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Genome-wide association study for radiographic vertebral fractures: A potential role for the 16q24 BMD locus

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Abstract

Vertebral fracture risk is a heritable complex trait. The aim of this study was to identify genetic susceptibility factors for osteoporotic vertebral fractures applying a genome-wide association study (GWAS) approach. The GWAS discovery was based on the Rotterdam Study, a population-based study of elderly Dutch individuals aged >55years; and comprising 329 cases and 2666 controls with radiographic scoring (McCloskey–Kanis) and genetic data. Replication of one top-associated SNP was pursued by de-novo genotyping of 15 independent studies across Europe, the United States, and Australia and one Asian study. Radiographic vertebral fracture assessment was performed using McCloskey–Kanis or Genant semi-quantitative definitions. SNPs were analyzed in relation to vertebral fracture using logistic regression models corrected for age and sex. Fixed effects inverse variance and Han–Eskin alternative random effects meta-analyses were applied. Genome-wide significance was set at $p < 5 \times 10^{-8}$. In the discovery, a SNP (rs11645938) on chromosome 16q24 was associated with the risk for vertebral fractures at $p=4.6\times10^{-8}$. However, the association was not significant across 5720 cases and 21,791 controls from 14 studies. Fixed-effects meta-analysis summary estimate was 1.06 (95% CI: 0.98–1.14; $p = 0.17$), displaying high degree of heterogeneity (I^2=57%; Q_{het} p =0.0006). Under Han–Eskin alternative random effects model the summary effect was significant ($p = 0.0005$). The SNP maps to a region previously found associated with lumbar spine bone mineral density (LS-BMD) in two large meta-analyses from the GEFOS consortium. A false positive association in the GWAS discovery cannot be excluded, yet, the low-powered setting of the discovery and replication settings (appropriate to identify risk effect size >1.25) may still be consistent with an effect size <1.10, more of the type expected in complex traits. Larger effort in studies with standardized phenotype definitions is needed to confirm or reject the involvement of this locus on the risk for vertebral fractures.

Keywords

Genome-wide association study; Vertebral fracture risk; Genetics of osteoporosis; GEFOS consortium; *FOXC2*

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2013.10.015.

Disclosures

All authors state that they have no conflicts of interest.

Introduction

Vertebral fractures are the most common osteoporotic fractures and represent a significant health issue [1,2]. Epidemiological measures derived from population-based studies vary between 1 and 3% per year for incidence and ~10 and 30% for the prevalence in elderly persons, varying by age, gender and geographic region [3-5]. Vertebral fractures are associated with a high morbidity [6-11], mortality [12,13] and a considerable financial burden. In the United States the costs of vertebral fractures were estimated to be 1.1 billion dollars in the year 2005, and are expected to rise by more than 50% by the year 2025 [14]. A recent report estimated the costs of vertebral fractures in Europe at 1.5billion euros in 2010 [15]. Furthermore, vertebral fractures are likely to become an increasingly important health issue with the increasing age of populations [1,14,15] and their association with increased risk of future osteoporotic fractures at other skeletal sites [7,16,17]. For all of these reasons, a better understanding of the genetic susceptibility to vertebral fracture has the potential to identify underlying biological mechanisms, improve risk prediction and lead to novel disease interventions.

Vertebral fracture risk is a heritable complex trait, also influenced by environmental, and gene–environment interactions [18,19]. A positive family history for vertebral fracture constitutes an independent risk factor for future fractures [20], emphasizing the importance of genetics in the pathogenesis of the disease. The hypothesis-free genome-wide association study (GWAS) approach has been particularly successful in identifying loci associated with many diseases and quantitative complex traits [21], including osteoporosis [18,22-24].

The aim of our study was to better understand the genetic architecture of radiographic vertebral fractures by conducting the first GWAS for this trait in a large population-based study of elderly Dutch individuals and pursuing replication in a large set of studies across Europe, the United States, Australia and Asia.

Methods

Datasets assessed

Sample discovery phase—The discovery sample was confined to the original Rotterdam Study cohort, a large population-based study of Dutch men and women aged 55 years and over (mean age at vertebral fracture assessment: 73.5 years). A detailed description of the Rotterdam Study has been reported previously [25]. In short, the study aimed to assess the incidence and determinants of disease and disability in elderly persons. The study has been approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam.

Sample replication phase—The Genetic Factors for Osteoporosis (GEFOS), Genetic Markers for Osteoporosis (GENOMOS) and Anglo-Australasian Osteoporosis Genetics Consortium (AOGC) are three consortia studying the genetic determinants of osteoporosis-related skeletal phenotypes in populations with available DNA and/or GWAS data [23,26-29]. Within this setting, 15 studies with both DNA samples and lateral morphometry-derived vertebral fracture data participated in the replication phase of this project

(Supplementary Table 1). More detailed descriptions are available in the Supplementary material.

The AOGC – Geelong Osteoporosis Study (AOGC-GOS) is a cohort drawn from the Geelong general population. Vertebral fracture imaging was performed in case of a clinical indication [30,31]. The AOGC – Sheffield (AOGC-SHEFFIELD) study constitutes a large population-based cohort of community-dwelling elderly women aged ≥75 years in Sheffield, UK [32]. AROS (Aarhus Osteoporosis Study) is a case–control study, including 462 osteoporotic patients (vertebral fracture and T-score < −2.5) and 336 controls [33]. AUSTRIOS is a prospective cohort study of elderly female patients above 70 recruited in 95 nursing homes in four counties in Austria. The AUSTRIOS-B cohort had vertebral fracture data available and was used for this project [34]. The Cantabria-Camargo (CABRIO-C) and Cantabria Case–Control (CABRIO-CC) studies are based in Northern Spain. CABRIO-C is a community-based study designed to evaluate the prevalence of metabolic bone diseases in postmenopausal women and men older than 50 years attending a primary care center in Santander [35,36]. CABRIO-CC is a clinic-based study of control individuals and patients with osteoporosis living in Cantabria, a region in Northern Spain [37,38]. The Calcium Intake Fracture Outcome Study (CAIFOS) is a randomized-controlled trial investigating calcium carbonate supplementation in ambulatory women older than 70 years recruited in Perth, Australia [39]. The Canadian Multicentre Osteoporosis Study (CaMoS) is a population-based prospective cohort of unrelated men and women followed for osteoporosis and osteoporotic fractures for the past 14 years [40-42]. The Danish Osteoporosis Prevention Study (DOPS) is a population-based study of perimenopausal women. The women were followed for 10years and approximately 35% were treated with hormone-replacement therapy (HRT) [43]. The Edinburgh Osteoporosis Study (EDOS) consists of a clinical referral population of patients assessed for evaluation of osteoporosis in Edinburgh, United Kingdom. The European Prospective Osteoporosis Study (EPOS) is the prospective phase of the European Vertebral Osteoporosis Study (EVOS) in which population-based samples had paired duplicate spinal films. Men and women from 36 centers in 19 European countries were recruited [5,44,45]. The Framingham Osteoporosis Study (FOS) is an ancillary study of the Framingham Study, a multigenerational family-based cohort study originally initiated to study the risk factors for cardiovascular disease [46-48]. Vertebral fracture assessment was done on multidetector computed tomography (CT) lateral scout views. The Korean osteoporosis study at Asan Medical Center (KorAMC) study is a hospital registered, cross-sectional study of postmenopausal Korean women in Seoul [49]. The Longitudinal Aging Study Amsterdam (LASA) is an ongoing multidisciplinary cohort study in older persons. A random sample of men and women aged 55years and over, stratified by age, sex, urbanization grade and expected 5-year mortality rate was drawn from the population register of Amsterdam, The Netherlands [50]. The Osteoporotic Fractures in Men Sweden (MrOS Sweden) study is a multicenter, prospective study including elderly men. Study subjects (men aged 69–80 years) were randomly identified using national population registers, contacted and asked to participate. Eligible subjects had to be able to walk without assistance, provide self-reported data, and sign an informed consent [51]. The Prospective Epidemiological Risk Factor (PERF) Study is based on subjects who were screened for or

