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RESEARCH ARTICLE

Bone mineral density and genetic markers involved in three connected pathways (focal adhesion, actin cytoskeleton regulation and cell cycle): the CUMAGAS-BMD information system

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Abstract

The focal adhesion, the actin cytoskeleton and cell-cycle are connected pathways and their genes are implicated in the pathogenesis of low BMD. Data from 211 studies that investigated the association between BMD and gene variants involved in these pathways were catalogued in a web-based information system and analyzed. In individual studies, significant association was found for 16 variants in lumbar spine, 11 in femoral neck and 5 in hip. In meta-analysis, significant results were shown for the variants COL1A1 rs1800012 (in lumbar spine and femoral neck), COL1A1 rs1107946 (in lumbar spine), TGFB1 rs1982073 (in femoral neck and hip) and TGFB1 rs1800469 (in lumbar spine).

Keywords: Bone mineral density, information system, meta-analysis, genetic polymorphism, focal adhesion, actin cytoskeleton regulation, cell cycle pathways

Introduction

Gene families and gene variants

A number of well-known structures display linkages to cytoskeletal proteins in anchoring functions that include desmosomes, tight junctions, adherens junctions, focal adhesions (FAs), and adhesions to extracellular matrix fibrils. All kinds of cell adhesions are involved in a variety of basic processes including cell motility, cell proliferation, cell differentiation, regulation of gene expression and cell survival in multicellular organisms. By guiding cells into their appropriate locations in cell attachment site of the body and by anchoring them there, adhesive interactions are thought to play a major role in the construction of the body plan of multicellular organisms during development (Ruoslahti & Obrink 1996). FAs, specifically, are the specialized structures where bundles of actin filaments are anchored to transmembrane receptors of the integrin family through a multi-molecular complex of junctional plaque proteins (Zaidel-Bar et al. 2004).

Adhesion is also important in the maintenance of the body plan. Recent studies indicate that these complexes also play important roles in intracellular signal transduction (Mitra et al. 2005). It is now clear that adhesion complexes containing cytoskeletal and plaque proteins are sites of clustering or aggregation of the components of many signaling pathways. According to KEGG (http://www.genome.jp/kegg/), "some of the constituents of FAs participate in the structural link between membrane receptors and the actin cytoskeleton, whereas others are signaling molecules, including different protein kinases

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and phosphatases, their substrates, and various adapter proteins. The signaling events culminate in reorganization of the actin cytoskeleton: a prerequisite for changes in cell shape and motility, and gene expression. Similar morphological alterations and modulation of gene expression are initiated by the binding of growth factors to their respective receptors, emphasizing the considerable crosstalk between adhesion- and growth factor-mediated signaling (Mitra et al. 2005). Mitotic cell cycle (CC) progression is accomplished through a reproducible sequence of events, DNA replication (S phase) and mitosis (M phase) separated temporally by gaps known as G1 and G2 phases. Eukaryotic cells respond to DNA damage by activating signaling pathways that promote CC arrest and DNA repair (Branzei et al. 2008)."

There is a strong connection among these pathways. FAs can regulate actin cytoskeleton, they can also signal for the arrest of CC or mitosis. On the other hand, actin cytoskeleton plays an important role in CC. As osteoporosis is an imbalance between excessive bone resorption and inadequate formation of new bone during remodeling (Gordon et al. 2008), these three pathways seem to play an important role.

There are a number of genes known to take part in these three pathways; ACTN3, AKT1, COL1A1, COL1A2, COMP, CTNNB1, FLNB, GRB2, IBSP, IGF1, IGF1R, ITGA1, ITGA2, ITGA2B, ITGB3, KDR, PDGFRA, PDGFRB, PIK3CA, RAP1A, SPP1, VEGFA VWF for the FA pathway; ACTN3, APC, CD14, CSK, F2, FGF1, FGF13, FGF2, FGF23, FGFR1, FGFR2, FGFR3, INS, ITGA1, ITGA2, ITGA2B, ITGB3, PDGFRA, PDGFRB, PIK3CA, PIP4K2B for the actin cytoskeleton regulation (ACS) pathway and ABL1, CREBBP, SMAD2, SMAD3, SMAD4, TGFB1, TGFB2 for CC.

Although these pathways are implicated in the pathogenesis of low bone mineral density (BMD), only a small number of gene variants involved in these pathways have been studied in association to the BMD. The most studied genes from these are the COL1A1 (variants rs1800012, rs1107946 and rs2412298) and the TGFB1 (variants rs1982073 and rs1800469). Association has been reported between *COL1A1* alleles, BMD, and osteoporotic fracture (Grant et al. 1996; Braga et al. 2000). The TGFB1 polymorphisms are associated with higher serum TGF-β1 levels and significantly lower bone mass (Grainger et al. 1999).

Disease

Osteoporosis is a common skeletal disease characterized by generalised reduction in BMD and micro-architectural deterioration of bone tissue, which leads to impaired skeletal strength and increased susceptibility to fracture (Johnson et al. 2009; Stewart & Ralston 2000). Osteoporosis has become a major health problem because of the alarming rise in its prevalence, especially due to the increasing life expectancy of the population, and its huge health implications and cost effects both financially and socially. There is evidence that genetic factors play an important role in osteoporosis and its associated phenotypes, including BMD (Williams & Spector 2006). Twin and family studies have indicated that between 25 and 85% of the variation in bone mass and other skeletal phenotypes is heritable. Recently, genome-wide linkage studies in human have identified loci on chromosomes 1p36, 1q21, 2p21, 5q33–35, 6p11–12, and 11q12–13 that show definite or probable linkage to BMD (Niu et al. 1999).

