKSCAN5</i>	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	<i>DUSP5</i>	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	<i>TACC2</i>	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	<i>PSMD13</i>	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	<i>CD44</i>	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	<i>LDLRAD3</i>	Novel Biology
11	69299537	rs4980659	11q13.3	C	G	0.52	0.033	1.16E-08	66537	intergenic	<i>CCND1</i>	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	<i>BLID</i>	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	<i>ATP2B1</i>	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	<i>MED13L</i>	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	<i>SMAD9</i>	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	<i>TMC05A</i>	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	<i>CYP19A1</i>	Estrogen biosynthesis 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	<i>SMAD3</i>	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	<i>TOM1L2</i>	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	<i>CEP112</i>	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	<i>TSHZ3</i>	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	<i>MAFB</i>	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	<i>ADAMTS5</i>	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	<i>RUNX1</i>	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	<i>ETS2</i>	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.