enrolled into randomized controlled clinical trial to identify genetic and other risk factors of diseases in the elderly in Copenhagen, Denmark [52].

Phenotyping

Osteoporosis-related skeletal phenotypes in the discovery sample—During the second follow-up visit between 1997 and 1999 all Rotterdam Study participants underwent radiographic screening. A trained research technician obtained lateral radiographs of the thoracolumbar spine following a standard protocol. Radiographs were evaluated morphometrically in Sheffield, UK, by the McCloskey–Kanis method as described previously [53]. Using this method, central collapse, anterior and posterior wedge, and crush deformities were identified based on a cut point of 3 standard deviation height reductions. All vertebral fractures were confirmed by visual interpretation by an expert in the field to rule out artifacts and other etiologies, such as pathological fractures. Cases were defined as those individuals who had at least one vertebral fracture, and controls were defined as those who were free of vertebral fractures. Bone mineral density (BMD) of the femoral neck (FN) and lumbar spine (LS) was measured by dual-energy X-ray absorptiometry (DXA), using a Lunar DPX-L densitometer (Lunar Radiation Corporation, Madison, WI, USA).

Other measurements (covariates) in the discovery sample—An extensive baseline home interview on medical history, risk factors for chronic diseases, and medication use was performed on all participants by trained interviewers. Smoking habits were coded as “current”, “former” and “never”. Self-reported age at natural menopause between 40 and 60 years, defined as 12 months after periods ceased, was collected retrospectively. Information on medication use included hormone replacement therapy and systemic corticosteroids. Alcohol intake was assessed from a validated semi-quantitative food-frequency questionnaire. Height and weight were measured with indoor clothing and no shoes. Body mass index (BMI) was calculated as weight (in kg) / height (in m^2).

Phenotyping replication phase—Vertebral fracture assessments differed by cohorts which applied either the McCloskey–Kanis [53] or the Genant semi-quantitative method [54]. Detailed description of the methods and cut-offs applied by each study is available in Table 1. Four of the replication studies used the McCloskey–Kanis method, which is similar to the discovery (Rotterdam Study), of which one study applied the same additional criterion of absolute height reduction. Phenotyping for covariates was similar to that of the discovery sample.

Genotyping

Genome-wide association data—The Rotterdam Study participants were genotyped using the Illumina Infinium HumanHap550 Beadchip in the Genetic Laboratory of Erasmus MC Department of Internal Medicine, The Netherlands, following manufacturers’ protocols and quality control standards.

Single nucleotide polymorphism (SNP) genotyping—The top associated SNP from the discovery phase (rs11645938) was genotyped in 15 studies within three main genotyping centers: deCODE Genetics in Reykjavik, Iceland, Queensland University in Brisbane

Australia and KBiosciences, Hertfordshire, U.K. (www.kbioscience.co.uk). Genotyping was carried out by personnel blinded to patient status in all centers. The samples genotyped by KBiosciences were part of the GENOMOS consortium DNA collection, and comprise most of the participating studies. For KBiosciences, a minimum of 1.5μl of DNA at 3.3ng/μl (when quantitated by PicoGreen analysis or 7 ng/μl if quantitated by spectrophotometry) was required for one SNP to be assayed using their proprietary KASPar PCR technique and Taqman (also used by Brisbane University for AOGC samples). Genotype calling was carried out using an automated system, the results of which were checked manually by study personnel using SNPviewer software (KBiosciences). deCODE used the same KASPar assay from KBiosciences to genotype the PERF study samples. To ensure genotyping validity across study centers, a reference plate was shipped from KBiosciences to the AOGC coordinating center. To ensure correct genotyping deCODE Genetics genotyped 92 HapMap samples for comparison with the KASPar assay, and both positive and negative samples were present on all genotyping plates. Additionally, duplicate SNP genotyping was performed in the Rotterdam Study (all samples) and CABRIO-C (random selection of 187 samples) and no discrepancies were found.

Statistical methods

Within the discovery cohort, we tested 2,543,887 genotyped or imputed (HapMap CEU release 22, build 36) [55,56] SNPs for association with risk of osteoporotic vertebral fractures using a logistic regression model (MACH2DAT) [57,58] adjusted for age, gender, and admixture principal components (PCs) derived using EIGENSTRAT to adjust for population substructure [59]. Potential effect modifiers for the relationship between genotype and vertebral fracture (i.e. height, weight, BMI, age at menopause, HRT use, corticosteroid use, >3 units alcohol use per day, current and ever smoking) were tested by adding them one at a time to the regression model and evaluating the change in both the effect estimate and significance. The GWAS was performed using a web-based interface (GRIMP) on scalable super-computing grid infrastructures [60]. At a genome-wide significant α-level of 5×10^{-8}, the design had 0.80 power to detect risk effect sizes (OR) of 1.8 to 2.1 for minor allele frequencies (MAF) of 20% to 10%, respectively.

Replication analyses

Except for the FOS and AOGC studies, all analyses were carried out centrally by the Rotterdam Coordinating Center. Again a logistic regression model adjusting for age and gender was used. Individuals with either missing genotype or phenotype data were excluded from analysis. Initially, fixed effects inverse variance meta-analysis was performed (METAL software [61]). The presence of statistically significant heterogeneity was assessed by Cochran's Q statistic (Q_{het} p) and the extent of the observed heterogeneity was measured by the I^2 metric. Han–Eskin alternative random effects meta-analysis was applied when the I^2 metric exceeded 50% as this model is optimized to detect associations under heterogeneity (Metasoft software [62]). SPSS 16.0, PLINK, and R software were used for the rest of the analyses. In addition, the Framingham Study analysis used population-based generalized estimating equation (GEE) approach correcting for correlations owing to family relationships and PCs. The replication setting incorporating 5720 cases and 21,791 controls

from 14 studies was powered to identify a variant with a MAF of 0.10 and risk effect size >1.25, associated at $p < 5\times10^{-8}$.