In addition, genome-wide association studies (GWAS) identified a considerable number of variants associated with osteoporosis or BMD, these variants include: rs10510628 in RBMS3 ($P = 2.83 \times 10^{-6}$), rs10514345 in GPR98 ($P = 2.15 \times 10^{-6}$), rs2165468 in PITRM1 ($P = 1.07 \times 10^{-6}$), rs10506701 in LOC100128674 ($P = 1.40 \times 10^{-6}$), rs9317284 in LOC647259 ($P = 2.44 \times 10^{-7}$), rs4087296 in MPHOSPH6 ($P = 3.11 \times 10^{-7}$), rs4811196 in CTNNBL1 ($P = 1.13 \times 10^{-6}$), rs6588313 in GPR177 ($P = 4.87 \times 10^{-6}$), rs17463551 in SOX6 ($P = 2.85 \times 10^{-6}$), rs13182402 in ALDH7A1 ($P = 8.53 \times 10^{-9}$), rs17131547 in TGFBR3 ($P = 1.49 \times 10^{-6}$), rs16945612, rs11859065, rs11864477, rs11860781 in ADAMTS18 ($P = 2.13 \times 10^{-8}$, 3.13×10^{-8}, 2.48×10^{-8}, 1.19×10^{-6}), rs4355801 in TNFRSF11B ($P = 7.6 \times 10^{-10}$), rs3736228 in LRP5 ($P = 6.3 \times 10^{-12}$), rs1021188 in RANKL ($P = 2 \times 10^{-14}$), rs851993 in ESR1 ($P = 3.3 \times 10^{-4}$), rs4811196 in CTNNBL1 ($P = 6.9 \times 10^{-4}$), rs3736228 in LRP5 ($P = 4.5 \times 10^{-3}$), rs2273061 in JAG1 ($P = 5.27 \times 10^{-8}$), rs3130340 in MHC ($P = 1.2 \times 10^{-7}$). (Kiel et al. 2007; Styrkarsdottir et al. 2008; Hsu et al. 2010; Guo et al. 2010; Xiong et al. 2009; Richards et al. 2008; Paternoster et al. 2010; Ichikawa et al. 2010; Kung et al. 2010). Furthermore, meta-analyses of GWAS revealed significance of the following variants: rs1430742 and rs2566755 in GPR177 ($P = 2.6 \times 10^{-13}$ and 3.3×10^{-13}, respectively), rs87938 in CTNNB1 ($P = 8.1 \times 10^{-10}$), rs1366594 in MEF2C ($P = 1.3 \times 10^{-13}$), rs1524058 in STARD3NL ($P = 1.1 \times 10^{-9}$), rs4729260 and rs7781370 in FLJ42280 ($P = 1.7 \times 10^{-10}$ and 1.1×10^{-9}, respectively), rs16921914 in DCDC5 ($P = 2.3 \times 10^{-9}$), rs7117858 in SOX6 ($P = 6.4 \times 10^{-10}$), rs10048146 in FOXL1 ($P = 1.7 \times 10^{-8}$), rs9303521 in CRHR1 ($P = 1.4 \times 10^{-8}$), rs11898505 in SPTBN1 ($P = 1.6 \times 10^{-8}$), rs1471403 in MEPE ($P = 1.5 \times 10^{-8}$), rs7932354 in ARHGAP1 ($P = 4.0 \times 10^{-9}$), rs228769 in HDAC5 ($P = 1.7 \times 10^{-8}$), rs7524102 and rs6426749 in ZBTB40 ($P = 7.6 \times 10^{-10}$ and 6.1×10^{-11}, respectively), rs2504063 in ESR1 ($P = 6.1 \times 10^{-11}$), rs2941740 in C6orf97 ($P = 2.0 \times 10^{-9}$), rs2062377 and rs11995824 in TNFRSF11B ($P = 3.5 \times 10^{-16}$ and $P = 1.1 \times 10^{-15}$, respectively), rs599083 in LRP5 ($P = 4.7 \times 10^{-8}$), rs2016266 in SP7 ($P = 1.3 \times 10^{-8}$), rs9533090 in AKAP11 ($P = 5.4 \times 10^{-25}$) and rs884205 in TNFRSF11A ($P = 9.4 \times 10^{-9}$), (Rivadeneira et al. 2009; Richards et al. 2009). Only one of the previous significant variants belongs to the three pathways: rs87938 in CTNNB1.

BMD is the most common assessment for diagnosing osteoporosis and is the most often used quantitative value in the design of genetic studies (Johnson et al. 2009). Although a genetic influence on osteoporotic risk is well established, the number of genes involved, the magnitude of their effects, and the way they may interact with each other and with other environmental risk factors are not well defined. However, the genes involved in

the FA, the ACS and the CC pathways have emerged as logical candidate genes for osteoporosis.

In order to explore the involvement of FA, ACS and CC gene polymorphisms (variants) in osteoporosis, we systematically searched for all available cohort studies that investigated the relationship between variants of the FA, ACS and CC family genes and BMD levels and created the CUMAGAS-BMD (Cumulative Meta-Analysis of Genetic Association Studies—Bone Mineral Density) information system. We catalogued all retrieved articles and estimated the risk effects of all individually investigated variants. Finally, the available data were synthesized using meta-analytic techniques in order to increase the power for detecting significant results and to decrease the uncertainty of the estimated genetic risk effects (Zintzaras & Lau 2008a).

Information system

CUMAGAS-BMD is a web-based database information system for meta-analysis of studies that investigate the relation between gene variants and BMD level located at http://biomath.med.uth.gr. CUMAGAS-BMD performs analysis and meta-analysis for all genetic models (dominant, recessive, additive and co-dominant) and provides information for various covariates (ethnicity, menopausal status, gender). A feature of CUMAGAS-BMD is the ability to carry out cumulative meta-analysis. In cumulative meta-analysis, studies were chronologically ordered by publication year, then, the pooled effect sizes are obtained at the end of each year, i.e. at each information step (Lau et al. 1992). Cumulative meta-analysis provides a frame work for updating a genetic effect from all studies as evidence accumulates. CUMAGAS-BMD has the capacity of continuous updating since authors of published studies have the privilege of entering or correcting their data into the system after a request (send an email to cumagas@med.uth.gr). Although the current study was restricted to variants involved in the FA, ACS and CC pathways, the system may accommodate genes of other pathways. The results produced form CUMAGAS-BMD were reproduced and validated using Meta-Analyst V.3 (Evidence-Based Practice Centers, Tufts Medical School, Boston, MA).

Methods

Selection of studies

All English language articles published before February 2011 were identified by extended computer based searches of the PubMed and HuGE PubLit database. The search criterion in PubMed included combination of the following terms: "variant-name" (refer to variants of the Gene Families and Gene Variants section), osteoporosis, and BMD. In the HuGE PubLit database, the disease term osteoporosis was used as search criterion.

The retrieved articles were then screened and read by two investigators (CD and EZ) in their entirety to assess their appropriateness for inclusion in the meta-analysis. All references cited in the articles were also reviewed to identify additional published articles not indexed in the PubMed and HuGE PubLit databases. Case reports, editorials and review articles were excluded. The search was restricted to articles in humans. Only studies that have used DNA-based analysis methods for genotyping were considered.

Articles providing data for different populations (according to ethnicity, menopausal status and gender) or different body sites (lumbar spine, femoral neck and hip) were considered as separate studies. In order to identify overlapping samples, the retrieved studies were appraised by examining the geographic location, author names and period of study. In overlapping studies, the study with the largest population was considered. The meta-analysis included cohort studies providing sufficient data for extracting the BMD mean, the corresponding standard deviation and number of subjects for the variants involved in the FA, ACS and CC pathways. Studies on children were excluded. Family based studies were also excluded due to different study design.

Data extraction

Data were extracted by two investigators (CD and TK), independently, and disagreements were resolved through consensus. Data extracted from each study included: first author, publication year, ethnicity of the study population, demographics, menopausal status, genotyping methodology and final results for outcomes of interest (BMD mean, standard deviation and number of subjects) for each genotype.

Data synthesis and analysis

The meta-analysis examined whether there was an association between FA, ACS and CC variant genotype and BMD. For each study, the mean value of BMD, and the corresponding standard deviation with the number of subjects, was evaluated for each genotype (mt-mutant type/mt, mt/wt-wild type, wt/wt). The meta-analysis estimated the pooled change in mean values of BMD ($\Delta\mu$) (standardized effect size) for the dominant (mt/mt+mt/wt vs. wt/wt), recessive (mt/mt vs. mt/wt+wt/wt), additive (mt/mt vs. wt/wt) and co-dominant (mt/wt vs. mt/mt+wt/wt) models (Zintzaras & Lau 2008a) using the inverse of the variance of $\Delta\mu$ as a weighting factor (Whitehead 2002; Hedges & Olkin 1985). Then, the 95% confidence interval of $\Delta\mu$ was calculated. Thus, $\Delta\mu$ was considered significant at $P<0.05$.

The heterogeneity between studies was tested using the Q-statistic. If $P_Q < 0.10$, then heterogeneity was considered statistically significant. Heterogeneity was quantified with the I^2 metric which takes values between 0 and 100% with higher values denoting greater degree of heterogeneity (Higgins & Thompson 2002). The pooled $\Delta\mu$ was estimated using random effects (RE) (DerSimonian and Laird) model (DerSimonian & Laird 1986). The RE model was chosen because is more conservative than the alternative fixed effects model which does not consider

the heterogeneity between studies. Separate analyses were considered for measurements of BMD made in lumbar spine, femoral neck and hip. The meta-analysis consisted of the main (overall) analysis, which includes all available data, and subgroup analysis. Subgroup analysis was used to evaluate patient related factors and to explain possible sources of heterogeneity. Subgroup analysis was performed by ethnicity, gender and menopausal status (Zintzaras et al. 2011). Subgroup meta-analysis was carried out when three or more studies existed. Sensitivity analysis, which examines the effect of excluding specific studies, was also conducted for models showed significance in the overall analysis (Zintzaras & Lau 2008a). A differential magnitude of effect in large vs. small studies was tested for the dominant model in the overall analysis using the Egger's test, and a result was considered statistically significant at $P_{bias} < 0.10$. The analysis was performed using CUMAGAS-BMD (http://biomath.med.uth.gr) (Zintzaras et al. 2010).

Results

Eligible studies

The literature review identified 287 titles in PubMed and HuGELit databases that met the search criteria. The search in HuGE PubLit traced articles already identified by PubMed. Figure 1 presents a flow chart of retrieved and excluded articles, with the reasons for exclusion (a list of the articles included in the analysis is provided as supplementary material). Data from 211 studies published in 66 articles that investigated the association between FA, ACS and CC variants and BMD met the inclusion criteria. The studies involved 54,736 patients in lumbar spine, 54,144 in femoral neck and 10,134 in hip. The search criterion identified nine GWAS, however, none of them met the inclusion criteria and/or provided extractable data for inclusion in CUMAGAS-BMD and in the subsequent meta-analysis (Kiel et al. 2007; Styrkarsdottir et al. 2008; Hsu et al. 2010; Guo et al. 2010; Xiong et al. 2009; Richards et al. 2008; Paternoster et al. 2010; Ichikawa et al. 2010; Kung et al. 2010).

Studies' characteristics

The characteristics of each study and the association results of variants are shown in the Supplementary Table. Briefly, 107 studies investigated lumbar spine, 85 femoral neck and 19 hip. Studies were conducted in various populations of different racial descent: 143 studies involved solely Whites, 62 studies recruited East Asians and 6 studies other populations. One hundred fifty-four studies provided data for females and 31 for males. One hundred twenty-four studies reported data for postmenopausal women and 14 for premenopausal women. Eighty of the studies that investigated femoral neck also investigated lumbar spine, 13 and 11 of the studies that investigated hip also investigated femoral neck and lumbar spine, respectively.

Individual studies' results

In lumbar spine, 41 studies showed significance under any genetic model for the variants: COL1A1 rs1800012 (17 studies), COL1A1 rs2412298 (2 studies), COL1A1 rs1107946 (4 studies), COL1A2 Chr.7:94033461 (14589C/T) (2 studies), FGFR1 rs6996321, TGFB1 rs55659002 (2 studies), TGFB1 rs8179181, TGFB1 rs1800469, TGFB1 rs1982073 (4 studies), TGFB1 rs13306709, IGF-I Chr2:102875166 [18 CA repeats (190 bps)wt(–)/mt(+)] and Chr2:102875166 [20 CA repeats (194 bps) wt(+)/mt(–)], IGFR1R rs2229765, ITGA rs2447867, TGFB1 Chr19:41837997, TGFB1 rs143680317.

In femoral neck, 35 studies showed significance for the variants: COL1A1 rs1800012 (19 studies), COL1A1 rs1107946 (5 studies), COL1A1 rs2412298, COL1A2 Chr7.:94038585, TGFB1 rs1800469, TGFB1 rs1982073 (3 studies), IGFR1R rs2229765, TGFB1 rs55659002, TGFB1 rs8179181, ITGArs2447867 and ITGA rs6450100. Finally, in hip, 8 studies derived significant association for the variants: COL1A1 rs1800012, IGF1 rs35767 (3 studies), COL1A1 rs1107946, TGFB1 rs55659002 and TGFB1 rs1982073 (2 studies).