Results

The description of the studies included in the discovery and replication phases is shown in Supplementary Table 1. Description of the vertebral fracture assessment done across studies is presented in Table 1 while baseline characteristics of the study populations are shown in Supplementary Table 2. In the discovery set, 329 of the 2995 Rotterdam Study participants had at least one vertebral fracture evident on the spinal radiographs. A genotyped SNP (rs11645938) on chromosome 16q24 (MAF = 10%) was associated at a genome-wide significant level ($p = 4.6 \times 10^{-8}$) with an increased risk of vertebral fractures (Fig. 1). Compared to the risk of non-carriers, the odds of the heterozygous carriers of the minor allele (C) was 1.7 times higher (95% confidence interval [CI] 1.3–2.3) and that of the homozygous carriers was 5.8 times higher (95% CI 2.7–12.8) (Supplementary Fig. 1). Fig. 2 shows the regional association plot of the locus, where a cluster of FOX genes maps ~200 kb from the associated SNP, containing *FOXF1, MTHFSD, FOXC2*, and *FOXL1*. Further adjusting for potential confounders did not influence either the effect estimate or the significance of the association between genotype and vertebral fracture risk. Similarly, the association remained significant after adjustment for either LS- or FN-BMD. Sex-stratified association analysis for the SNP, showed similar effect estimates (OR heterozygote men: 1.8 [95% CI: 1.2–2.8] and OR heterozygote women: 1.6 [95% CI: 1.1–2.3]; OR homozygote women: 8.4 [95% CI: 3.0–23.0] and OR homozygote men 3.3 [95% CI: 0.9–12.7]).

The associated SNP rs11645938 was successfully genotyped in 14 of the replication studies (5722 vertebral fracture cases and 21,793 controls; MAF ~8–12%) while it was found to be monomorphic in the Korean population of the KorAMC study (Table 2). The summary effect estimate for vertebral fracture risk obtained from the meta-analysis was 1.06 (95% CI: 0.98–1.14; p=0.17) and the effect estimate displayed high degree of heterogeneity with I^2=57% and Q_{het} p =0.0006 (Fig. 3). When considering a Han–Eskin alternative random effects meta-analysis model the summary effect was significant (p = 0.0005). When applying more stringent genotyping criteria (call rate > 95%; Hardy–Weinberg equilibrium p > 0.05) the association became significant in both the fixed (p=0.045) and Han–Eskin alternative random effects meta-analysis (p=0.0002). When further restricting analyses only to those studies that used the McCloskey–Kanis assessment a consistent, nonetheless not a statistically significant, effect direction was observed (replication p=0.29).

Discussion

To our knowledge, this is the first GWAS for radiographically determined vertebral fracture. A marker on chromosome 16q24 was genome-wide significantly associated with vertebral fracture in the Rotterdam Study discovery set. However, this association was not significant in a replication effort including 15 studies world-wide using conventional statistical analysis techniques.

Work by Stankiewicz et al. implicated deletions/mutations in this 16q24 locus in the VACTERL association (Vertebral anomalies, Anal atresia, Cardiovascular anomalies, TracheoEsophageal fistula, Renal and Radial anomalies, Limb defects), a non-random association of birth defects that includes vertebral defects [63]. *FOXC2*, mapping ~200kb upstream from the associated SNP, is highly expressed in human bone tissue, and its expression is regulated by bone morphogenetic proteins [64]. The gene is involved in osteoblast differentiation through activation of canonical Wnt/β-catenin signals [65], and in mice Foxc2 functions as a transcription factor essential for axial skeletogenesis [66]. The vertebral fracture associated SNP maps to a region previously found to be associated with LS-BMD in a meta-analysis of 19,125 individuals [23] and further replicated in 83,894 individuals [22]. However, the vertebral fracture SNP was not associated with either LS- or FN-BMD in our study and this signal was independent of the one previously reported for the BMD SNP rs10048146 ($r^2 = 0.002$).

Despite the underlying biological plausibility supporting this association and even with identifying a genome-wide significant signal in the discovery GWAS, replication in independent studies is still needed [21,67,68]. Subsequently, de-novo direct genotyping of rs11645938 in 5720 cases and 21,791 controls, from multiple independent studies around the world, did not provide robust evidence for replication of the association. Therefore, there is a high likelihood of the signal being a false-positive signal. It is expected that discoveries at underpowered settings would have low positive predictive value for true findings and this applies even for signals that pass a stringent genome-wide significance threshold [69]. However, other considerations might have also contributed to an apparent lack of replication of a potentially true association, and these will serve to inform the design of future GWAS of the vertebral fracture phenotype.

Signals in underpowered settings are likely to display inflated effects due to the “winner’s curse” phenomenon, where the effect estimate observed in the first study overestimates the actual risk observed at the general population level [70-72]. According to a post-hoc calculation for the replication phase, the current design had merely 0.42 power to detect an OR of 1.2. The study sample should have included more than 8000 cases to achieve 0.80 power, and we know that typically GWAS of complex traits even requires close to 30,000 cases to identify truly associated SNPs with moderate allele frequencies (e.g. MAF = 0.10) in a powered setting. Previous efforts have pointed out that SNPs with MAF <10% tend to be difficult to replicate due to the lack of statistical power [78]. Thus, we cannot yet exclude the possibility that the identified association has a very small, yet genuine effect [73]. Larger-scale GWAS meta-analyses for osteoporotic vertebral fractures are seriously needed.

GWA studies rely on the principle of linkage disequilibrium (LD) where markers are tested under the assumption they tag an underlying causal genetic variant. When the linkage disequilibrium structure in the region differs across populations this may result in decreased power and lack of replication [74-77]. The rs11645938 marker is not in LD with any other marker contained in HapMap and only in moderate LD with one marker ($r^2 = 0.41$ with rs11647070) from the 1000 Genomes Project. This observation led us to conclude that existing GWAS without the rs11645938 on their arrays would be poorly imputed, which was the case in the FOS and AOGC studies, and therefore to overcome this, de-novo

genotyping of the marker was performed in these studies. However, strictly speaking, genotyping in the Australian AOGC and CAIFOS studies did not attain conventional criteria for unknown reasons. Further, the SNP is monomorphic in Asian populations.