One study (Ralston et al. 2006) was a mega study (20,786 subjects) and produced significant results for COL1A1 rs1800012 variant in lumbar spine and femoral neck. In the meta-analysis, a sensitivity analysis was carried out excluding this study to investigate its effect in the pooled effect size.

Meta-analysis main results and subgroup analysis

Table 1 shows the results of the main and subgroup analyses for the association between FA, ACS and CC variants and BMD.

Lumbar spine

In total, five variants were investigated in three or more studies and their results were meta-analyzed: COL1A1 rs1800012, COL1A1 rs1107946, COL1A1 rs2412298, TGFB1 rs1982073 and TGFB1 rs1800469. Significant results were shown only for the variants COL1A1 rs1800012, COL1A1 rs1107946 and TGFB1 rs1800469.

In particular, overall there was a significant association for the dominant model of the variant COL1A1 rs1800012 [$\Delta\mu = -0.15$ (–0.22 to –0.081)] with the heterogeneity between studies being significant ($I^2 = 86\%$ and $P_Q < 0.01$). In subgroup analyses, Whites, females postmenopausal and premenopausal showed also significance. The co-dominant and additive model produced similar pattern of results (except the postmenopausal subgroup for the additive model). The recessive model did not show significance for any population. Sensitivity analysis (exclusion of the mega study) did not change the significance of results and effect size in the overall analysis for the dominant model of COL1A1 rs1800012 polymorphism [$\Delta\mu = -0.17$ (95% CI = –0.25 to –0.09) with $I^2 = 86\%$ and $P_Q < 0.01$].

For the variant COL1A1 rs1107946, the recessive and additive models showed significant results for the

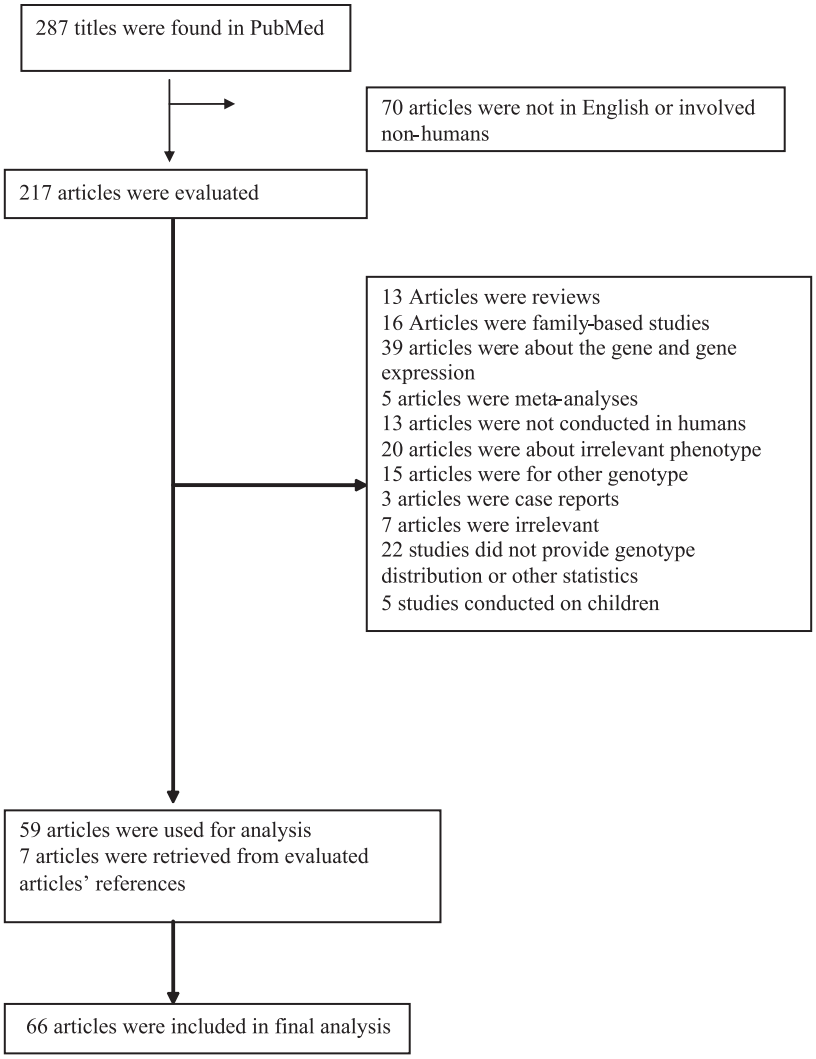

Figure 1. Flow chart of retrieved studies and studies excluded, with specification of reasons.

postmenopausal subgroup [$\Delta\mu = 0.20$ (0.017–0.39) and $\Delta\mu = -0.21$ (–0.39 to –0.020), respectively] and lack of heterogeneity ($I^2 = 0\%$ and $P_Q > 0.66$). The opposite signs for $\Delta\mu$ in the two models indicate the high dominance of the risk allele T (Falconer & Mackay 1996). However, this result was based on a small number of studies and therefore safe conclusions can not be drawn.

Finally, the variant TGFB1 rs1800469 derived significant result for the additive model in E. Asian postmenopausal females [$\Delta\mu = -0.26$ (–0.45 to –0.077) with $I^2 = 0\%$ and $P_Q = 0.71$]; however, the number of studies was only three. There was a differential magnitude of effect in large vs. small studies in all variants for the variants COL1A1 rs1800012 and COL1A1 rs2412298 ($P_{bias} < 0.10$).

Femoral neck

Four variants were investigated in three or more studies and their results were meta-analyzed: COL1A1 rs1800012, COL1A1 rs1107946, COL1A1 rs2412298 and TGFB1 rs1982073. Significant results were shown for the variants COL1A1 rs1800012 and TGFB1 rs1982073.

For the COL1A1 rs1800012 polymorphism, in the main analysis, the dominant model produced significant association with BMD [$\Delta\mu = -0.12$ (–0.18 to –0.060)] and large heterogeneity ($I^2 = 81\%$, $P_Q < 0.01$). In subgroup analyses, Whites, female and postmenopausal derived also significant associations. The sensitivity analysis for mega study did not change the results [$\Delta\mu = -0.13$ (–0.20 to –0.061) with $I^2 = 79\%$ and $P_Q < 0.01$]. The additive and co-dominant models produced the same pattern of results with the dominant model. However, the recessive model produced significant results, overall [$\Delta\mu = 0.19$ (0.10–0.27)] and for the sub-groups, but, with $\Delta\mu > 0$, indicating the high dominance of the risk allele T (Falconer & Mackay 1996).

The TGFB1 rs1982073 polymorphism showed significant association for the dominant model overall and in female (postmenopausal): $\Delta\mu = -0.38$ (–0.71 to –0.046) with $P_Q < 0.01$ and $\Delta\mu = -0.54$ (–1.02 to –0.054) with $P_Q < 0.01$, respectively. There was a differential magnitude of effect in large vs. small studies for the variants COL1A1 rs1800012 and TGFB1 rs1982073 ($P_{bias} < 0.10$).

Table 1. Meta-analysis results for (a) lumbar spine, (b) femoral neck and (c) hip are presented. The random effects mean change ($\Delta\mu$) in BMD and the respective 95% confidence interval (CI) are shown. The heterogeneity metrics (P_Q, I^2) and significance level of the differential magnitude of effect in large versus vs. small studies (P_{bias}) are also shown for the dominant, recessive, additive and co-dominant models.

Gene, polymorphism	Model	Population	Studies included in meta-analysis	Number of subjects	$\Delta\mu$ (95% CI)	$\Delta\mu$ (95% CI)	I^2 (%), P_Q	P_{bias}
(a) Lumbar spine								
COL1A1, rs1800012 (2046G/T)	Dominant model	All	49*	43,726	−0.15 (−0.22 to −0.081)	−0.15 (−0.22 to 0.081)	86, <0.01	0.01
		Whites	46	43,361	−0.15 (−0.22 to −0.075)	−0.15 (−0.22 to 0.075)	87, <0.01	0.01
		Female	34	17,956	−0.21 (−0.32 to −0.094)	−0.21 (−0.32 to 0.094)	90. <0.01	0.09
		Postmenopausal	23	12,025	−0.37 (−0.71 to −0.029)	−0.37 (−0.71 to 0.029)	99, <0.01	0.08
		Premenopausal	6	867	−0.21 (−0.35 to −0.065)	−0.21 (−0.35 to 0.065)	0, 0.60	0.91
		Male	6	3026	−0.053 (−0.13 to 0.023)	−0.053 (−0.13 to 0.023)	0, 0.45	0.93
	Recessive model	All	39	42,280	0.077 (−0.061 to 0.22)	0.077 (−0.061 to 0.22)	76, <0.01	0.51
		Whites	37	41,969	0.074 (−0.062 to 0.21)	0.074 (−0.062 to 0.21)	76, <0.01	0.51
		Female	27	16,880	0.032 (−0.17 to 0.23)	0.032 (−0.17 to 0.23)	78, <0.01	0.62
		Postmenopausal	20	11,337	0.0002 (−0.42 to 0.42)	0.0002 (−0.42 to 0.42)	93, <0.01	0.62
		Male	5	2991	−0.030 (−0.26 to 0.21)	−0.030 (−0.26 to 0.21)	22, 0.28	0.75
	Additive model	All	39	42,280	−0.18 (−0.28 to −0.073)	−0.18 (−0.28 to 0.073)	54, <0.01	0.26
		Whites	37	41,969	−0.17 (−0.27 to −0.072)	−0.17 (−0.27 to 0.072)	51, <0.01	0.26
		Female	27	16,880	−0.17 (−0.29 to −0.057)	−0.17 (−0.29 to 0.057)	35, 0.04	0.16
		Postmenopausal	20	11,337	−0.25 (−0.60 to 0.095)	−0.25 (−0.60 to 0.095)	89, <0.01	0.24
		Male	5	2991	0.013 (−0.24 to 0.27)	0.013 (−0.24 to 0.27)	29, 0.23	0.72
	Co-dominant model	All	39	42,280	−0.15 (−0.24 to −0.071)	−0.15 (−0.24 to 0.071)	89, <0.01	<0.01
		Whites	37	41,969	−0.15 (−0.23 to −0.067)	−0.15 (−0.23 to 0.067)	89, <0.01	0.01
		Female	27	16,880	−0.21 (−0.34 to −0.080)	−0.21 (−0.34 to 0.080)	92, <0.01	0.08
		Postmenopausal	20	11,337	−0.421 (−0.81 to −0.027)	−0.421 (−0.81 to 0.027)	99, <0.01	0.10
		Male	5	2991	−0.063 (−0.14 to 0.016)	−0.063 (−0.14 to 0.016)	0, 0.49	0.85
COL1A1, rs1107946 (−1997G/T)	Dominant model	All	9	13,512	0.021 (−0.040 to 0.082)	0.021 (−0.040 to 0.082)	56, 0.02	0.87
		Whites	7	11,276	0.021 (−0.044 to 0.086)	0.021 (−0.044 to 0.086)	50, 0.06	0.86
		Female	6	9618	0.046 (−0.033 to 0.13)	0.046 (−0.033 to 0.13)	61, 0.03	0.76
		Postmenopausal	4	5497	0.011 (−0.10 to 0.13)	0.011 (−0.10 to 0.13)	62, 0.05	0.70
	Recessive model	All	9	13,512	0.009 (−0.12 to 0.14)	0.009 (−0.12 to 0.14)	42, 0.09	0.13
		Whites	7	11,276	0.072 (−0.13 to 0.27)	0.072 (−0.13 to 0.27)	49, 0.07	0.24
		Female	6	9618	0.021 (−0.18 to 0.22)	0.021 (−0.18 to 0.22)	62, 0.02	0.22
		Postmenopausal	4	5497	0.20 (0.017 to 0.39)	0.20 (0.017 to 0.39)	0, 0.74	0.48
	Additive model	All	9	13,512	−0.017 (−0.16 to 0.13)	−0.017 (−0.16 to 0.13)	53, 0.03	0.17
		Whites	7	11,276	−0.073 (−0.28 to 0.13)	−0.073 (−0.28 to 0.13)	52, 0.05	0.27
		Female	6	9618	−0.012 (−0.24 to 0.21)	−0.012 (−0.24 to 0.21)	67, 0.01	0.20
		Postmenopausal	4	5497	−0.21 (−0.39 to −0.020)	−0.21 (−0.39 to 0.020)	0, 0.66	0.62
	Co-dominant model	All	9	13,512	0.019 (−0.033 to 0.070)	0.019 (−0.033 to 0.070)	36, 0.13	0.86
		Whites	7	11,276	0.028 (−0.029 to 0.085)	0.028 (−0.029 to 0.085)	33, 0.18	0.80
		Female	6	9618	0.038 (−0.024 to 0.10)	0.038 (−0.024 to 0.10)	36, 0.17	0.48
		Postmenopausal	4	5497	0.036 (−0.077 to 0.15)	0.036 (−0.077 to 0.15)	59, 0.06	0.56
COL1A1, rs2412298 (−1663 ins/delT)	Dominant model	All (Whites)	4	5524	−0.046 (−0.11 to 0.015)	−0.046 (−0.11 to 0.015)	10, 0.34	0.64
		Female	3	4952	−0.032 (−0.090 to 0.027)	−0.032 (−0.090 to 0.027)	0, 0.55	na
	Recessive model	All (Whites)	4	5524	0.079 (−0.23 to 0.38)	0.079 (−0.23 to 0.38)	75, 0.01	0.73
		Female	3	4952	−0.038 (−0.36 to 0.29)	−0.038 (−0.36 to 0.29)	75, 0.02	na
	Additive model	All (Whites)	4	5524	−0.094 (−0.41 to 0.22)	−0.094 (−0.41 to 0.22)	76, 0.01	0.77
		Female	3	4952	0.028 (−0.30 to 0.35)	0.028 (−0.30 to 0.35)	75, 0.02	na
	Co-dominant model	All (Whites)	4	5524	−0.023 (−0.081 to 0.035)	−0.023 (−0.081 to 0.035)	0, 0.75	0.04
		Female	3	4952	−0.016 (−0.078 to 0.045)	−0.016 (−0.078 to 0.045)	0, 0.66	na
TGFB1, rs1982073 (869T/C)	Dominant model	All	10	2940	−0.018 (−0.10 to 0.065)	−0.018 (−0.10 to 0.065)	2, 0.42	0.87
		E. Asians	7	1660	0.045 (−0.071 to 0.16)	0.045 (−0.071 to 0.16)	1, 0.42	0.61