Despite the fact that all studies used radiological assessments, a critical issue to bear in mind is the phenotype definition, considering that diverse methods and cut-offs exist for the assessment of vertebral fractures [79]. Phenotype measurement differences are a known possible source of heterogeneity, which might be reflected in our study by the great variation in vertebral fracture prevalences among the studies. Noticeably, prevalence estimates varied between 6% and 49% in the cohort studies. Furthermore, quantitative scoring is based on morphometry alone, which may result in inclusion of deformities into the phenotype definition that are not truly vertebral fractures [80]. These non-fracture deformities are frequently labeled as Genant grade 1 or “mild vertebral fractures,” when, in fact, they may be normal variations in vertebral shape. Therefore, many studies assign an expert to filter out these non-fracture deformities. Nevertheless, this triage procedure may not have been sufficiently standardized, and this could have introduced the statistically significant heterogeneity between studies. Several methods exist to explore the existence of associations in heterogeneous data and when we applied a Han–Eskin random effects model, more stringent genotyping criteria or sensitivity analyses for phenotype definition, the results became more consistent. Perhaps selecting Genant grade 2 and 3 types including “moderate” and “severe” vertebral fractures [81] could provide a better phenotype definition for future genetic studies. In fact, Liu and colleagues demonstrated that the heritability of a stricter phenotype (when only more severe deformities counted) was higher than considering all vertebral deformities together [19]. Therefore, phenotype standardization among meta-analysis participants can be a key in replication [71,82]. Unfortunately, data harmonization was not possible because severity grading or qualitative standardized reading to enable data harmonization was not available for most of the studies included in our analysis. This consideration, along with the relatively small sample sizes across replication studies, is a major hurdle to be overcome in future studies focusing on radiographic vertebral fractures. Clinical vertebral fracture is an alternative phenotype definition for future genetic studies, though achieving sufficient sample sizes will be also challenging; considering that only a small fraction of vertebral fractures come to clinical attention (i.e. are symptomatic). In addition, it would be valuable to gain more insight into incident vertebral fractures. Nevertheless, definition of incident vertebral fractures is accompanied by different and possibly greater precision errors than identification of prevalent vertebral fractures. On the other hand, by comparing images at different follow-ups, the radiological reader has the opportunity to correct possible misclassifications, including misattributions of baseline deformities as fracture cases caused by erroneous vertebral height readings due to for example superimposition of other structures or magnification errors [83-86].

In conclusion, although a GWAS in the population-based Rotterdam Study identified a marker mapping to the 16q24 (*FOXC2*) BMD locus as being genome-wide significantly associated with radiographic vertebral fracture in that population, this could not be conclusively replicated by de-novo genotyping across 15 studies worldwide. A false positive association in the GWAS discovery cannot yet be excluded. However, these results from a

low-powered setting may still be consistent with a small true effect size as is common in complex traits. Larger efforts in subsequent GWAS for radiographic vertebral fracture with standardized phenotype definitions may confirm or reject the involvement of this locus on the risk for vertebral fractures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Szulc P, Bouxsein ML. Overview of osteoporosis: epidemiology and clinical management. Vertebral fracture initiative resource document. 2011
2. Brunton S, Carmichael B, Gold D, Hull B, Kauffman T, Papaioannou A, et al. Vertebral compression fractures in primary care: recommendations from a consensus panel. J Fam Pract. 2005; 54:781–8. [PubMed: 16144592]
3. Samelson EJ, Hannan MT, Zhang Y, Genant HK, Felson DT, Kiel DP. Incidence and risk factors for vertebral fracture in women and men: 25-year follow-up results from the population-based Framingham study. J Bone Miner Res. 2006; 21:1207–14. [PubMed: 16869718]
4. Van der Klift M, De Laet CE, McCloskey EV, Hofman A, Pols HA. The incidence of vertebral fractures in men and women: the Rotterdam Study. J Bone Miner Res. 2002; 17:1051–6. [PubMed: 12054160]
5. Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res. 2002; 17:716–24. [PubMed: 11918229]
6. Burger H, Van Daele PL, Grashuis K, Hofman A, Grobbee DE, Schutte HE, et al. Vertebral deformities and functional impairment in men and women. J Bone Miner Res. 1997; 12:152–7. [PubMed: 9240738]
7. Ross PD. Clinical consequences of vertebral fractures. Am J Med. 1997; 103:30S–42S. discussion 42S-43S. [PubMed: 9302895]
8. Nevitt MC, Ettinger B, Black DM, Stone K, Jamal SA, Ensrud K, et al. The association of radiographically detected vertebral fractures with back pain and function: a prospective study. Ann Intern Med. 1998; 128:793–800. [PubMed: 9599190]
9. Gold DT. The clinical impact of vertebral fractures: quality of life in women with osteoporosis. Bone. 1996; 18:185S–9S. [PubMed: 8777086]
10. Oleksik A, Lips P, Dawson A, Minshall ME, Shen W, Cooper C, et al. Health-related quality of life in postmenopausal women with low BMD with or without prevalent vertebral fractures. J Bone Miner Res. 2000; 15:1384–92. [PubMed: 10893688]
11. Oleksik AM, Ewing S, Shen W, van Schoor NM, Lips P. Impact of incident vertebral fractures on health related quality of life (HRQOL) in postmenopausal women with prevalent vertebral fractures. Osteoporos Int. 2005; 16:861–70. [PubMed: 15558238]
12. Bliuc D, Nguyen ND, Milch VE, Nguyen TV, Eisman JA, Center JR. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. JAMA. 2009; 301:513–21. [PubMed: 19190316]
13. Ensrud KE, Thompson DE, Cauley JA, Nevitt MC, Kado DM, Hochberg MC, et al. Prevalent vertebral deformities predict mortality and hospitalization in older women with low bone mass. J Am Geriatr Soc. 2000; 48:241–9. [PubMed: 10733048]
14. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res. 2007; 22:465–75. [PubMed: 17144789]
15. Ström, O.; Borgström, F.; Kanis, JA.; Compston, J.; Cooper, C.; McCloskey, EV., et al. Osteoporosis: burden, health care provision and opportunities in the EU. Arch Osteoporos. 2011. http://dx.doi.org/10.1007/s11657-011-0060-1
16. Melton LJ III, Atkinson EJ, Cooper C, O'Fallon WM, Riggs BL. Vertebral fractures predict subsequent fractures. Osteoporos Int. 1999; 10:214–21. [PubMed: 10525713]