Table 1. Continued on next page

Table 1. Continued

Gene, polymorphism	Model	Population	Studies included in meta-analysis	Number of subjects	Δμ (95% CI)	Δμ (95% CI)	I^2 (%), P_Q	P_{bias}
		Whites	3	1280	−0.077 (−0.19 to 0.035)	−0.077 (−0.19 to 0.035)	0, 0.63	na
		Female (Postmen.)	8	2118	0.005 (−0.10 to 0.11)	0.005 (−0.10 to 0.11)	12, 0.34	0.90
	Recessive model	All	10	2940	0.029 (−0.13 to 0.19)	0.029 (−0.13 to 0.19)	64, <0.01	0.33
		E. Asians	7	1660	−0.037 (−0.20 to 0.13)	−0.037 (−0.20 to 0.13)	50, 0.06	0.15
		Whites	3	1280	0.17 (−0.17 to 0.51)	0.17 (−0.17 to 0.51)	76, 0.02	na
		Female (Postmen.)	8	2118	0.065 (−0.14 to 0.27)	0.065 (−0.14 to 0.27)	70, <0.01	0.44
	Additive model	All	10	2940	−0.047 (−0.22 to 0.13)	−0.047 (−0.22 to 0.13)	56, 0.02	0.55
		E. Asians	7	1660	0.044 (−0.14 to 0.23)	0.044 (−0.14 to 0.23)	32, 0.18	0.22
		Whites	3	1280	−0.21 (−0.55 to 0.14)	−0.21 (−0.55 to 0.14)	71, 0.02	na
		Female (Postmen.)	8	2118	−0.060 (−0.29 to 0.17)	−0.060 (−0.29 to 0.17)	63, 0.01	0.72
	Co-dominant model	All	10	2940	−0.005 (−0.096 to 0.085)	−0.005 (−0.096 to 0.085)	27, 0.20	0.41
		E. Asians	7	1660	−0.008 (−0.13 to 0.12)	−0.008 (−0.13 to 0.12)	33, 0.18	0.40
		Whites	3	1280	0.004 (−0.15 to 0.16)	0.004 (−0.15 to 0.16)	39, 0.19	na
		Female (Postmen.)	8	2118	0.032 (−0.076 to 0.14)	0.032 (−0.076 to 0.14)	27, 0.21	0.57
TGFB1, rs1800469 (−1348C/T)	Dominant model	All	4	1493	−0.063 (−0.18 to 0.050)	−0.063 (−0.18 to 0.050)	0, 0.62	0.32
		E. Asians (Female/ Postmen.)	3	902	−0.13 (−0.29 to 0.025)	−0.13 (−0.29 to 0.025)	0, 0.89	na
	Recessive model	All	4	1493	0.078 (−0.19 to 0.34)	0.078 (−0.19 to 0.34)	70, 0.02	0.71
		E. Asians (Female/ Postmen.)	3	902	0.20 (−0.0007 to 0.39)	0.20 (−0.0007 to 0.39)	30, 0.24	na
	Additive model	All	4	1493	−0.12 (−0.39 to 0.16)	−0.12 (−0.39 to 0.16)	60, 0.06	0.99
		E. Asians (Female/ Postmen.)	3	902	−0.26 (−0.45 to −0.077)	−0.26 (−0.45 to 0.077)	0, 0.71	na
	Co-dominant model	All	4	1493	0.023 (−0.095 to 0.14)	0.023 (−0.095 to 0.14)	17, 0.31	0.76
		E. Asians (Female/ Postmen.)	3	902	0.085 (−0.046 to 0.22)	0.085 (−0.046 to 0.22)	0, 0.44	na

na,: non-applicable.