17. Black DM, Arden NK, Palermo L, Pearson J, Cummings SR. Study of Osteoporotic Fractures Research Group. Prevalent vertebral deformities predict hip fractures and new vertebral deformities but not wrist fractures. J Bone Miner Res. 1999; 14:821–8. [PubMed: 10320531]
18. Ralston SH, Uitterlinden AG. Genetics of osteoporosis. Endocr Rev. 2010; 31:629–62. [PubMed: 20431112]
19. Liu CT, Karasik D, Zhou Y, Hsu YH, Genant HK, Broe KE, et al. Heritability of prevalent vertebral fracture and volumetric bone mineral density and geometry at the lumbar spine in three generations of the Framingham study. J Bone Miner Res. 2011; 27:954–8. [PubMed: 22222934]
20. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, et al. A family history of fracture and fracture risk: a meta-analysis. Bone. 2004; 35:1029–37. [PubMed: 15542027]
21. Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med. 2010; 363:166–76. [PubMed: 20647212]
22. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet. 2012; 44:491–501. [PubMed: 22504420]
23. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009; 41:1199–206. [PubMed: 19801982]
24. Richards JB, Zheng HF, Spector TD. Genetics of osteoporosis from genome-wide association studies: advances and challenges. Nat Rev Genet. 2012; 13:576–88. [PubMed: 22805710]
25. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011; 26:657–86. [PubMed: 21877163]
26. Langdahl BL, Uitterlinden AG, Ralston SH, Trikalinos TA, Balcells S, Brandi ML, et al. Large-scale analysis of association between polymorphisms in the transforming growth factor beta 1 gene (TGFB1) and osteoporosis: the GENOMOS study. Bone. 2008; 42:969–81. [PubMed: 18284942]
27. Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, et al. Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. PLoS Med. 2006; 3:e90. [PubMed: 16475872]
28. Duncan EL, Danoy P, Kemp JP, Leo PJ, McCloskey E, Nicholson GC, et al. Genome-wide association study using extreme truncate selection identifies novel genes affecting bone mineral density and fracture risk. PLoS Genet. 2011; 7:e1001372. [PubMed: 21533022]
29. Sims AM, Shephard N, Carter K, Doan T, Dowling A, Duncan EL, et al. Genetic analyses in a sample of individuals with high or low BMD shows association with multiple Wnt pathway genes. J Bone Miner Res. 2008; 23:499–506. [PubMed: 18021006]
30. Henry MJ, Pasco JA, Korn S, Gibson JE, Kotowicz MA, Nicholson GC. Bone mineral density reference ranges for Australian men: Geelong Osteoporosis Study. Osteoporos Int. 2010; 21:909–17. [PubMed: 19707703]
31. Henry MJ, Pasco JA, Nicholson GC, Seeman E, Kotowicz MA. Prevalence of osteoporosis in Australian women: Geelong Osteoporosis Study. J Clin Densitom. 2000; 3:261–8. [PubMed: 11090233]
32. McCloskey EV, Beneton M, Charlesworth D, Kayan K, deTakats D, Dey A, et al. Clodronate reduces the incidence of fractures in community-dwelling elderly women unselected for osteoporosis: results of a double-blind, placebo-controlled randomized study. J Bone Miner Res. 2007; 22:135–41. [PubMed: 17042717]
33. Harslof T, Husted LB, Carstens M, Stenkjaer L, Langdahl BL. Genotypes and haplotypes of the estrogen receptor genes, but not the retinoblastoma-interacting zinc finger protein 1 gene, are associated with osteoporosis. Calcif Tissue Int. 2010; 87:25–35. [PubMed: 20508921]
34. Dobnig H, Piswanger-Solkner JC, Roth M, Obermayer-Pietsch B, Tiran A, Strele A, et al. Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass, and fracture risk. J Clin Endocrinol Metab. 2006; 91:3355–63. [PubMed: 16735485]

35. Martinez J, Olmos JM, Hernandez JL, Pinedo G, Llorca J, Obregon E, et al. Bone turnover markers in Spanish postmenopausal women: the Camargo cohort study. Clin Chim Acta. 2009; 409:70–4. [PubMed: 19737549]
36. Olmos JM, Hernandez JL, Martinez J, Pariente E, Llorca J, Gonzalez-Macias J. Bone turnover markers in Spanish adult men: the Camargo cohort study. Clin Chim Acta. 2010; 411:1511–5. [PubMed: 20594548]
37. Riancho JA, Valero C, Naranjo A, Morales DJ, Sanudo C, Zarrabeitia MT. Identification of an aromatase haplotype that is associated with gene expression and postmenopausal osteoporosis. J Clin Endocrinol Metab. 2007; 92:660–5. [PubMed: 17118999]
38. Zarrabeitia MT, Hernandez JL, Valero C, Zarrabeitia A, Amado JA, Gonzalez-Macias J, et al. Adiposity, estradiol, and genetic variants of steroid-metabolizing enzymes as determinants of bone mineral density. Eur J Endocrinol. 2007; 156:117–22. [PubMed: 17218734]
39. Prince RL, Devine A, Dhaliwal SS, Dick IM. Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double-blind, placebo-controlled trial in elderly women. Arch Intern Med. 2006; 166:869–75. [PubMed: 16636212]
40. Richards JB, Leslie WD, Joseph L, Siminoski K, Hanley DA, Adachi JD, et al. Changes to osteoporosis prevalence according to method of risk assessment. J Bone Miner Res. 2007; 22:228–34. [PubMed: 17129177]
41. Richards JB, Papaioannou A, Adachi JD, Joseph L, Whitson HE, Prior JC, et al. Effect of selective serotonin reuptake inhibitors on the risk of fracture. Arch Intern Med. 2007; 167:188–94. [PubMed: 17242321]
42. Tenenhouse A, Joseph L, Kreiger N, Poliquin S, Murray TM, Blondeau L, et al. Estimation of the prevalence of low bone density in Canadian women and men using a population-specific DXA reference standard: the Canadian Multicentre Osteoporosis Study (CaMos). Osteoporos Int. 2000; 11:897–904. [PubMed: 11199195]
43. Mosekilde L, Hermann AP, Beck-Nielsen H, Charles P, Nielsen SP, Sorensen OH. The Danish Osteoporosis Prevention Study (DOPS): project design and inclusion of 2000 normal perimenopausal women. Maturitas. 1999; 31:207–19. [PubMed: 10340280]
44. Ismail AA, O'Neill TW, Cockerill W, Finn JD, Cannata JB, Hoszowski K, et al. Validity of self-report of fractures: results from a prospective study in men and women across Europe. EPOS Study Group European Prospective Osteoporosis. Study Group Osteoporos Int. 2000; 11:248–54.
45. O'Neill TW, Felsenberg D, Varlow J, Cooper C, Kanis JA, Silman AJ. The prevalence of vertebral deformity in European men and women: the European Vertebral Osteoporosis Study. J Bone Miner Res. 1996; 11:1010–8. [PubMed: 8797123]
46. Dawber TR, Meadors GF, Moore FE Jr. Epidemiological approaches to heart disease: the Framingham study. Am J Public Health Nations Health. 1951; 41:279–81. [PubMed: 14819398]
47. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 1979; 110:281–90. [PubMed: 474565]
48. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, et al. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. Am J Epidemiol. 2007; 165:1328–35. [PubMed: 17372189]
49. Koh JM, Oh B, Lee JY, Lee JK, Kimm K, Park BL, et al. Association of FLT3 polymorphisms with low BMD and risk of osteoporotic fracture in postmenopausal women. J Bone Miner Res. 2007; 22:1752–8. [PubMed: 17620055]
50. Deeg DJ, van Tilburg T, Smit JH, de Leeuw ED. Attrition in the Longitudinal Aging Study Amsterdam. The effect of differential inclusion in side studies. J Clin Epidemiol. 2002; 55:319–28. [PubMed: 11927198]
51. Mellstrom D, Johnell O, Ljunggren O, Eriksson AL, Lorentzon M, Mallmin H, et al. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res. 2006; 21:529–35. [PubMed: 16598372]

52. Bagger YZ, Rasmussen HB, Alexandersen P, Werge T, Christiansen C, Tanko LB. Links between cardiovascular disease and osteoporosis in postmenopausal women: serum lipids or atherosclerosis per se? Osteoporos Int. 2007; 18:505–12. [PubMed: 17109061]
53. McCloskey EV, Spector TD, Eyres KS, Fern ED, O'Rourke N, Vasikaran S, et al. The assessment of vertebral deformity: a method for use in population studies and clinical trials. Osteoporos Int. 1993; 3:138–47. [PubMed: 8481590]
54. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993; 8:1137–48. [PubMed: 8237484]
55. The International HapMap Project. Nature. 2003; 426:789–96. [PubMed: 14685227]
56. Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, et al. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467:52–8. [PubMed: 20811451]
57. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annu Rev Genomics Hum Genet. 2009; 10:387–406. [PubMed: 19715440]
58. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–34. [PubMed: 21058334]
59. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–9. [PubMed: 16862161]
60. Estrada K, Abuseiris A, Grosveld FG, Uitterlinden AG, Knoch TA, Rivadeneira F. GRIMP: a web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. Bioinformatics. 2009; 25:2750–2. [PubMed: 19700477]
61. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–1. [PubMed: 20616382]
62. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet. 2011; 88:586–98. [PubMed: 21565292]
63. Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Bejjani BA, et al. Genomic and genic deletions of the FOX gene cluster on 16q24.1 and inactivating mutations of FOXF1 cause alveolar capillary dysplasia and other malformations. Am J Hum Genet. 2009; 84:780–91. [PubMed: 19500772]
64. Nifuji A, Miura N, Kato N, Kellermann O, Noda M. Bone morphogenetic protein regulation of forkhead/winged helix transcription factor Foxc2 (Mfh1) in a murine mesodermal cell line C1 and in skeletal precursor cells. J Bone Miner Res. 2001; 16:1765–71. [PubMed: 11585339]
65. Kim SH, Cho KW, Choi HS, Park SJ, Rhee Y, Jung HS, et al. The forkhead transcription factor Foxc2 stimulates osteoblast differentiation. Biochem Biophys Res Commun. 2009; 386:532–6. [PubMed: 19540201]
66. Winnier GE, Hargett L, Hogan BL. The winged helix transcription factor MFH1 is required for proliferation and patterning of paraxial mesoderm in the mouse embryo. Genes Dev. 1997; 11:926–40. [PubMed: 9106663]
67. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007; 447:1087–93. [PubMed: 17529967]
68. Hoover RN. The evolution of epidemiologic research: from cottage industry to "big" science. Epidemiology. 2007; 18:13–7. [PubMed: 17179754]
69. Panagiotou OA, Ioannidis JP. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol. 2012; 41:273–86. [PubMed: 22253303]
70. Ioannidis JP. Why most discovered true associations are inflated. Epidemiology. 2008; 19:640–8. [PubMed: 18633328]
71. Kraft P, Zeggini E, Ioannidis JP. Replication in genome-wide association studies. Stat Sci. 2009; 24:561–73. [PubMed: 20454541]
72. Zhong H, Prentice RL. Correcting "winner's curse" in odds ratios from genomewide association findings for major complex human diseases. Genet Epidemiol. 2010; 34:78–91. [PubMed: 19639606]

73. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet. 2003; 33:177–82. [PubMed: 12524541]
74. Montpetit A, Nelis M, Laflamme P, Magi R, Ke X, Remm M, et al. An evaluation of the performance of tag SNPs derived from HapMap in a Caucasian population. PLoS Genet. 2006; 2:e27. [PubMed: 16532062]
75. Chen F, Stram DO, Le Marchand L, Monroe KR, Kolonel LN, Henderson BE, et al. Caution in generalizing known genetic risk markers for breast cancer across all ethnic/racial populations. Eur J Hum Genet. 2011; 19:243–5. [PubMed: 21102626]
76. He J, Wilkens LR, Stram DO, Kolonel LN, Henderson BE, Wu AH, et al. Generalizability and epidemiologic characterization of eleven colorectal cancer GWAS hits in multiple populations. Cancer Epidemiol Biomarkers Prev. 2011; 20:70–81. [PubMed: 21071539]
77. Sim X, Ong RT, Suo C, Tay WT, Liu J, Ng DP, et al. Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. PLoS Genet. 2011; 7:e1001363. [PubMed: 21490949]
78. Stromberg U, Bjork J, Vineis P, Broberg K, Zeggini E. Ranking of genome-wide association scan signals by different measures. Int J Epidemiol. 2009; 38:1364–73. [PubMed: 19734549]
79. Oei L, Rivadeneira F, Ly F, Breda SJ, Zillikens MC, Hofman A, et al. Review of radiological scoring methods of osteoporotic vertebral fractures for clinical and research settings. Eur Radiol. 2013; 23(2):476–86. [PubMed: 22892721]
80. Puisto V, Heliovaara M, Impivaara O, Jalanko T, Kroger H, Knekt P, et al. Severity of vertebral fracture and risk of hip fracture: a nested case–control study. Osteoporos Int. 2011; 22:63–8. [PubMed: 20195843]
81. Jiang G, Eastell R, Barrington NA, Ferrar L. Comparison of methods for the visual identification of prevalent vertebral fracture in osteoporosis. Osteoporos Int. 2004; 15:887–96. [PubMed: 15071725]
82. Kerkhof HJ, Meulenbelt I, Akune T, Arden NK, Aromaa A, Bierma-Zeinstra SM, et al. Recommendations for standardization and phenotype definitions in genetic studies of osteoarthritis: the TREAT-OA consortium. Osteoarthritis Cartilage. 2011; 19:254–64. [PubMed: 21059398]
83. Reeve J, Lunt M, Felsenberg D, Silman AJ, Scheidt-Nave C, Poor G, et al. Determinants of the size of incident vertebral deformities in European men and women in the sixth to ninth decades of age: the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res. 2003; 18:1664–73. [PubMed: 12968676]
84. Felsenberg D, Silman AJ, Lunt M, Armbrecht G, Ismail AA, Finn JD, et al. Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res. 2002; 17:716–24. [PubMed: 11918229]
85. Lunt M, Ismail AA, Felsenberg D, Cooper C, Kanis JA, Reeve J, et al. Defining incident vertebral deformities in population studies: a comparison of morphometric criteria. Osteoporos Int. 2002; 13:809–15. [PubMed: 12378370]
86. Lunt M, Gowin W, Johnell, Armbrecht G, Felsenberg D. A statistical method to minimize magnification errors in serial vertebral radiographs. Osteoporos Int. 2001; 12:909–13. [PubMed: 11804017]