(b) Femoral neck

COL1A, rs1800012 (2046G/T)	Dominant model	All	46*	44,100	−0.12 (−0.18 to −0.060)	81, <0.01	0.03
		Whites	43	43,735	−0.12 (−0.18 to −0.065)	81, <0.01	0.03
		Female	35	19,114	−0.17 (−0.25 to −0.079)	84, <0.01	0.15
		Postmenopausal	24	13,183	−0.11 (−0.20 to −0.034)	72, <0.01	0.87
		Premenopausal	6	867	−0.13 (−0.31 to 0.051)	30, 0.21	0.50
	Recessive model	All	37	42,690	0.19 (0.10 to 0.27)	35, 0.02	0.46
		Whites	35	42,379	0.19 (0.10 to 0.27)	37, 0.02	0.45
		Female	28	18,038	0.20 (0.078 to 0.32)	43, 0.01	0.83
		Postmenopausal	21	12,495	0.19 (0.011 to 0.37)	60, <0.01	0.77
	Additive model	All	37	42,690	−0.23 (−0.33 to −0.12)	55, <0.01	0.22
		Whites	35	42,379	−0.23 (−0.34 to −0.12)	57, <0.01	0.21
		Female	28	18,038	−0.25 (−0.40 to −0.10)	61, <0.01	0.82
		Postmenopausal	21	12,495	−0.22 (−0.39 to −0.039)	58, <0.01	0.79

Table 1. Continued on next page

Table 1. Continued

Gene, polymorphism	Model	Population	Studies included in meta-analysis	Number of subjects	Δμ (95% CI)	I^2 (%), P_Q	P_{bias}
	Co-dominant model	All	37	42,690	−0.10 (−0.16 to −0.033)	82, <0.01	0.05
		Whites	35	42,379	0.009 (−0.008 to 0.025)	0, 0.99	0.02
		Female	28	18,038	−0.15 (−0.25 to −0.052)	86, <0.01	0.14
		Postmenopausal	21	12,495	−0.092 (−0.18 to −0.005)	73, <0.01	0.8
COL1A1, rs1107946 (−1997G/T)	Dominant model	All	8	12,907	0.18 (−0.23 to 0.58)	99, <0.01	0.63
		Whites	6	10,671	−0.022 (−0.11 to 0.068)	72, <0.01	0.36
		Female	5	9013	0.40 (−0.21 to 1.01)	99, <0.01	0.51
		Postmenopausal	3	4892	−0.009 (−0.18 to 0.16)	81, <0.01	Na
	Recessive model	All	7	12,333	0.28 (−0.44 to 1.01)	98, <0.01	0.92
		Whites	5	10,097	0.10 (−0.13 to 0.33)	58, 0.05	0.09
		Female	5	9013	−0.021 (−0.44 to 0.40)	91, <0.01	0.07
		Postmenopausal	3	4892	0.23 (−0.22 to 0.67)	75, 0.02	Na
	Additive model	All	7	12,333	−0.12 (−0.99 to 0.75)	99, <0.01	0.78
		Whites	5	10,097	−0.11 (−0.35 to 0.13)	62, 0.03	0.12
		Female	5	9013	0.15 (−0.68 to 0.98)	98, <0.01	0.26
		Postmenopausal	3	4892	−0.24 (−0.70 to 0.23)	76, <0.01	Na
	Co-dominant model	All	7	12,333	0.32 (−0.050 to 0.70)	99, <0.01	0.41
		Whites	5	10,097	0.020 (−0.055 to 0.095)	57, 0.05	0.91
		Female	5	9013	0.35 (−0.18 to 0.88)	99, <0.01	0.56
		Postmenopausal	3	4892	0.021 (−0.14 to 0.18)	76, 0.02	Na
COL1A1, rs2412298 (−1663 ins/delT)	Dominant model	All (Whites, female)	3	4952	−0.023 (−0.10 to 0.055)	30, 0.24	Na
	Recessive model	All (Whites, female)	3	4952	0.065 (−0.13 to 0.26)	34, 0.22	Na
	Additive model	All (Whites, female)	3	4952	−0.061 (−0.27 to 0.15)	41, 0.18	Na
	Co-dominant model	All (Whites, female)	3	4952	−0.014 (−0.075 to 0.048)	0, 0.59	Na
TGFB1, rs1982073 (869T/C)	Dominant model	All	8	2782	−0.38 (−0.71 to −0.046)	92, <0.01	0.23
		Whites	3	1789	−0.11 (−0.33 to 0.11)	74, 0.02	Na
		E. Asians	5	993	−0.56 (−1.27 to 0.15)	95, <0.01	0.11
		Female (Postmen.)	6	1960	−0.54 (−1.02 to −0.054)	93, <0.01	0.3
	Recessive model	All	7	1685	0.13 (−0.082 to 0.34)	69, <0.01	0.02
		E. Asians	5	993	0.15 (−0.062 to 0.36)	60, 0.04	0.04
		Female (Postmen.)	5	863	0.22 (−0.038 to 0.48)	67, <0.02	0.1
	Additive model	All	7	1685	−0.45 (−0.95 to 0.049)	91, <0.01	0.02
		E. Asians	5	993	−0.57 (−1.23 to 0.10)	92, <0.01	0.07
		Female (Postmen.)	5	863	−0.69 (−1.39 to 0.022)	92, <0.01	0.11
	Co-dominant model	All	7	1685	−0.13 (−0.34 to 0.094)	78, <0.01	0.48
		E. Asians	5	993	−0.18 (−0.50 to 0.15)	85, <0.01	0.31
		Female (Postmen.)	5	863	−0.20 (−0.53 to 0.14)	83, <0.01	0.83
(c) Hip							
COL1A, rs1800012 (2046G/T)	Dominant model	All (Whites)	7*	3212*	−0.068 (−0.161 to 0.026)	15, 0.32	0.13
		Female (Postmen.)	4	2504	−0.046 (−0.13 to 0.039)	0, 0.41	0.05
	Recessive model	All (Whites)	7	3212*	0.042 (−0.14 to 0.22)	0, 0.91	0.02
		Female (Postmen.)	4	2504	0.06 (−0.18 to 0.29)	0, 0.82	0.08

Table 1. Continued on next page

Table 1. Continued

Gene, polymorphism	Model	Population	Studies included in meta-analysis	Number of subjects	Δμ (95% CI)	I^2 (%), P_Q	P_{bias}
	Additive model	All (Whites)	7	3212*	−0.088 (−0.27 to 0.095)	0, 0.96	<0.01
		Female (Postmen.)	4	2504	−0.069 (−0.305 to 0.17)	0, 0.80	0.02
	Co-dominant model	All (Whites)	7	3212*	−0.09 (−0.20 to 0.022)	33, 0.18	0.14
		Female (Postmen.)		2504	−0.041 (−0.13 to 0.047)	0, 0.49	0.11
IGF1, rs35767 (−1245C/T)	Dominant model	All	4	3484	−0.03 (−0.30 to 0.24)	93, < 0.01	0.27
	Recessive model	All	4	3484	−0.029 (−0.20 to 0.14)	52, 0.10	0.32
	Additive model	All	4	3484	0.053 (−0.23 to 0.34)	80, <0.01	0.51
	Co-dominant model	All	4	3484	−0.08 (−0.29 to 0.13)	89, <0.01	0.40
TGFB1, rs1982073 (869T/C)	Dominant model	All (Postmen.)	3	1436	−0.20 (−0.31 to −0.1)	0, 0.80	na

*4 studies (2445 subjects) included in all three BMD phenotypes analyses (lumbar spine, femoral neck, and hip) for COL1A1 rs1800012, 44 studies (43,513 subjects) included in both analyses for lumbar and femoral neck BMD, 1 study (180 subjects) included both in lumbar and hip BMD analysis and 2 studiesy (587 subjects) included in femoral neck and hip BMD analysis.