Abbreviations

BMD	bone mineral density
GWAS	genome-wide association study
SNP	single nucleotide polymorphism
GEFOS	Genetic Factors for Osteoporosis

GENOMOS	Genetic Markers for Osteoporosis
AOGC	Anglo-Australasian Osteoporosis Genetics Consortium
AOGC-GOS	Anglo-Australasian Osteoporosis Genetics Consortium — Geelong Osteoporosis Study
AROS	Aarhus Osteoporosis Study
CABRIO-C	Cantabria-Camargo study
CABRIO-CC	Cantabria Case–Control study
CAIFOS	Calcium Intake Fracture Outcome Study
CaMoS	Canadian Multicentre Osteoporosis Study
DOPS	Danish Osteoporosis Prevention Study
HRT	hormone-replacement therapy
EDOS	Edinburgh Osteoporosis Study
EPOS	European Prospective Osteoporosis Study
EVOS	European Vertebral Osteoporosis Study
FOS	Framingham Osteoporosis Study
CT	computed tomography
KorAMC	Korean osteoporosis study at Asan Medical Center
LASA	Longitudinal Aging Study Amsterdam
MrOS Sweden	Osteoporotic Fractures in Men Sweden
PERF	Prospective Epidemiological Risk Factor
FN	femoral neck
LS	lumbar spine
DXA	dual-energy X-ray absorptiometry
BMI	body mass index
OR	odds ratio
MAF	minor allele frequency
PCs	principal components
GEE	generalized estimating equation
VACTERL	Vertebral anomalies, Anal atresia, Cardiovascular anomalies, TracheoEsophageal fistula, Renal and Radial anomalies, Limb defects

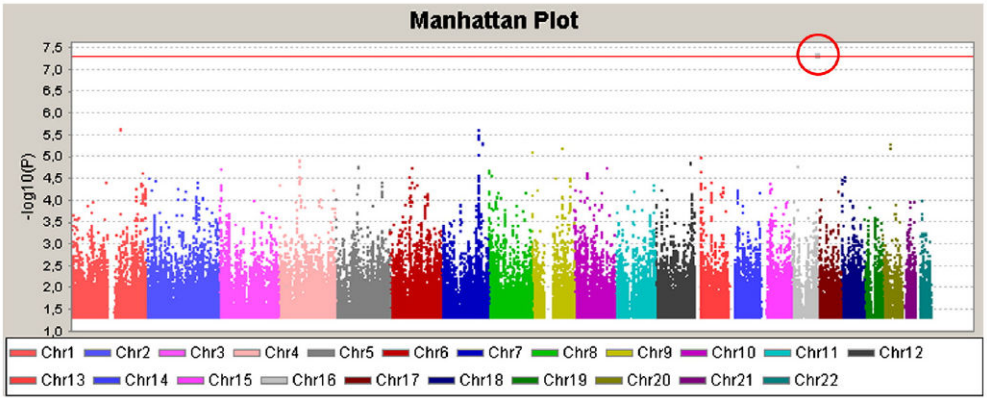

Fig. 1.

Manhattan plot of negative logarithm *p*-values plotted by chromosome, showing that a SNP on chromosome 16q24 was associated at a genome-wide significant level with osteoporotic vertebral fractures ($p=4.6 \times 10^{-8}$) in the Rotterdam Study (encircled).

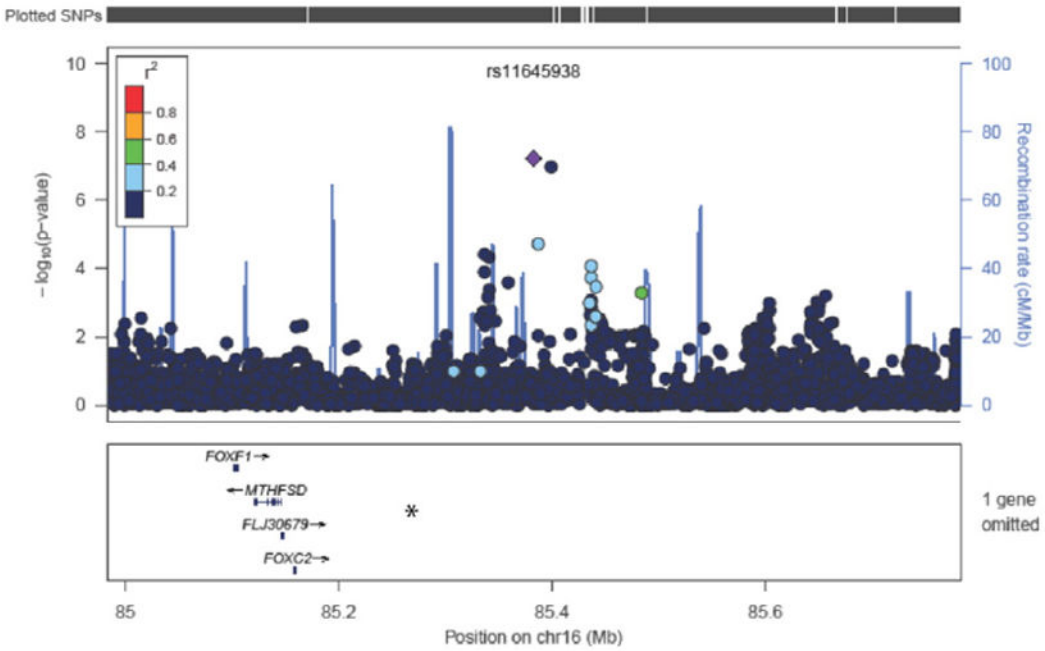

Fig. 2.

Regional association plot showing position on chromosome 16 and association *p*-values of the analyzed SNPs in the Rotterdam Study with neighboring genes. Included are genotyped, HapMap II and 1000 Genomes imputed SNPs. The rectangle is the SNP of interest, and the circles represent neighboring SNPs with their respective correlation with the top marker. The spikes depict the recombination rates. The position of the rs10048146 SNP that has previously been found as associated with lumbar-spine bone mineral density is indicated with *.