Hip

Three variants were investigated in three or more studies and their results were meta-analyzed: COL1A1 rs1800012, IGF1 rs35767 and TGFB1 rs1982073. Significant results were shown in the overall analysis for the dominant model of TGFB1 rs1982073 polymorphism [$\Delta\mu = -0.20$ (−0.31 to −0.10) with $I^2 = 0\%$ and $P_Q = 0.80$].

Discussion

The purpose of this project was to catalogue all currently available data from the FA, ACS and CC family genes in BMD, to assess comprehensively the involvement of these data with low BMD and to provide evidence of risk variants. To achieve this, we created a web-based database information system called CUMAGAS-BMD for performing cumulative meta-analysis of each variant implicated in BMD susceptibility. By synthesizing the data from many studies, there is a considerable gain in improving power for detecting significant results. However, in the presence of large between-study heterogeneity, the results should be interpreted with caution (Zintzaras & Lau 2008a; Trikalinos et al. 2008).

Five variants were meta-analyzed in lumbar spine and significance was shown for the variants COL1A1 rs1800012, COL1A1 rs1107946 and TGFB1 rs1800469. The two COL1A1 variants (rs1800012 and rs1107946) are in strong linkage disequilibrium (D′ = 0.96) (Stewart, 2006). For femoral neck, four variants were meta-analyzed and significance was produce for the variants COL1A1 rs1800012 and TGFB1 rs1982073. Three variants were meta-analyzed for hip and significance was shown only for the variant COL1A1 rs1800012. However, none of the studied markers showed association in the published GWAS. A large heterogeneity between studies was shown. In general, there was a consistency of genetic effects overall and across different populations (postmenopausal, female, Whites) and, consequently, the subgroup analyses did not explain the heterogeneity. However, the findings of the present study are not genome-wide significant (conventionally at $P < 5 \times 10^{-8}$); nevertheless, such a correction is quite stringent for a meta-analysis. Moreover, a multiple test adjustments is not strictly required in the present study since meta-analysis of GAS is considered an exploratory study, in which data are synthesized with a specific objective (to reduce the uncertainty of effect) but without a prespecified key hypothesis (Zintzaras & Lau 2008a).

CUMAGAS-BMD represents a combination between an evidence-based approach and an electronic information system to systematically search, review and synthesize the rapidly emerging body of genetic studies in BMD, with the advantage of continuous updating. Available evidence was catalogued and where appropriate, synthesized with meta-analytic techniques, including cumulative meta-analysis, and thus, directing the research in the field. Recently Zintzaras et al. (2011) published a meta-analysis of genetic association studies (GAS) that investigated the association between variants in the FA pathway and osteoporosis as a dichotomous (qualitative) trait and introduced CUMAGAS-OSTEOprosis, an information system for cataloguing and synthesizing all relevant GAS. The meta-analysis involved 72 studies (from 39 articles) and 46 of those studies (from 20 articles) investigated also the association with BMD and therefore they were included in the present meta-analysis. The meta-analysis on the qualitative trait (Zintzaras et al. 2011) derived significant results ($P < 0.05$) for the variants COL1A1

rs1800012, COL1A1 rs1107946 and ITGB3 rs5920. The current meta-analysis identified two of the previous variants (COL1A1 rs1800012 and COL1A1 rs1107946) as potential markers for osteoporosis. CUMAGAS has been expanded to additional complex phenotypes such chronic lymphocytic leukemia, peripheral arterial disease, hypertension, osteoarthritis (Kitsios & Zintzaras 2010; Zintzaras & Kitsios 2009; Zintzaras & Zdoukopoulos 2009; Zintzaras et al. 2010). It has also the capacity to incorporate the findings from emerging GWAS; however, integration of GWAS will depend upon the public availability of their data.

In general, individual genetic studies have small sample sizes and thus, there is insufficient power to detect the minor contributing role of variants (Zintzaras & Lau 2008a; Zintzaras & Lau 2008b). In order to increase power, data should be collected form different centers and the development of consortia may help for this purpose (Zintzaras & Lau 2008b). The need for data sharing and joint analysis of studies, including GWAS, has already been highlighted by initiatives (GAIN Collaborative Research Group, Manolio et al. 2007). Although it has been conducted a mega study (Ralston et al. 2006), in general studies in BMD have modest sample sizes to detect weak associations. However, the results all studies should be utilized in order to provide more evidence for different populations.

In the present data synthesis, we did not take into account possible effect modifiers (e.g. age, health status and life style) that influence the effect size and modulate the BMD levels; therefore, an unadjusted analysis was performed. However, if the individual patients' data would be available, adjusted estimates for the effect modifiers could also be derived. In addition, the subjects in each study cover a wide spectrum of osteoporosis, in terms of duration, demographics and other clinical manifestations. The development of low BMD levels is influenced by interaction of many genes and by environmental factors (such as particular nutrients), which have been evaluated in association to specific genetic background (Clayton & McKeigue 2001). In synthesizing the data, we did not consider potential gene—gene and gene—environment interactions (which are also subject to availability in the published studies) and this may have reduced the power of the findings and introduced heterogeneity, but it is unlikely to inflate the number of false positive results.

In conclusion, there is evidence of implication of FA, ACS and CC family genes in BMD and consequently in osteoporosis. With the implementation of the CUMAGAS-BMD, summary effect estimates were calculated in the context of 12 meta-analyses for genetic variants and BMD. The resulting evidence provided insights regarding the role of numerous candidate genes on BMD susceptibility. The ongoing research in BMD genetics will shed light on new gene—gene and gene—environment interactions studies and more conclusive claims about the role of these genes in BMD may derive from these data. The CUMAGAS-BMD information system would be a useful resource for reviewing and interpreting the findings of accumulating genomic epidemiology research in BMD.

Declaration of interest

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