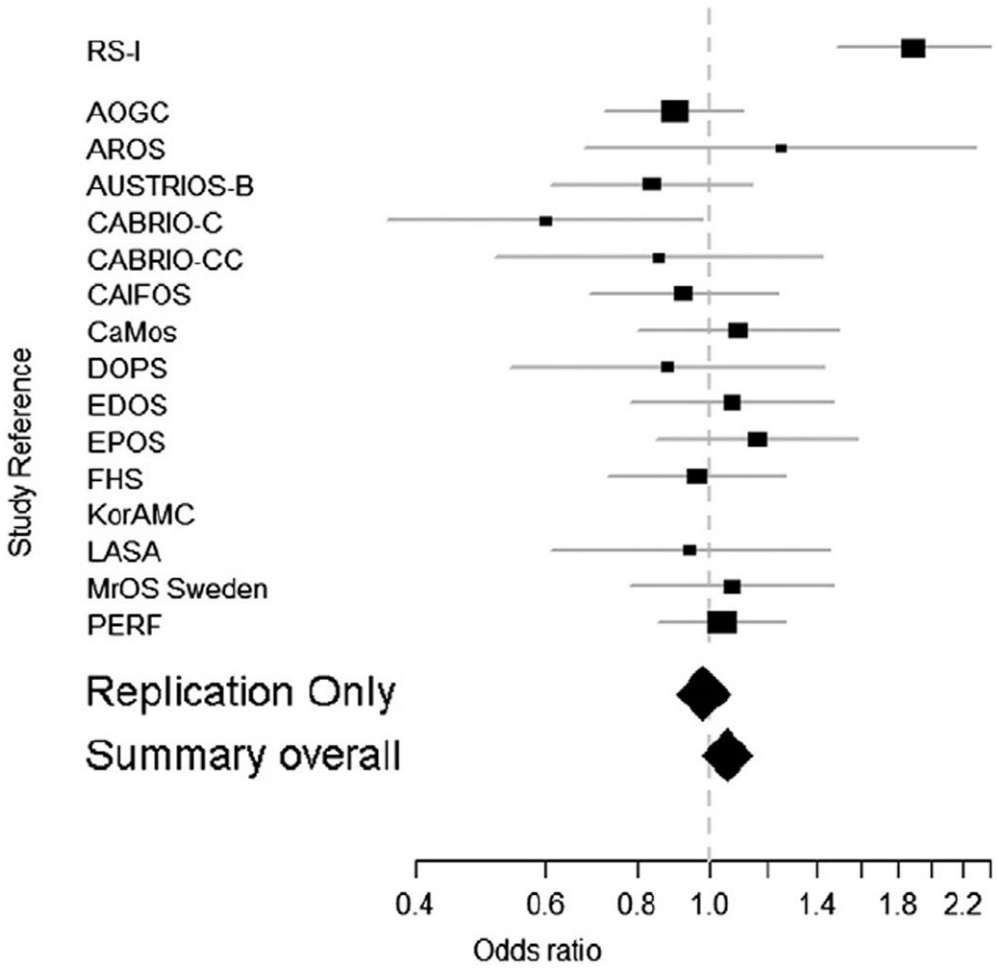

Fig. 3.

Forest plot showing meta-analysis results of vertebral fracture risk for rs11645938 in discovery and replication studies. Effect estimates represented by squares are displayed on a logarithmic scale, with horizontal lines corresponding to 95% confidence intervals. The center line of the diamond stands for the overall summary measure, and its horizontal line indicates the 95% confidence interval.

Table 1

Vertebral fracture assessment.

Study	Morphometry method used	Number of vertebral fracture cases	Number of vertebral fracture controls	Prevalence or case:control ratio[b]	Comparison setting		Cut-off values used		Fractures confirmed by expert[a]
					Relative to population reference	Absolute height reduction[c]	McCloskey–Kanis	Genant	
							3 SD relative reduction[d]	Difference in vertebral height ratios of at least:	
RS-I	McCloskey–Kanis	329	2666	0.11	Yes	Yes	Yes	NA	Yes
AOGC	McCloskey–Kanis	686	3411	0.17	Yes	No	Yes	NA	Yes
AROS	Genant	335	130	1:0.39[b]	No	No	NA	20%	Yes
AUSTRIOS-B	Genant	803	1261	0.39	No	No	NA	20%	Yes
CABRIO-C	Genant	195	1185	0.14	No	No	NA	20%	Yes
CABRIO-CC	Genant	220	354	1:1.61[b]	No	No	NA	20%	Yes
CAIFOS	McCloskey–Kanis	428	600	0.42	Yes	No	Yes	NA	Yes
CaMoS	McCloskey–Kanis	243	1785	0.12	Yes	No	NA	NA	Yes
DOPS	Genant	108	1605	0.06	No	No	NA	20%	Yes
EDOS	Genant	495	523	0.49	No	No	NA	20%	Yes
EPOS	McCloskey–Kanis	313	1779	0.15	Yes	Yes	Yes	NA	Yes
FOS	Genant	417	2291	0.15	No	No	NA	20%	Yes
KorAMC	Genant	101	1193	0.08	No	Yes	NA	20%	Yes
LASA	Genant	237	268	0.47	No	No	NA	20%	Yes
MrOS Sweden[e]	Genant	309	2613	0.11	No	No	NA	20%	No
PERF	Genant	830	2793	0.23	No	No	NA	20%	No

[a] E.g. radiologist/clinician, to rule out artifacts and other etiologies, such as pathological fractures.

[b] Prevalence in population-based studies, case:control ratio in case–control studies.

[c] Any of the three vertebral heights (anterior, central, or posterior) shows a minimum decrease of at least 4 mm.

[d] 3 SD relative reduction of 2 out of 3 ratios: (ha/hp; hm/hp; hp/hp predicted).

[e] Prevalent X-ray verified vertebral fractures only available for about 1425 subjects.

Table 2

Descriptive information about genotyping of the rs11645938 SNP and association statistics per study.

Study	SNP call rate	*p*-Value Hardy–Weinberg equilibrium	Minor allele frequency	Effect estimate (beta)	Standard error	*p*-Value
RS-I	99.9%	0.52	9.65%	0.669	0.122	4.6E–08
AOGC	93.7%	0.83	9.74%	−0.11	0.11	0.33
AROS	99.3%	0.79	9.76%	0.22	0.31	0.61
AUSTRIOS-B	97.0%	0.96	11.74%	−0.18	0.16	0.26
CABRIO-C	99.1%	0.84	7.71%	−0.51	0.25	0.04
CABRIO-CC	99.1%	0.61	8.98%	−0.16	0.26	0.53
CAIFOS	99.2%	0.02	10.01%	−0.02	0.15	0.91
CaMoS	99.0%	0.12	9.57%	0.09	0.16	0.50
DOPS	98.6%	0.17	10.01%	−0.13	0.25	0.59
EDOS	99.4%	0.99	9.45%	0.07	0.16	0.65
EPOS	99.6%	0.80	9.62%	0.15	0.16	0.75
FOS	97.6%	0.86	9.97%	−0.04	0.14	0.78
KorAMC	97.8%	NA	0.00%	NA	NA	NA
LASA	100.0%	0.82	11.16%	−0.06	0.22	0.79
MrOS Sweden	98.6%	0.49	9.77%	0.07	0.16	0.69
PERF	100.0%	0.79	9.45%	0.04	0.10	0.70